The Structural Basis of Ligand Perception and Signal Activation by Receptor Kinases

Ulrich Hohmann, Kelvin Lau, and Michael Hothorn

Structural Plant Biology Laboratory, Department of Botany and Plant Biology, University of Geneva, 1211 Geneva, Switzerland; email: ulrich.hohmann@unige.ch, kelvin.lau@unige.ch, michael.hothorn@unige.ch

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Abstract

Plants have evolved a family of unique membrane receptor kinases to orchestrate the growth and development of their cells, tissues, and organs. Receptor kinases also form the first line of defense of the plant immune system and allow plants to engage in symbiotic interactions. Here, we discuss recent advances in understanding, at the molecular level, how receptor kinases with lysin-motif or leucine-rich-repeat ectodomains have evolved to sense a broad spectrum of ligands. We summarize and compare the established receptor activation mechanisms for plant receptor kinases and dissect how ligand binding at the cell surface leads to activation of cytoplasmic signaling cascades. Our review highlights that one family of plant membrane receptors has diversified structurally to fulfill very different signaling tasks.

Contents

INTRODUCTION 110
LIGAND BINDING AND RECEPTOR ACTIVATION BY LYSIN-MOTIF-CONTAINING RECEPTOR KINASES 113
ARCHITECTURE OF PLANT RECEPTOR KINASES WITH LEUCINE-RICH-REPEAT ECTODOMAINS 116
LIGAND RECOGNITION BY ISLAND-DOMAIN-CONTAINING LEUCINE-RICH-REPEAT RECEPTOR KINASES 119
LIGAND RECOGNITION IN PEPTIDE-BINDING LEUCINE-RICH-REPEAT RECEPTOR KINASES 121
LEUCINE-RICH-REPEAT RECEPTOR KINASE ACTIVATION BY SHAPE-COMPLEMENTARY CO-RECEPTOR KINASES 124
STRUCTURAL ASPECTS OF RECEPTOR-KINASE-CONTROLLED CYTOPLASMIC SIGNALING EVENTS 127
FUTURE DIRECTIONS 129

INTRODUCTION

As multicellular organisms, plants must generate and respond to many internal signaling cues to coordinate their growth and development. Their sessile lifestyle also requires them to recognize various signal inputs from the outside world in response to an ever-changing environment. Many of these signals are sensed at the cell surface, and plants have evolved a unique family of membrane receptor kinases (RKs, also called receptor-like kinases) that are capable of transmitting extracellular signals across membranes. *Arabidopsis* has approximately 600 RKs, all of which are composed of an extracellular ligand-binding domain, a single membrane-spanning helix, and a cytoplasmic kinase domain (111) (**Figure 1**). The extracellular ligand-binding domains of plant RKs can differ drastically in size and architecture. In this review, we focus on RKs with lysin-motif (LysM) and leucine-rich-repeat (LRR) ectodomains, as both of these families have been structurally characterized.

Receptor kinases (RKs): proteins that feature an extracellular ligand-binding domain, a single membrane-spanning helix, and a cytoplasmic kinase domain

Lysin motif (LysM): a domain, approximately 40 amino acids long, that is involved in the binding of *N*-acetyl-D-glucosamine and related carbohydrate ligands

Leucine-rich repeats (LRRs): repeat units, approximately 22 amino acids long, with a hydrophobic core that can stack onto each other to build larger assemblies

Plant RKs carry out four major tasks (**Figure 2**). First, they sense extracellular signals—which could be native or foreign small molecules, peptides, or protein ligands—with high specificity and selectivity (ligand binding). Second, their recognition of ligands at the cell surface leads to profound changes in the activity of the cytoplasmic kinase domain of the receptor (receptor activation). Third, the kinase module of the activated RK becomes capable of regulating the activity of cytoplasmic signaling cascades, which generate the final signaling output (downstream signaling). And fourth, RKs provide protein interaction sites and signature motifs that render them subject to multiple levels of regulation and that integrate them into larger signaling networks (modulation of RK activity) (**Figure 2**).

