# LRR-containing receptors regulating plant development and defense

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Development 131, 251-261
Published by The Company of Biologists 2004
doi:10.1242/dev.00998

## Summary

**Despite the presence of more than 400 genes that encode receptor-like kinases (RLKs) in the *Arabidopsis thaliana* genome, very little is known about the range of biological processes that they control, or the mechanisms by which they function. This review focuses on the most recent findings from studies of several leucine-rich-repeat (LRR) class RLKs in *A. thaliana*, and their implications for our understanding of plant receptor function and signaling. We compare the biological functions of plant and animal LRR-containing receptors, and the potential commonalities in the signaling mechanisms employed.**

## Introduction

One of the fundamental mechanisms by which cells communicate in multicellular organisms is through the secretion of ligands that then bind to cell surface receptors possessing protein kinase catalytic activities. Given that plant cells are separated from one another by cell walls, it was assumed for decades that most cell-to-cell communication would occur via the cytoplasmic bridges called plasmodesmata. However, in 1990, Walker and Zhang identified the first plant receptor-like kinase (RLK) in maize (Walker and Zhang, 1990) and, since then, many RLKs have been identified from the flowering plant *Arabidopsis thaliana* and other plant species. These findings demonstrate that, like other eucaryotes, plant cells are able to perceive external signals at the plasma membrane.

An extensive phylogenic analysis of the RLKs in *A. thaliana* has revealed that more than 400 genes encode putative plant receptor kinases (PRKs) (reviewed by Tichtinsky et al., 2003; Shiu and Bleecker, 2001a; Shiu and Bleecker, 2001b), defined as proteins that contain an extracellular domain, a single-pass transmembrane domain and a cytoplasmic serine/threonine (ser/thr) protein kinase domain (Shiu and Bleecker, 2001a; Shiu and Bleecker, 2001b). The PRKs can be classified on the basis of their extracellular domains (Shiu and Bleecker, 2002). Leucine-rich repeat (LRR)-containing PRKs represent the largest group of PRKs in the *A. thaliana* genome, with 216 members. These represent 13 subfamilies (LRR I to XIII), which can be classified according to the organization of the LRRs in the extracellular domain (Shiu and Bleecker, 2002). Plant LRR-RLKs possess a functional cytoplasmic kinase domain, and all of the plant LRR-RLKs analyzed to date possess ser/thr kinase activity. The phenotypes associated with mutations in various LRR-PRKs show that they play roles in diverse processes during growth and development (Table 1A).

In animals, numerous studies have reported functions for LRR-containing receptors in neuronal development, pattern formation, differentiation and growth of gonads and thyroid glands, and antifungal responses (Table 1B). These receptors contain LRRs in the extracellular portion of the protein, and either possess a transmembrane domain(s) or are anchored to the plasma membrane by a glycosylphosphatidylinositol (GPI) linkage. However, unlike plant LRR-RLKs, animal LRR-containing receptors do not contain a cytoplasmic kinase domain, but instead are thought to transduce their signal through the activation of co-receptors. Nevertheless, the LRR motifs found in the extracellular part of these receptors are alike in plants and animals, indicating that they could share similar mechanisms of ligand binding and activation.

This review will focus on the developmental processes that are affected by mutations in LRR-containing receptors, describing what we know about the ligands, the receptors and the pathways that are activated during some signaling pathways in plants. Out of the 216 LRR-RLKs in *A. thaliana*, only 10 or so have known functions, and only four have been extensively studied. Presented in Table 1 are many of the LRR receptors of plants, and of other organisms, for which functions have been attributed. Recent studies, in plants and animals, have led to a better understanding of a number of signaling pathways that involve LRR-containing receptors, and a comparison of the recent advances gives us some insights into the conserved and divergent signaling mechanisms within plants, and between plants and animals.

## The CLAVATA pathway

The development of higher plants is largely postembryonic, and plant embryos contain few of the organs found in the adult plant. Plant embryos are simple in structure (Fig. 1). The apical end of the embryo contains a shoot meristem, which produces the above-ground organs and tissue of the plant, namely the stems, the leaves and the flowers. The basal end of the embryo contains a root meristem, which gives rise to the root system. Plant organs are formed from shoot and root meristems during post-embryonic development. Cytohistological examination of shoot meristems has revealed that the shoot apex is partitioned into radial domains (Fig. 2D) (Steeves and Sussex, 1989). This organization defines at least three functionally distinct zones of the shoot meristem: the central zone (CZ), the peripheral

zone (PZ) and the rib zone (RZ). The CZ is composed of stem cells with low mitotic activity. This CZ is surrounded at the meristem flanks by the PZ (Fig. 2D), where progeny of the stem cells divide more rapidly than those at the center and are incorporated into organ primordia. Underneath these two zones, the RZ gives rise to the internal part of the stems. As the size and shape of the shoot meristem are defined early in embryogenesis and remain relatively constant during normal development, the meristem has to maintain a tight balance between the proliferation of stem cells at the CZ and the targeting of these cells towards differentiation at the periphery. Very similar and evolutionaily related zones to those found in the shoot meristem are found within flower meristems during the initiation of flower organs. Within the last few years, genetic analyses, along with gene expression and biochemical data, have uncovered the importance of cell-cell interactions, and the involvement of an LRR-RLK, in maintaining the size homeostasis of meristems.

In *A. thaliana*, loss-of-function mutations in any of the three *CLAVATA* (*CLV*) genes (*CLV1*, *CLV2* and *CLV3*) cause an ectopic accumulation of stem cells in the CZ, and a progressive enlargement of the shoot meristem (Fig. 2). Very similar defects are found within *clv* flowers, in which an early accumulation of stem cells leads to additional organs formed of each type and the continued proliferation of the flower beyond the normally terminal carpels. These *clv* mutant