Briefly, some RKs play essential roles in plant growth and development. The LRR-RK BRASSINOSTEROID INSENSITIVE 1 (BRI1) senses the growth-promoting brassinosteroids to regulate, for example, cell elongation and cell division (25, 46, 71, 132). Cell expansion and proliferation are also signaled by the pentameric peptide hormone phytosulfokine, which is recognized by PHYTOSULFOKINE RECEPTOR (PSKR) (81–83). Root development is in part coordinated by ROOT MERISTEM GROWTH FACTOR (RGF) peptides, which are sensed by the ROOT MERISTEM GROWTH FACTOR RECEPTOR (RGFR) family of

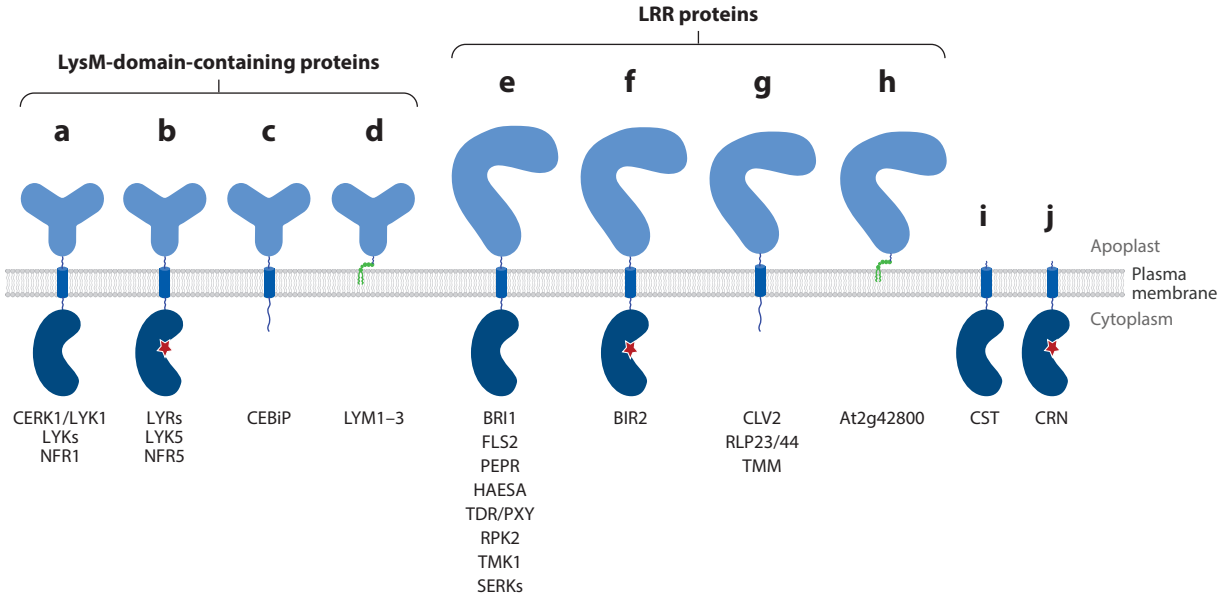


Figure 1

Architecture of plant receptor kinases (RKs), receptor-like proteins (RLPs), and receptor-like cytoplasmic kinases (RLCKs). RKs (schemes *a*, *b*, *e*, and *f*) consist of an extracellular ligand-binding domain (*light blue shapes*, *top*); a single membrane-spanning helix (*blue cylinders*, *middle*); and a cytoplasmic kinase domain (*dark blue kidney shapes*, *bottom*), which can be a catalytically impaired pseudokinase (*dark blue kidney shape with red star*). Loop regions (*blue lines*) connect the different domains. RLPs (schemes *c*, *d*, *g*, and *h*) lack a cytoplasmic kinase domain. Most RLPs have a single transmembrane helix and can have a longer unstructured cytoplasmic loop, as illustrated by schemes *c* and *g*, but some lack any transmembrane element and are attached to the membrane with a glycosylphosphatidylinositol (GPI) anchor (shown in *green*), as illustrated by schemes *d* and *h*. RLCKs (schemes *i* and *j*) are composed of a transmembrane helix and a cytoplasmic kinase or pseudokinase domain but lack an extracellular domain. Although the overall architecture of RKs and RLPs is the same, the nature of the extracellular domain varies among different RK and RLP subfamilies. Schemes *a–d* represent proteins that contain an extracellular domain consisting of three lysin motifs (LysMs), and schemes *e–h* depict leucine-rich-repeat (LRR) proteins; the schemes show the various possible combinations of extracellular domains, transmembrane helices, cytoplasmic kinase domains, and GPI anchors in these proteins.