**Table 1. Function of LRR-RLKs in plants and LRR-containing receptors in animals**

**A. Function of LRR-RLKs in plants**

| | Genes | Functions | Organism | References |
|---|---|---|---|---|
| Development | CLAVATA1 (CLV1) | Meristem differentiation | *Arabidopsis* | (Clark et al., 1997) |
| | Phytosulfokine receptor (PSK receptor) | Peptide hormone binding | Tomato | (Matsubayashi et al., 2002) |
| | ERECTA (ER) | Overall plant shape | *Arabidopsis* | (Torii et al., 1996) |
| | Ipomocea nil receptor protein kinase 1 (INRPK1) | Short-day photoperiodic floral induction | Ipomocea nil | (Bassett et al., 2000) |
| | HAESA/RLK5 | Floral organ abscission | *Arabidopsis* | (Jinn et al., 2000) |
| | Excess microsporocytes 1 (EMS1)/Extra sporogenous cells (EXS) | Endosperm and pollen development | *Arabidopsis* | (Zhao et al., 2002a; Canales et al., 2002) |
| | Somatic embryogenesis receptor 1 (AtSERK1) | Ovule development and early embryogenesis | *Arabidopsis* | (Hecht et al., 2001) |
| | Brassinosteroid insensitive 1 (BRI1) and BRI1 associated receptor kinase 1 (BAK1) | Perception of BR | *Arabidopsis* | (Li and Chory, 1997; Li et al., 2002; Nam and Li, 2002) |
| | VASCULAR HIGHWAY1 (VH1) | Leaf patterning | *Arabidopsis* | (Clay and Nelson, 2002) |
| | Systemin receptor (SR160)/CURL3/tBRI1 | Perception of BR and systemin | Tomato | (Montoya et al., 2002; Scheer and Ryan, 2002) |
| Symbiosis | Nodule autoregulation receptor kinase (GmNARK)/ Hyper autoregulation of nodulation receptor 1 (HAR1) | Autoregulation of nodulation | Soybean *Lotus japonicus* | (Searle et al., 2003; Krusell et al., 2002; Nishimura et al., 2002) |
| | Symbiosis receptor-like kinase (SYMRK)/ Nodulation receptor kinase (NORK) | Root nodule formation | *Lotus japonicus* *Medicago sativa* | (Stracke et al., 2002; Endre et al., 2002) |
| Host defense | FLAGELLIN SENSITIVE 2 (FLS2) | Plant defense/pathogen recognition | *Arabidopsis* | (Gomez-Gomez and Boller, 2000) |
| | Xa21 | Fungal perception00 | Rice | (Song et al., 1995) |

**B. Function of LRR-containing receptors in animals**

| | Genes | Functions | Organism | References |
|---|---|---|---|---|
| Development | Tartan Capricious (Caps) Connectin | Neuromuscular connections in embryos/ cell interaction during DV boundary formation | *Drosophila* | (Chang et al., 1993; Shishido et al., 1998; Meadows et al., 1994) |
| | Nyctalopin (NYX) | Retina development | Human | (Bech-Hansen et al., 2000; Pusch et al., 2000) |
| | Windpipe (wdp) | Trachea development? | *Drosophila* | (Huff et al., 2002) |
| | Gp150 | Development of eyes, wings and sensory organs | Animals | (Fetchko et al., 2002) |
| | Nogo receptor (NgR) | Neuronal regeneration after injury | Mammals | (Fournier et al., 2001) |
| | NgR homologue 1 (NgRH1) | ? | | (Pignot et al., 2003) |
| | NgR homologue 2 (NgRH2) | ? | | |
| | Slitrk (Slit/Trk family) | Neuritogenesis | Mammals | (Aruga and Mikoshiba, 2003) |
| | LRR transmembrane proteins (LRRTMs) | Development of CNS? | Mammals | (Lauren et al., 2003) |
| | LRR induced by β-amyloid (Lib) | ? | Mammals | (Satoh et al., 2002) |
| | Toll receptor | Axis formation and host defense | *Drosophila* | (Hashimoto et al., 1988; Lemaitre et al., 1996) |
| Host defense | Synleurin | Increase sensitivity to cytokines and lipopolysaccharides | Human | (Wang et al., 2003) |
| | Toll-like receptors (TLRs) | Host defense | Mammals | (Dunne and O'Neill, 2003) |
| | RP105/CD180 | TLR of B lymphocytes | Mammals | (Miyake et al., 1995) |

phenotypes result in club-shaped (*clavatus* in Latin) fruits (Fig. 2). Although mutations of all three genes lead to similar phenotypes, the *clv1* null mutant has a subtle phenotype, whereas the phenotype of the *clv3* null mutant is more severe. The *CLV* loci encode signal transduction components. CLV1 is an LRR-RLK, which possesses an extracellular domain containing 21 LRRs, a transmembrane domain and a functional cytoplasmic ser/thr kinase domain (Clark et al., 1997; Williams et al., 1997; Stone et al., 1998). It is predicted that CLV1 is targeted to the plasma membrane, but its subcellular localization has not yet been demonstrated. CLV2 is a receptor-like protein, composed of 21 LRRs in the predicted extracellular part of the receptor (which contains an 'island' of non-LRR sequence between the 17th and the 18th repeats) and a short predicted cytoplasmic tail that has no known sequence motifs (Jeong et al., 1999; Kayes and Clark, 1998). CLV3 is a small 96-amino acid polypeptide that may act as a ligand for the CLV1 and/or CLV2 receptors (Fletcher et al., 1999; Rojo et al., 2002).

Molecular genetic studies have indicated that the primary function of proteins encoded by the *CLV* loci is to restrict the expression domain of a stem-cell promoting factor called *WUSCHEL* (*WUS*), which encodes a homeodomain transcription factor (Laux et al., 1996; Mayer et al., 1998). *WUS* is necessary for stem cell specification; *wus* mutants repeatedly form shoots and flowers that lack stem cells, and, as a consequence, lack extended organogenesis (Laux et al., 1996). The *wus* mutation is fully epistatic to *clv* mutations, indicating that WUS acts downstream of the CLV1 pathway. *WUS* is expressed in a small group of cells situated below the CZ of the shoot and flower meristems, called the organizing center (OC) (Mayer et al., 1998). The mechanism by which *WUS* expression in the underlying OC leads to stem cell specification in overlying CZ cells is unclear. In shoot apices, *WUS* overexpression is sufficient to induce stem cell identity, and also *CLV3* gene expression in adjacent cells (Brand et al., 2000; Schoof et al., 2000). The induction of *CLV3* by *WUS* expression forms a putative feedback loop that regulates the size of the stem cell population in the shoot meristem, in which CLV3 restricts expression of *WUS* and *WUS* induces the expression of *CLV3* (Schoof et al., 2000).