LRR-RKs (comprising RGFR1–3) (95, 109, 115). The LRR-RKs CLAVATA 1 (CLV1), BARELY ANY MERISTEM 1–3 (BAM1–3), RECEPTOR-LIKE PROTEIN KINASE 2 (RPK2), and TRACHEARY ELEMENT DIFFERENTIATION FACTOR RECEPTOR/PHLOEM INTERCALATED WITH XYLEM (TDR/PXY) sense small, posttranslationally modified CLV3/EMBRYO SURROUNDING REGION (ESR)–RELATED (CLE) peptide hormones, including CLV3, to maintain plant stem cell populations in the shoot and in the root (24, 30, 32, 33, 45, 88, 89, 106, 114, 145). A family of secreted peptide hormones with structural similarities to CLEs, the INFLORESCENCE DEFICIENT IN ABSCISSION–LIKE (IDL) peptides, trigger abscission and cell separation processes in plants by binding to the LRR-RKs HAESA and HAESA-LIKE 2 (HSL2) (15, 67, 100, 126). Larger peptide hormones, such as EPIDERMAL PATTERNING FACTORs (EPFs), specify stomatal patterning by binding to the LRR-RKs ERECTA and ERECTA-LIKE 1 (ERL1) (43, 69, 70, 112). Finally, TRANSMEMBRANE KINASE 1 (TMK1), a founding member of the plant RK family (19), regulates plant growth by interacting with AUXIN BINDING PROTEIN 1 (ABP1) (27, 137).

Pattern recognition receptors form a specialized class of plant RKs that are able to sense microbe-, pathogen-, and damage-associated molecular patterns as a first line of defense in the plant

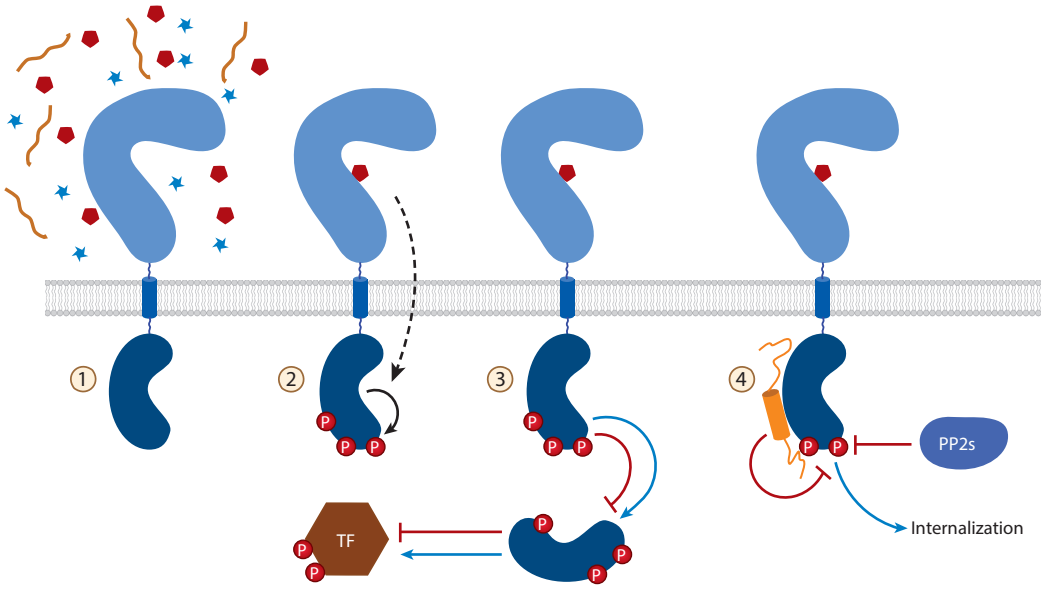


Figure 2

The four steps of plant receptor kinase (RK) signaling. (①) RKs sense ligands using their extracellular domain. Among many possible ligands present in the extracellular space, RKs specifically sense native or foreign small molecules, peptides, and/or proteins. (②) Binding of a ligand to the extracellular domain activates the receptor by changing the activity of its cytoplasmic kinase domain. (③) Subsequently, the kinase module activates (*blue arrow*) or inactivates (*red bars*) cytoplasmic components to generate a signaling output, ultimately by influencing the activity of transcription factors (TFs). (④) RK activity is regulated at various levels. For example, protein interaction sites allow for regulation by inhibitory proteins (shown in *orange*), the kinase domain can be inactivated by protein phosphatases (PP2s), and the localization of the RK can be altered by endocytosis, leading to recycling or degradation. As in **Figure 1**, each protein architecture shows the extracellular domains (*light blue shapes*, *top*), transmembrane helices (*blue cylinders*, *middle*), cytoplasmic kinase domains (*dark blue kidney shapes*, *bottom*), and loop regions connecting the different domains (*blue lines*). Potential ligands are shown as red pentagons, green stars, and orange sticks, and sites of protein phosphorylation are shown as red circles.

immune system (2, 3). The LRR-RK FLAGELLIN SENSITIVE 2 (FLS2) senses a conserved part of the bacterial flagellin monomer (22, 23, 37). Danger signals, such as the endogenous Pep peptides, are transmitted by the RK PEP RECEPTOR 1 (PEPR1) (66, 122, 138, 139).

Plant pattern recognition receptors may also contain the carbohydrate-binding LysMs in their ectodomains. These modules are also present in viral, bacterial, fungal, and animal proteins and are able to recognize carbohydrates that contain *N*-acetyl-D-glucosamine (NAG) (13). Plant RKs with LysM-containing ectodomains are involved in sensing symbiotic signals [NAG-containing nodulation (Nod) factors] to establish, for example, rhizobia and mycorrhiza, and are involved in plant immune responses by recognizing the NAG-containing chitin and peptidoglycan cell walls of fungal and bacterial invaders (2, 34). The *Lotus japonicus* NODULATION FACTOR RECEPTOR 1/5 (NFR1/5) and *Medicago truncatula* LYSM DOMAIN–CONTAINING RECEPTOR-LIKE KINASE-RELATED 3 (MtLYR3) participate in the recognition of Nod factors consisting of four or five β-1,4-linked NAGs with an alkyl chain at the C-2 position of the nonreducing terminal glucosamine residue (11, 35, 73, 78, 79, 98, 121, 135). LysM-containing RKs, such as CHITIN ELICITOR RECEPTOR KINASE 1 (CERK1), can specifically interact with NAG-containing chitin oligomers to trigger plant immune responses (53, 54, 87, 127).

Erratum

Over the past five years, the three-dimensional structures of several plant RK ectodomains (LysM and LRR) and kinase domains have been reported. Here, we review what these atomic