Several recent studies have revealed key aspects of CLV signaling. An elegant study carried out by Fletcher and co-workers has shown that for CLV3 to function, it must be secreted into the extracellular space within the meristem (Rojo et al., 2002). They assessed the importance of the signal peptide and the secretion of CLV3 for the proper function of CLV3, using *CLV3-GFP* fusion cDNAs driven by the constitutive viral 35S promoter. These constructs induced the constitutive production of CLV3-GFP fusion proteins. In *clv3* transgenic plants possessing a version of this construct in which the putative signal peptide of the CLV3-GFP fusion

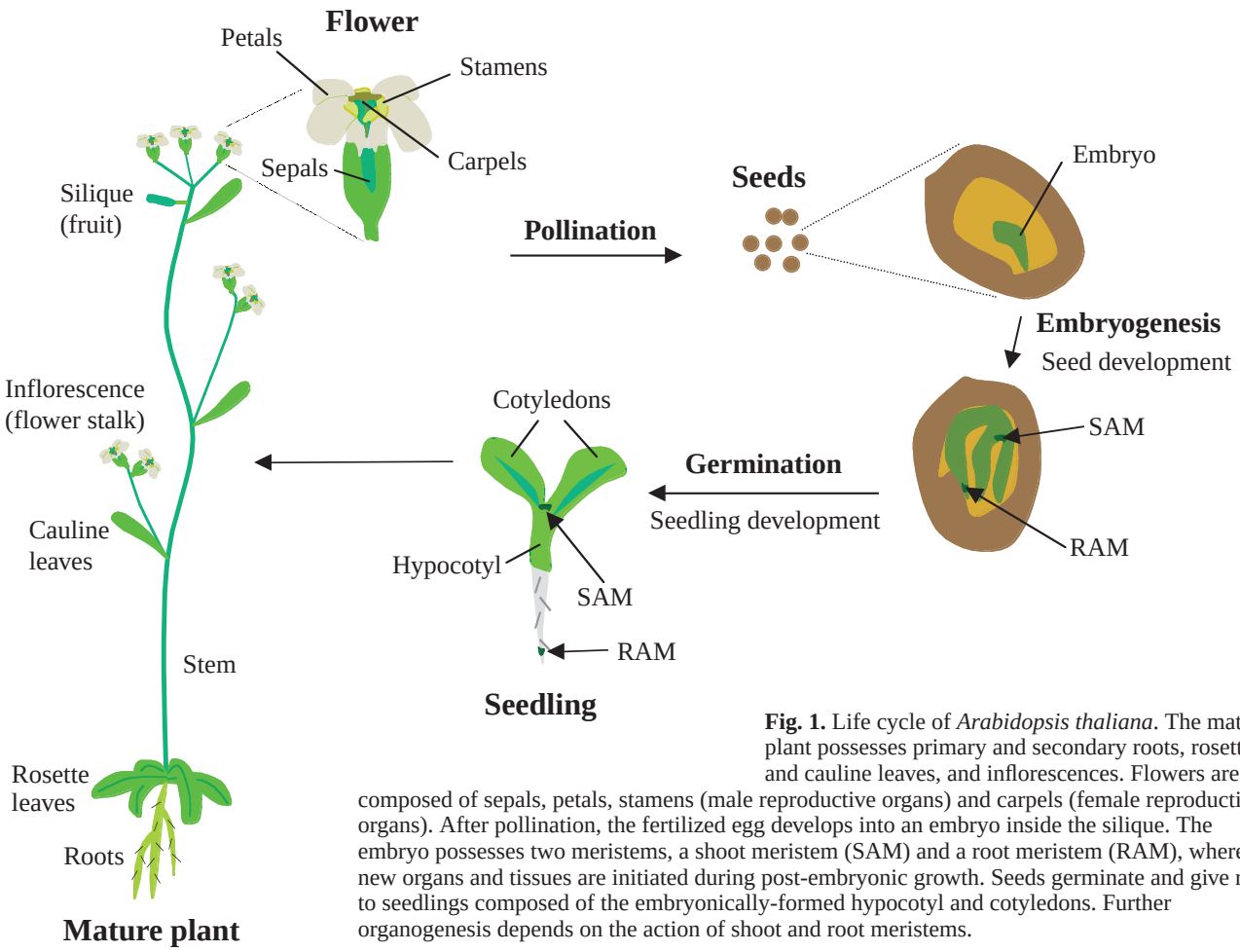


**Fig. 1.** Life cycle of *Arabidopsis thaliana*. The mature plant possesses primary and secondary roots, rosette and cauline leaves, and inflorescences. Flowers are composed of sepals, petals, stamens (male reproductive organs) and carpels (female reproductive organs). After pollination, the fertilized egg develops into an embryo inside the silique. The embryo possesses two meristems, a shoot meristem (SAM) and a root meristem (RAM), where new organs and tissues are initiated during post-embryonic growth. Seeds germinate and give rise to seedlings composed of the embryonically-formed hypocotyl and cotyledons. Further organogenesis depends on the action of shoot and root meristems.

protein is deleted, the protein is localized in the cell cytoplasm, and no rescue of the *clv3* phenotype is observed. However, when a wild-type full-length *CLV3-GFP* fusion cDNA is used, the protein is detected outside of the cells in the apoplastic space, and it is able to suppress the accumulation of meristematic cells in the *clv3* mutant plants. Targeting CLV3, through the secretory system, to the vacuole by use of a vacuolar sorting signal from barley lectin also blocked CLV3 function (Rojo et al., 2002). These experiments demonstrate that CLV3 must be secreted into the extracellular space within the meristem in order to function.

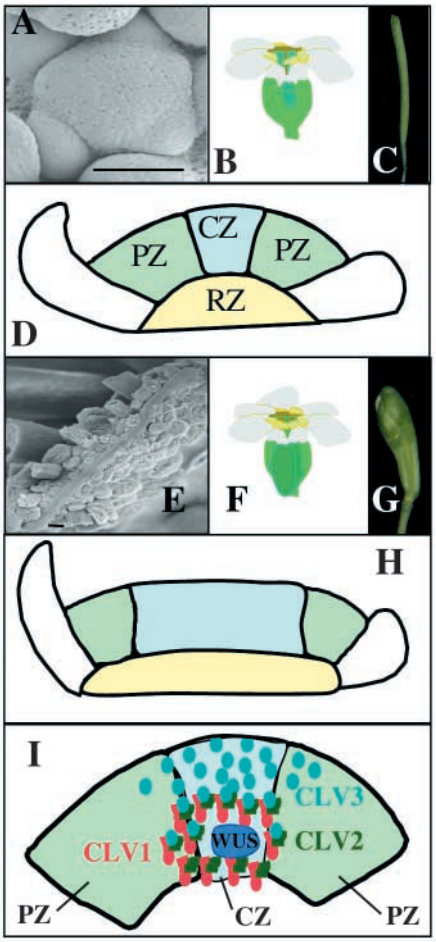


**Fig. 2.** The shoot meristem. Scanning electron microscopy of a wild-type shoot meristem (A) reveals little morphological differentiation, except for the distinct organ primorida (op), whereas molecular and detailed histological studies have revealed important subdomains (PZ, peripheral zone; CZ, central zone; RZ, rib zone) of the shoot meristem (D). The flower meristem gives rise to a regular number of floral organs and organ types (B), eventually forming the fruit (also called the silique; C). In plants carrying a mutation in one of the *CLV* genes, the shoot meristem can be massively enlarged (E), as a result of expansion of the population of stem cells (H). *clv* flower meristems also accumulate stem cells, resulting in the formation of additional organs of each type (F), and leading to distorted, club-shaped fruits (G). (I) A model for CLV signaling. The CLV3 protein (blue) is secreted from the stem cells and diffuses to the underlying cell layers, where CLV1 (red) is expressed. There, CLV1 binds to CLV3 and activates signaling to repress *WUS* expression, as well as to sequester CLV3 to prevent it from diffusing to the *WUS*-expressing region. CLV2 (green) is a putative co-receptor with CLV1. Scale bar in A and E: 50 μm.

Experiments performed by Lenhard and Laux have gone further to determine whether or not CLV3 proteins can move within the shoot meristem (Lenhard and Laux, 2003). They observed that when a *CLV3-GFP* fusion construct was expressed under the control of the endogenous *CLV3* promoter and transformed into *clv3* mutant plants, CLV3-GFP could diffuse within the shoot meristem to positions several cells away from the stem cell that secreted it, and could suppress the *clv3* mutant phenotype. Furthermore, expression of CLV3 protein from an epidermis-specific promoter not only suppresses the mutant phenotype of *clv3* plants, but also results in a *wus*-like phenotype with early termination of the meristem. This indicates that the CLV3 protein can diffuse from the epidermal cells to the center of the meristem where *WUS* expression is normally maintained. This long-range effect was not observed in a *clv1* mutant background, indicating that the activity of CLV3, synthesised under the control of an epidermis-specific promoter, is dependent on functional CLV1. To test whether the CLV1 receptor was able to sequester the CLV3 protein, expression of both *CLV1* and *CLV3-GFP* was driven by an epidermis-specific promoter. In this experiment, strong CLV3-GFP fluorescence was restricted to the epidermis, which was in contrast to the more diffuse fluorescence observed when CLV3-GFP was expressed alone. This showed that CLV1 can restrict the movement of CLV3-GFP, and indicated that in wild-type plants, CLV3 does not reach the OC because of its sequestration by CLV1 outside of this region. The implications of these studies are that CLV1 both responds to the CLV3 signal and limits its diffusion, making CLV1 both a positive mediator of the CLV3 signal, and a key factor to limit where CLV3 acts.

The next experiment was designed to test whether overexpression of CLV3 from the stem cells would decrease the *WUS* expression domain and the size of the meristem. Indeed, the expression of five copies of *CLV3* under its own promoter in otherwise wild-type plants reduced the meristem size by more than 20% compared with the control, and correlated with a smaller *WUS*-expression domain. The small difference in the *WUS* expression domain measured between wild-type and transgenic meristems indicated that the proposed regulatory feedback loop between *WUS* and *CLV3* is able to prevent the meristem termination in this experiment. Furthermore, to confirm that the reduction in meristem size was due to an excess of CLV3 protein secreted by the stem cells, the researchers also expressed *CLV1* under the *CLV3* promoter in the transgenic plants expressing five copies of *CLV3*. CLV1 was expected to immediately sequester CLV3, preventing it from functioning and, indeed, in this case, the meristem size was increased by 20% (Lenhard and Laux, 2003). These experiments showed that CLV3 proteins, although produced in the stem cells, can move away to neighboring cells, where they activate the CLV1 pathway to repress *WUS* expression.

Another recent study strongly indicates the presence of an additional receptor kinase(s) that functions redundantly with CLV1 during the regulation of meristem development (Diévart et al., 2003). Isolation of insertional alleles within *clv1* mutant plants revealed that *clv1* null alleles display fairly weak phenotypes. All of the strong and intermediate *clv1* alleles characterized to date contain missense mutations, and these

alleles are therefore most likely to be dominant negative. Consistent with this hypothesis, co-suppression of the intermediate *clv1-1* allele in transgenic plants partially rescued the mutant phenotype, such that the co-suppressed plants appears phenotypically similar to *clv1* null mutants, which are weak in phenotype. Thus, suppressing expression of the dominant-negative clv1-1 isoforms reduced the severity of the mutant phenotype. Because the *clv3* null allele exhibits the strongest phenotype among the *clv* alleles, and because *clv1* null alleles are rather weak in phenotype, it is likely that there is an additional receptor kinase(s) capable of relaying the CLV3 signal in the absence of CLV1. This redundant receptor could be inactivated by the presence of clv1 dominant-negative isoforms, indicating receptor multimerization during the activation of CLV1 signaling. Interestingly, *clv1* dominant-negative alleles exhibit major differences from dominant-negative alleles characterized among animal receptor kinases, including the ability of missense mutations in the extracellular domain to act in a dominant-negative manner.

Both genetic and biochemical studies have been used to try to determine the components of the signaling pathway that function downstream of CLV1. Many animal receptor kinases associate with small GTPases of the Ras superfamily to relay signal to downstream targets such as a mitogen-activated protein kinase (MAPK) pathway. Although there is no evidence for a Ras ortholog in plants, there is a plant-specific family of small Rho-related GTPases, termed Rop (Li et al., 1998; Winge et al., 1997). Co-immunoprecipitation experiments have revealed that CLV1 may be associated in a complex with one Rop protein (Trotochaud et al., 1999). Although the detected protein is antigenically-related to a known Rop protein in *A. thaliana*, it remains to be determined whether the detected protein is indeed a member of the Rop family and whether the binding to CLV1 is direct or mediated by linker proteins.