HOW TO READ STRUCTURAL REPRESENTATIONS

Protein structures can be visualized in several different ways, with each view presenting different types of information. A surface view shows the whole solvent-excluded outer surface of a molecule, be it a protein and all side chains (**Figure 3*b***) or a ligand (**Figure 8*a***). This view is suitable for showing a molecule's overall shape and the proximity between proteins or between proteins and their ligands. Ribbon diagrams are used to bring out secondary structure features, with α-helices shown as either tubes or coiled ribbons, β-strands shown as flat arrows, and loops shown as smoothed lines (**Figure 3*a*,*d***). Side chains are generally omitted from ribbon diagrams. Important residues can be added, such as the cysteines that form disulfide bonds in **Figure 3*d*** (which shows these in a bonds representation). A C_α trace follows the C_α backbone atoms (**Figure 3*c*,*e***). Again, side chains in a bonds representation can be added, and dotted lines can be used to point out hydrogen bonds or salt bridges (**Figure 3*c***). The degree of similarity between the three-dimensional folds of two structures or domains is assessed by calculating the r.m.s.d. (root-mean-square deviation) for C_α atoms between two superimposed models; an r.m.s.d. of 0 Å (1 Å = 0.1 nm) would signify identical structures.

models can tell us about how plant RKs interact with their ligands, how ligand binding at the cell surface leads to receptor activation in the cytoplasm, how plant RKs interact with downstream signaling cascades, and how the activity of RKs can be modulated (**Figure 2**).

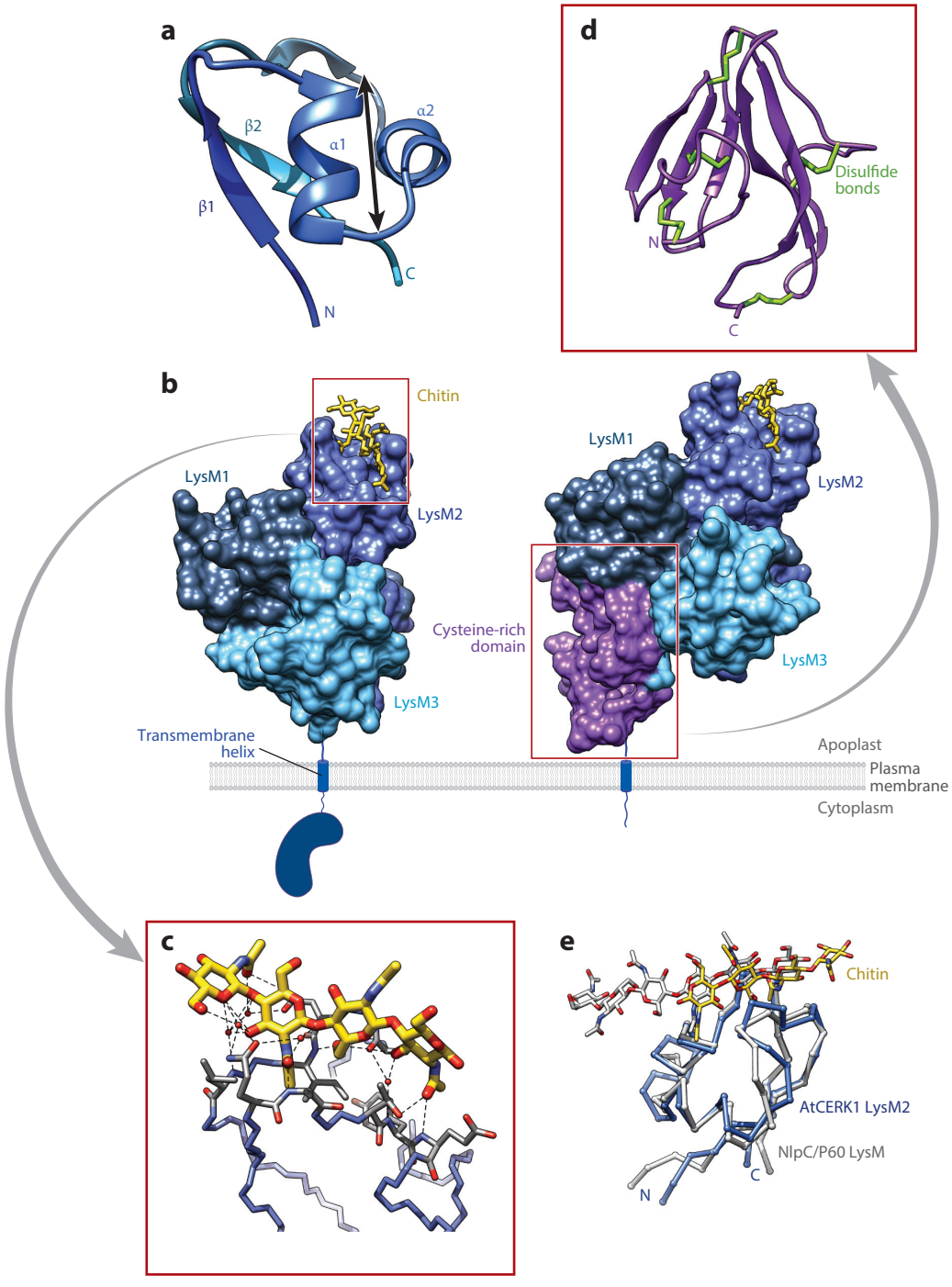
LIGAND BINDING AND RECEPTOR ACTIVATION BY LYSIN-MOTIF-CONTAINING RECEPTOR KINASES