Two different type-2C protein phosphatases (PP2C) negatively regulate the CLV1 pathway: the kinase associated protein phosphatase (KAPP) and the protein phosphatase POLTERGEIST (POL). Overexpression of KAPP, which binds the kinase domain of several RLKs in vivo and in vitro by its forkhead-associated domain (FHA), results in a Clv1$^{-}$ phenotype (Braun et al., 1997; Gomez-Gomez et al., 2001; Li et al., 1999; Shah et al., 2002; Stone et al., 1994; Trotochaud et al., 1999; van der Knaap et al., 1999; Williams et al., 1997). Conversely, suppression of KAPP expression reduces the severity of *clv1* mutant plants. These experiments indicate that KAPP is a negative regulator of the CLV1 pathway (Williams et al., 1997; Stone et al., 1998). Moreover, the interaction of KAPP with RLKs, such as CLV1, is dependent on the phosphorylation status of the RLK (Shah et al., 2002; Stone et al., 1998; Williams et al., 1997). Recently, studies on the *A. thaliana* somatic embryogenesis receptor kinase1 (AtSERK1) have indicated that KAPP interacts with the phosphorylated activation loop of the AtSERK1 receptor and is an integral part of the AtSERK1 endocytosis mechanism (Shah et al., 2002). These results indicate that KAPP could play a similar role in several RLK signaling pathways, including CLV1 (Williams et al., 1997; Stone et al., 1998), FLS2 (see below) (Gomez-Gomez et al., 2001) and HAESA, an RLK regulating abscission (Stone et al., 1994; Jinn et al., 2000).

The second protein phosphatase, POL, also regulates the CLV1 pathway (Yu et al., 2003; Yu et al., 2000). *POL* encodes a predicted nuclear-localized PP2C with a putative N-terminal regulatory domain, and, on the basis of phylogenetic analyses, POL represents a new subclass of plant PP2Cs (Yu et al., 2003). POL protein synthesized in *E. coli* displays phosphatase catalytic activity, but only if an amino-terminal domain is removed (Yu et al., 2003). The ubiquitous expression of *POL* in many plant tissues indicates that POL may function to regulate signal transduction in multiple developmental pathways. There is evidence to indicate that POL affects the activity of WUS, a target of the CLV1 pathway. Although *pol* and *wus* are normally both recessive mutations, *wus* is incompletely dominant in a *pol* mutant background, indicating that *POL* and *WUS* may function closely in the same pathway. Two models have been proposed for the regulation of the CLV1 pathway by POL. One scenario consistent with genetic analyses is that POL acts as a downstream negative regulator of CLV1 signaling. Alternatively, POL could function as an activator of CLV target gene(s), and is itself inactivated by CLV signaling. More components of CLV signaling will need to be isolated to resolve these questions of the mechanism of POL function. POL and KAPP seem to act at different stages of the CLV1 signaling pathway, with KAPP thought to dephosphorylate the CLV1 receptor at the plasma membrane and POL potentially dephosphorylating a downstream intermediate of the signaling pathway.

Work on the CLV1 pathway is progressing rapidly in the identification of signaling components, and in establishing the hierarchy of those components. However, challenges remain, as many components have yet to be identified and, more importantly, very few direct protein interactions have been assessed in detail.

### The BRI1 pathway

Steroid hormones are very important for physiological and developmental regulation, both in animals and plants. In animals, steroid hormones are recognized by ligand-dependent steroid nuclear receptors that promote the transcription of specific target genes. The existence of receptors at the plasma membrane involved in the rapid response to steroid hormone, also known as non-genomic signaling, was postulated many years ago (reviewed by Losel et al., 2003). However, considerable controversy still remains over the nature or presence of receptors that mediate the steroid response at the plasma membrane.

In *A. thaliana*, no homologs of steroid nuclear receptors are known. However, steroid receptors at the plasma membrane have recently been identified. Exogenous application of brassinosteroid (BR) to plants induces a large range of phenotypes, including stem elongation, cell expansion of young aerial tissues (such as hypocotyl and petioles), xylogenesis, leaf bending and ethylene biosynthesis (Clouse, 2001). Plants unable to produce or perceive BR (see below) exhibit phenotypes such as dwarfism, dark green leaves, reduced male fertility and prolonged life span, and they respond inappropriately when grown in the dark (Fig. 3A).

To identify components of the BR signaling pathway, genetic screens have been performed to isolate mutants that are unable to respond to the exogenous application of BRs, particularly the most bioactive compound, the steroid hormone brassinolide (BL). The first mutant, *brassinolide insensitive 1*

(*bri1*), was identified in *A. thaliana* by its BR-deficient phenotype and its failure to be rescued by exogenous BL treatment (Clouse et al., 1996; Li and Chory, 1997). *BRI1* is globally expressed in all plant tissues and its plasma membrane localization made it a good candidate for a receptor that transduces the BR signal across the plasma membrane in plants (Friedrichsen et al., 2000). Biochemical and genetic analyses have shown that both the extracellular domain of the BRI1 receptor and, in particular, its 70 amino acid 'island' located in between the 21st and 22nd LRRs, are required for perception of BL. In the first of these experiments, chimeric receptors were generated that contained fusions of the extracellular domain of BRI1 and cytoplasmic signaling sequences of the XA21 receptor, a rice LRR receptor kinase for disease resistance. These chimeric receptors were able to activate defense response genes when BL was applied exogenously to rice cell suspension culture (He et al., 2000). This revealed that the extracellular domain of BRI1 was responsible for its role in perceiving BL. Interestingly, a mutation in the extracellular 'island' domain of the chimeric BRI1/XA21 receptor abolished this defense response, demonstrating the importance of this domain for BL perception. In another experiment, specific BL-binding activity was detected after BRI-GFP fusion proteins from transgenic plants were immunoprecipitated using anti-GFP antibodies (Wang et al., 2001); this indicates that BRI1 is either the BL receptor, or a rate-limiting component of the BL receptor complex.

Consistent with the BRI1-XA21 chimeric receptor studies indicating a central role for the 'island' domain in BL perception, only mutations in the 'island' domain greatly reduced the association of BRI1 with BL. Moreover, BL association with BRI1 activates BRI1 phosphorylation in vivo and requires the kinase activity of BRI1. Together, these results suggest that BRI1 is the primary receptor for BR in *A. thaliana*. However, it has not yet been shown that BRI1 is able to bind BR directly. Further studies will be needed to determine whether another component, such as a steroid-binding protein, is also required to mediate steroid binding to BRI1.

At least two putative steroid-binding proteins, which may be secreted, have been identified in the *A. thaliana* genome (Arabidopsis Genome Initiative, 2000; Li et al., 2001a). Interestingly, a screen for suppressors of the weak, partial loss-of-function *bri1-5* allele has revealed that overexpression of a type II serine carboxypeptidase, *BRS1*, can suppress the *bri1* phenotype (Li et al., 2001a). This suppression is specific to the BRI1 receptor, and depends on the presence of BR, on a functional BRI1 kinase domain and on BRS1 protease activity. BRS1 is proposed to regulate an early event in BRI1 signaling, and putative steroid-binding proteins could be substrates of BRS1. It is possible that if BRS1 is required to activate a BR-binding protein, then elevated expression of BRS1 might increase the concentration of bound

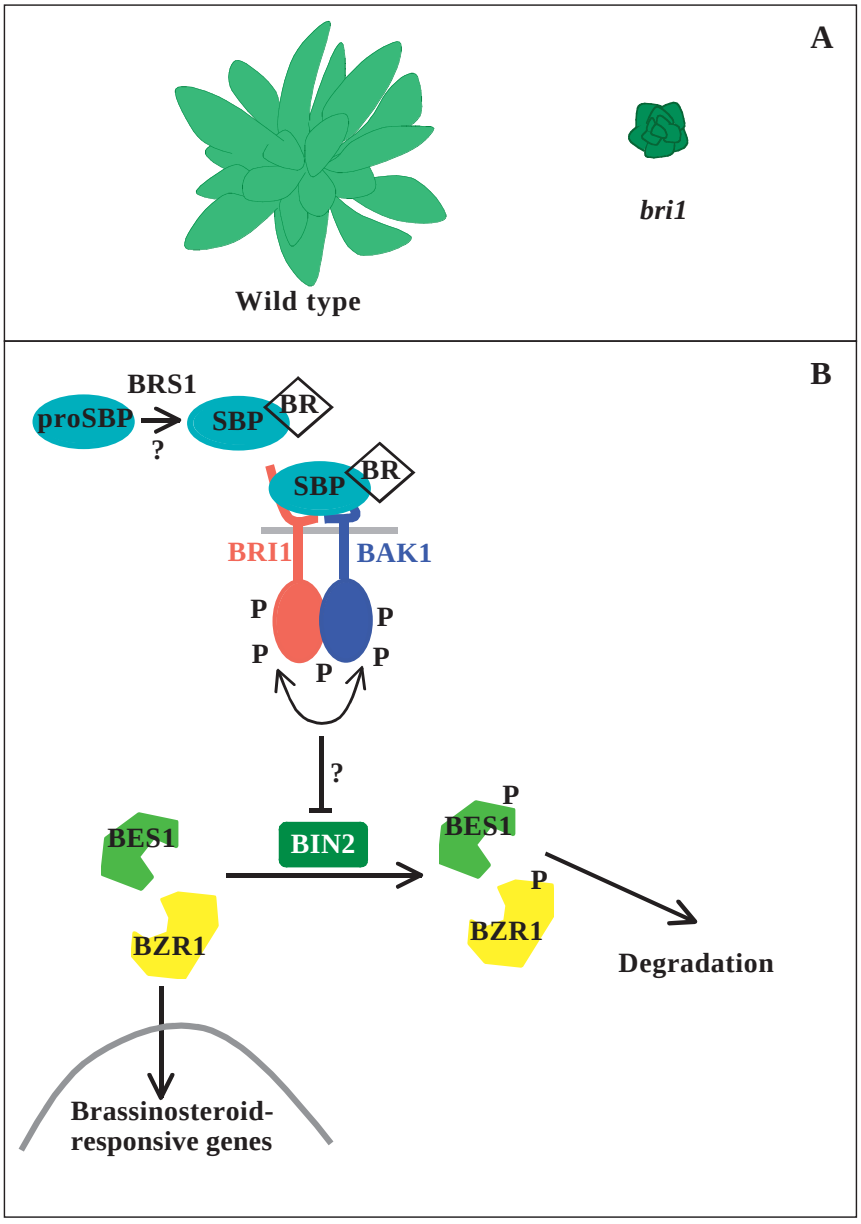


**Fig. 3.** *bri1* mutant plants and signaling. (A) Representation of wild type and the severe dwarf phenotype observed in *bri1* mutant plants. Rosette leaves of *bri1* plants are 'cabbage-like', because of defects in stem elongation, and cell expansion of hypocotyls and petioles. (B) Model for the BRI1 signaling pathway. The type II serine carboxypeptide BRS1 activates a putative steroid binding protein (proSBP), which then associates with the brassinosteriod BR. SBP-BR interacts with the LRR-RLK BRI1 (red) and the receptor BAK1 (dark blue). BAK1/BRI1 then inactivate the GSK3-like kinase BIN2, which is an upstream regulator of BES1 and BRZ1. In the absence of BR, BIN2 is constitutively active, and phosphorylates BES1 and BRZ1, leading to their degradation. When BES1 and BRZ1 are not phosphorylated, they are localized to the nucleus where they activate transcription of brassinosteroid responsive genes.

BR, thus overcoming the reduce signaling capability of the bri1-5 isoform.

Last year, a role for another LRR-RLK, the BRI1-associated receptor kinase (BAK1), was demonstrated by a genetic gain-of-function screen and biochemical analyses (Li et al., 2002; Nam and Li, 2002). BAK1 is a member of the somatic embryogenesis receptor kinase (AtSERK) family, which consists of five genes in *A. thaliana*. BAK1 (AtSERK3) possesses four leucine zippers and five LRRs in its extracellular domain. Direct physical interaction between BRI1 and BAK1 was observed both in a yeast two-hybrid system and in vivo in *A. thaliana* (Li et al., 2002; Nam and Li, 2002). Although *BAK1* overexpression suppresses the phenotypes of weak *bri1* alleles, the *bak1* null phenotype is weaker than the *bri1* null phenotype, indicating that BAK1 is partially dispensable for BRI1 signaling and/or that *BAK1* homologous genes functionally overlap with BAK1. BRI1 could potentially form heterodimers with other AtSERK family members in the absence of BAK1. Two models have been proposed for BRI1/BAK1 activation (Fig. 3B). In the first model, ligand-bound BRI1 activates BAK1, which then phosphorylates downstream components to regulate gene expression (Li et al., 2002). A second model proposes that BRI1 and BAK1 form an inactive heterodimer, which is then stabilized and activated by the binding of BR, allowing the transphosphorylation of the kinase domains to activate downstream components (Nam and Li, 2002). Whether BAK1 binds to the brassinosteroid hormone itself is unclear. Further analyses might investigate the mechanisms, sites and timing of phosphorylation of BRI1 and BAK1 in response to BR binding in vivo, and will help distinguish between these hypotheses.