LysM domains are conserved carbohydrate-binding modules that are approximately 40 amino acids long and are found in all kingdoms of life (2, 13). The first LysM-domain structure from *Escherichia coli* revealed a conserved fold characterized by the presence of two α-helices packed against the same surface of a small antiparallel β-sheet (5) (**Figure 3*a***; see also the sidebar titled How to Read Structural Representations). LysM signatures are present in several plant RKs (such as AtCERK1) (5, 34, 42, 54), in receptor-like proteins (RLPs) [such as *Oryza sativa* CHITIN ELICITOR BINDING PROTEIN (OsCEBiP)] (23, 24, 49, 62), and in cell surface glycosylphosphatidylinositol (GPI)–anchored proteins (8) (**Figure 1**).

The extracellular domain of the LysM-RK AtCERK1 features three LysM domains, which tightly pack against each other to form a cloverleaf-shaped assembly that is further stabilized by interdomain disulfide bonds (77) (**Figure 3*b***). AtCERK1 binds chitin in vitro (53), and the second LysM domain (LysM2) provides a binding pocket for the chitin oligomer $(NAG)_5$ (77) (**Figure 3*b***). Different chitin oligomer dissociation constants (K_d) have been reported for AtCERK1 and AtLYK5, ranging from the low micromolar to the millimolar range (18, 77). In the AtCERK1 crystal structure, $(NAG)_5$ binds in an extended conformation and makes extensive hydrogen bond interactions, mainly with backbone atoms in the AtCERK1 LysM2 binding site, several of which are mediated by water molecules (77) (**Figure 3*c***). A highly similar assembly of three LysM domains is also present in OsCEBiP, with LysM2 again providing the chitin-binding site (76) (**Figure 3*b***). In addition to its cloverleaf-shaped LysM assembly, OsCEBiP features a C-terminal antiparallel β-domain of unknown function (cysteine-rich domain; see **Figure 3*b*,*d***) (76). It is unclear whether the remaining LysM domains in AtCERK1 and OsCEBiP also bind chitin or other NAG-containing ligands, as reported for symbiotic plant LysM-RKs (79) and for fungal effector proteins (29). It also remains to be determined what types of carbohydrates are sensed by OsCERK1, which does not detectably bind chitin oligomers in vitro (76).

Receptor-like proteins (RLPs): proteins that have the same extracellular architecture as RKs but lack a cytoplasmic kinase domain

Glycosylphosphatidylinositol linositol (GPI)–anchored proteins: proteins with a GPI anchor at the C terminus that are attached to the plasma membrane without a membrane helix


a
β2
α1
α2
β1
C
N
d
Disulfide
bonds
N
C
b
Chitin
LysM1
LysM2
LysM3
Cysteine-rich
domain
Transmembrane
helix
Apoplast
Plasma
membrane
Cytoplasm
c
e
Chitin
AtCERK1 LysM2
NlpC/P60 LysM
C
N

All plant LysM domains show a high degree of structural conservation with bacterial LysM proteins, such as the bacterial endopeptidase NlpC/P60 (134) (**Figure 3*e***). It can thus be assumed that the extracellular ligand-binding domains of plant LysM receptors have evolved from ancestral LysM proteins, as has been suggested for the ectodomains of plant cytokinin receptors (50).