To date, no direct substrate for the BRI1/BAK1 complex has been described. However, several proteins with roles downstream of the BRI1 receptor in the BR signaling pathway have been uncovered. BRI1-EMS-SUPPRESSOR 1 (BES1) and BRASSINAZOLE RESISTANT 1 (BRZ1) are two proteins that share 88% identity with each other and are part of a protein family that has six known members in *A. thaliana* (He et al., 2002; Wang et al., 2002; Yin et al., 2002). Except for a putative nuclear localization signal in the amino-terminal portion of both BES1 and BRZ1, these proteins do not possess significant similarities to known proteins (Zhao et al., 2002b). A number of studies have sought to determine the roles of BES1 and BRZ1 in the BRI1 signaling pathway. Semi-dominant and dominant mutations in the sequences coding for the PEST domains of *BES1* and *BRZ1*, respectively, give rise to mutant plants that, in the dark, are resistant to the BR biosynthesis inhibitor brassinazole. Although these mutant plants exhibit the same phenotypes as one another in the dark, BES1 and BRZ1 seem to play different roles in the light. When grown in the light, the gain-of-function *bes1-D* mutants display constitutive BR response phenotypes which include extensive elongation of leaves and stems. By contrast, gain-of-function *brz1-D* mutants exhibit a semi-dwarf phenotype, possibly due to the activation of a feedback mechanism that inhibits the normal BR signaling pathway.

BES1 and BRZ1 activity may be regulated at the protein level (Fig. 3B) (He et al., 2002; Wang et al., 2002; Yin et al., 2002; Zhao et al., 2002b). BL treatment increases both the accumulation of, and the nuclear localization of, unphosphorylated BES1 and BRZ1 proteins. This effect is dependent on BRI1, which indicates that BES1 and BRZ1 are downstream specific, positive regulators of the BR signaling pathway. One could infer from these results that, in the absence of BL, a negatively acting kinase phosphorylates BES1 and BRZ1, leading to their degradation. Several studies indicate that this upstream repressor of BES1 and BRZ1 is the ser/thr kinase BRASSINOSTEROID-INSENSITIVE 2 (BIN2) (Li and Nam, 2002; Li et al., 2001b). Gain-of-function mutations of this GSK3/SHAGGY-like kinase result in plants that exhibit a BR-deficient phenotype suppressed by *bes1-D*, suggesting that BIN2 is likely to function upstream of BES1 (Yin et al., 2002). In addition, BRZ1 and BES1 both possess multiple consensus sequences (S/TxxxS/T) known to be phosphorylated by GSK3/Shaggy kinases. Finally, BIN2 interacts in yeast cells with BES1 and BRZ1, and phosphorylates both proteins in vitro. These data indicate that in the absence of BR, constitutively activated BIN2 phosphorylates BES1 and BRZ1, which is likely to lead to the degradation of phosphorylated BES1 and BRZ1 proteins (Fig. 3B). In the presence of BR, activation of the BRI1 complex at the plasma membrane leads to the inhibition of the BIN2 kinase activity. This, in turn, permits the accumulation of unphosphorylated BES1 and BRZ1 proteins that can translocate to the nucleus to activate their specific BR responsive targets. In agreement with the *bes1* and *brz1* mutant phenotypes, microarray analyses show that BES1 activates the transcription of genes that encode the cell-wall modifying enzymes required for cell elongation, and BRZ1 activates the transcription of genes involved in the BR negative-feedback pathway that inhibits BR-induced cell elongation in a light-dependent manner (Wang et al., 2002; Yin et al., 2002).

The model proposed for the BR pathway activation by BES1 and BRZ1 is reminiscent of the mechanism described for the Wingless/Wnt signaling pathway. In models of both systems, the signal (Wnt in animals versus BR in plants) activates a plasma-membrane receptor (Frizzled versus BR1 and BAK1) that activates signaling by inactivating a GSK3-like kinase (Shaggy versus BIN2), which normally functions to repress, through phosphorylation and degradation, downstream effector(s) (β-catenin versus BES1 and BZR1) (reviewed by Cadigan and Nusse, 1997). Clearly remarkable progress has been made on both identifying the components of BRI1 signaling, as well as some of the key protein interactions. Pressing questions include understanding the nature of BL interaction with BRI1 (e.g., is it mediated by a BL-binding protein?), and the mechanism by which BRI1 and BAK1 regulate BIN2 activity.

## LRR-containing receptors and defense responses in plants and animals

The *Drosophila melanogaster* Toll receptor and its mammalian homologs, the Toll-like receptors (TLR), have extracellular LRR domains structurally similar to many plant RLKs. However, Toll differs from plant RLKs in that its cytoplasmic domain is composed of about 200 amino acids called the Toll/interleukin 1 receptor (TIR) domain (Hashimoto et al., 1988; Takeda et al., 2003). The *Toll* pathway was first identified in *D. melanogaster* on the basis of its role in dorsoventral patterning during early embryogenesis (Hashimoto et al., 1988). Genetic and molecular analyses are consistent with a model in which a proteolytic cascade activated on the ventral

side of the pre-cellular embryo processes the secreted cytokine Spätzle, a member of the cysteine knot family of growth factors, which activates the Toll receptor (Lemaitre et al., 1996; Weber et al., 2003). Toll activation recruits a complex containing at least three proteins (Sun et al., 2002), the adaptor MyD88/Krapfen (Kra) (Charatsi et al., 2003), the scaffolding Tube protein (Letsou et al., 1991; Letsou et al., 1993), and the cytoplasmic ser/thr protein kinase Pelle (Hecht and Anderson, 1993). Interestingly, the kinase domain of Pelle is the animal protein kinase most similar to the cytoplasmic ser/thr kinase domains of plant LRR-RLKs (Shiu and Bleecker, 2001b). Several studies have demonstrated that Kra binds to Toll by their respective TIR domains, and that Kra, Tube and Pelle form an heterotrimeric complex bound by their death domains, which are widely involved in protein-protein interactions (Sun et al., 2002). This aggregation triggers phosphorylation and degradation of the IκB-like inhibitor Cactus, so that the Rel/NFκB transcription factor Dorsal is released and translocates to the nucleus (Galindo et al., 1995; Grosshans et al., 1994). In the nucleus, Dorsal directs the expression of a number of downstream factors (Thisse et al., 1991).

In adult *D. melanogaster*, the Toll pathway is also involved in innate immunity (Lemaitre et al., 1996). The mechanism of intracellular signaling that occurs upon fungal and bacterial infection is essentially the same as that described above for dorsoventral axis formation, with the exception that Spätzle is generated in response to fungal products through the activation of the serine protease Persephone (Ligoxygakis et al., 2002), and that the Rel factor translocated in the nucleus is the Dorsal-related immunity factor (Dif), which upregulates the transcription of antimicrobial genes (Ip et al., 1993). In humans, ten Toll-like receptors are implicated in the recognition of pathogen-associated molecular patterns (PAMPs) (Dunne and O'Neill, 2003). The ligands identified include bacterial lipopeptides, peptidoglycan, dsRNA, fibronectin and flagellin. These ligands possess the same functional domains as the ligands of their Toll homolog in *D. melanogaster*, and all Toll-like receptors use TIR domain-containing adaptors to transduce the signal through the activation of two Interleukin-1 receptor-associated kinases (IRAKs), homologs of the Pelle ser/thr kinase cytoplasmic protein in the fly (Cao et al., 1996a). IRAKs phosphorylate and activate the tumor necrosis factor (TNF) and the receptor-associated factor 6 (TRAF6) (Cao et al., 1996b). Activated TRAF6, in turn, activates the mitogen-activated protein kinases (MAPK) and the mitogen-activaged protein kinase kinase kinase (MAPKKK), called transforming growth factor β-activated kinase (TAK1). TAK1 can phosphorylate the IκBα kinase complex, as well as MKK (which are MAPKKs), leading to activation of the p38 MAPKs and c-Jun N-terminal kinase (JNK) (Takaesu et al., 2000).

Interestingly, the animal developmental/defense response pathways described above have some parallels with certain signaling pathways in plants. Both plants and animals use LRR-containing receptors to detect specific pathogenic peptides, both use Pelle-family kinases to relay signals from the receptors, and both use MAPK pathway kinases to activate downstream defense responses. A screen for mutants insensitive to flagellin (major antigens of several bacteria) upon bacterial infection led to the discovery of the *FLS2* locus in *A. thaliana* (Gomez-Gomez et al., 1999). The *FLS2* gene codes for an LRR-RLK (Gomez-Gomez and Boller, 2000). Mutations in both the LRR and the kinase domains of FLS2 affect the binding activity of the most conserved domain of flagellin, the peptide flg22 (Gomez-Gomez et al., 2001). This binding activity is rescued when a wild-type FLS2 receptor is expressed in the *fls2* mutant plants, indicating that FLS2 is the receptor for the flg22 peptide, and that the kinase activity of the FLS2 receptor is necessary for the binding of flg22. The kinase-associated protein phosphatase KAPP interacts with the kinase domain of FLS2 in a yeast two-hybrid assay, and plants overexpressing KAPP are insensitive to flagellin treatment (Gomez-Gomez et al., 1999). These data indicate that KAPP is a negative regulator of the FLS2 signaling pathway. New data have shown that FLS2 directly or indirectly activates a cascade of phosphorylation, implicating the MAPK pathway proteins MEKK1, MKK4/MKK5 and MPK3/MPK6 in this signaling pathway (Asai et al., 2002). The targets of this signal include the well-described defense genes, such as *PAL1*, *GST1*, *PR1* and *PR5*, induced in many plant species, as well as the *WRKY22/WRKY29* transcription factors and LRR-RLK FRK1 (FLG22-induced receptor-like kinase 1). Interestingly, the activation of the MAPK cascade confers resistance to bacteria and fungi, indicating a convergence of the signal induced by different pathogens to this specific cascade. Moreover, the results also indicate the presence of a MAPK-independent pathway, which could be calcium-dependent, for the activation of other defense-responsive factors.

## Conclusions

One important feature of signal transduction in animals is that receptors are usually the starting point of a complex array of signaling pathways, where cross-talk, feedback loops, branch points and multi-component signaling complexes converge to enable the transcription of target genes in the nucleus. There is no reason to think that this feature is not also true in plants. Thus, for the three pathways described in this review, the CLV1, BRI1 and FLS2 pathways, the simple linear models of their function are likely to represent, at best, only a portion of very complex signaling circuitries. Already for the CLV1 pathway, characterization of the signaling regulator POL has revealed a WUS-independent CLV1 pathway regulating meristem development (Yu et al., 2003; Yu et al., 2000).

The RLK pathways may be further complicated by functional redundancy between receptors. There are 216 LRR-RLKs in the *A. thaliana* genome, but only a handful have been associated with a biological function. One possible reason that the function of so many receptors is unknown is that multiple receptors may functionally overlap with one another, such that mutant versions of the receptors would not show up in straightforward genetic screens. Indeed, receptors with functional overlap are strongly implicated in both the CLV1 and ERECTA pathways (Table 1) (Diévart et al., 2003; Shpak et al., 2003). Additional functional redundancy has been detected at the level of ligand function. The putative CLV1 ligand CLV3 is part of a family that contains at least 20 CLV3-like (CLE) members (Sharma et al., 2003). CLE members are expressed in several tissues during development, and some of them, such as CLV3, are secreted into the periplast. One of them, CLE40, when ectopically expressed in the meristem of transgenic plants, is functionally equivalent to CLV3 (Hobe et al., 2003). However, in wild-type plants, its level of expression

is ubiquitous and low, and the loss of *CLE40* in *A. thaliana* enhances root waving. Thus, a CLV3-like protein plays a role in root development, indicating that a CLV1-like receptor may receive and transduce the CLE40 signal in roots.

Studies on gene families that encode ligands, receptors and signaling components are likely to yield much new information on the diversity and specificity of pathways downstream LRR-RLKs in plants. Open questions still remain as to the function of more than 200 LRR-RLKs that are as yet without an attributed signaling mechanism. We know little about whether mechanisms of signaling identified to date for receptors such as BRI1, FLS2 and CLV1 are common among those receptors that remain unknown. We might hope, however, that many receptors will use common downstream components and mechanisms to relay signals from the plasma membrane to the nucleus. As we have described, the protein phosphatase KAPP appears to play a role in negatively regulating the signaling of many receptors. Moreover, the gene encoding the protein phosphatase POL, which regulates CLV1 signaling, is expressed in many tissues in *A. thaliana*, exhibits mutant phenotypes outside of the meristem, and has sequence similarity with several other *POL*-like genes, indicating that the function of this family of PP2C could be conserved downstream of multiple LRR-RLKs (Yu et al., 2003; Yu et al., 2000). A close BRI1 homolog, VH1, appears to regulate vascular development in *A. thaliana* (Clay and Nelson, 2002). One wonders whether VH1 regulates vascular development through BIN2 or a BIN2-related protein. Will other pathogen responses use similar signaling mechanisms to those that are being uncovered for FLS2? These and related questions will certainly occupy researchers for years to come.

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