Pseudokinases: catalytically inactive kinase domains that are thought to mediate protein-protein interactions or act as dummy substrates

Mechanistic studies of AtCERK1 also provide insight into the activation mechanism of LysM-RKs. AtCERK1 crystal structures suggest that the LysM2 domain specifically reads out a chitin tetramer and that the receptor does not undergo significant conformational changes upon ligand binding (77). Associated quantitative biochemical assays have revealed that AtCERK1 cannot discriminate between chitin tetramers and longer fragments (77). Unlike the tetramer, however, these longer chitin oligomers efficiently trigger AtCERK1-mediated immune responses (53, 77, 97, 142). Consistently, chitin octamers but not shorter fragments promote ligand-induced homodimerization of the AtCERK1 ectodomains in vitro (77). LysM-domain oligomerization on longer NAG-containing carbohydrate oligomers has been visualized in crystal structures of bacterial and fungal LysM-containing proteins (29, 134), but an active LysM-RK signaling complex remains to be characterized.

Dimerization of the AtCERK1 ectodomains upon chitin sensing may bring the cytoplasmic kinase domains of the receptor in close proximity and allow for their transphosphorylation and subsequent activation of downstream immune responses (77). The proposed receptor activation mechanism for AtCERK1 is reminiscent of the one previously suggested for human Toll-like immune receptors (TLRs). In the case of TLR3, an oligomeric ligand (a viral RNA molecule) promotes homodimerization of the TLR LRR ectodomains, leading to dimerization of the cytoplasmic Toll/interleukin-1 receptor (TIR) domain of the receptor and subsequent recruitment of a cytoplasmic kinase signaling complex (9, 74) (**Figure 4*a*,*d***). However, recent evidence also supports heterodimerization models of AtCERK1 with the high-affinity chitin RK AtLYK5 (18) (**Figure 4*c***). The roles of LysM-containing RLPs such as OsCEBiP remain to be clarified; although OsCEBiP interacts with chitin tetramers with micromolar affinity via its LysM2 domain, whether OsCEBiP by itself can undergo ligand-dependent dimerization is not known (44, 76). In planta experiments have suggested that LysM-RLPs (such as OsCEBiP) or LysM-RKs with cytoplasmic pseudokinase modules may interact with active LysM-RKs in larger signaling complexes, the significance of which remains to be characterized (18, 107) (**Figure 4*b***).

Overall, the structural, biochemical, and genetic evidence suggests that LysM-RKs use an individual LysM domain to sense NAG-containing carbohydrate oligomers. Larger carbohydrate oligomers can promote ligand-induced homodimerization and possibly also heterodimerization

Figure 3

Structures of lysin-motif (LysM)–containing proteins. These domains are conserved across all kingdoms of life and show similar modes of ligand binding. (*a*) Ribbon diagram of a LysM domain with the typical βααβ topology [Protein Data Bank (PDB) ID 1E0G; **http://www.rcsb.org**]. The two β-strands are in an antiparallel arrangement, with both α-helices on one side. The arrow indicates the potential ligand-binding cleft between the α-helices. (*b*) Surface views of AtCERK1 (PDB ID 4EBZ, *left*) and OsCEBiP (PDB ID 5JCE, *right*), each of which contains three LysM domains (LysM1–3) arranged in a cloverleaf-shaped assembly. OsCEBiP has an additional C-terminal cysteine-rich domain (shown in *magenta*). Chitin (shown in *yellow* in a bonds representation) binds to LysM2 in both proteins. (*c*) Bonds representation of chitin (shown in *yellow*) binding to AtCERK1 LysM2 (C_α trace, shown in *blue* along with interacting residues in a bonds representation in *gray*), which is mediated by hydrogen bonds (*black dotted lines*), mainly between the ligand and protein backbone atoms. Water molecules are shown as red spheres. (*d*) Ribbon diagram of the OsCEBiP cysteine-rich domain (shown in *magenta*), a new fold of unknown function. Six β-strands, arranged in two β-sheets of three strands each, fold into a β-barrel. The domain is stabilized by cysteine residues that contribute to the formation of several disulfide bonds (shown in *green* in a bonds representation). (*e*) The LysM-domain fold and the mode of ligand recognition, which are conserved between plant and bacterial proteins. Superposition of AtCERK1 LysM2 and NlpC/P60 (PDB ID 4UZ3, C_α traces) with their respective ligands (shown in bonds representations) shows that they align with an r.m.s.d. (root-mean-square deviation) of ~0.9 Å, comparing 35 corresponding C_α atoms.

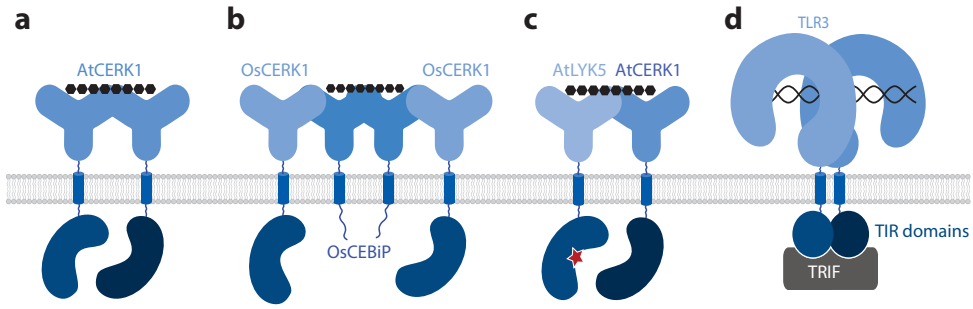


Figure 4

Suggested receptor activation models for receptor kinases (RKs) that contain a lysin motif (LysM). (*a*) A chitin oligomer of at least seven *N*-acetyl-D-glucosamine (NAG) units (*black hexagons*) leads to dimerization of the RK AtCERK1. Both proteins bind to the ligand with their respective LysM2 domains. Each receptor molecule binds to distinct NAG units, so that the chitin oligomer ties the two proteins together. (*b*) Like AtCERK1, the receptor-like protein (RLP) OsCEBiP dimerizes upon chitin oligomer binding. In contrast to AtCERK1, two CEBiPs bind to opposing sides of the same chitin oligomer. Because studies have suggested that OsCERK1 heterodimerizes with OsCEBiP, ligand binding might lead to the formation of a tetrameric or higher-order signaling complex. (*c*) Upon ligand binding, heterodimerization of AtCERK1 and another chitin-binding LysM-RK, such as AtLYK5, may initiate downstream signaling. (*d*) Chitin-mediated homodimerization of AtCERK1 might resemble double-stranded-RNA-driven TLR3 homodimerization in animal innate immunity. In both cases, a polymeric ligand leads to homodimerization of the extracellular domains, which in turn brings the intracellular parts of the proteins in close proximity to initiate downstream signaling.

of LysM receptors, leading to activation of their cytoplasmic kinase modules and subsequent activation of plant immune responses or symbiotic interactions.

ARCHITECTURE OF PLANT RECEPTOR KINASES WITH LEUCINE-RICH-REPEAT ECTODOMAINS

RKs with LRR ectodomains form the largest subgroup of plant RKs, with ~200 LRR-RKs in *Arabidopsis* (111). Proteins containing LRR domains are found in all kingdoms of life (64). Animal cell surface receptors with LRR ectodomains have been characterized in molecular detail (9). Plant LRR domains are composed of individual repeat units that are 22–23 amino acids long. Leucines and other apolar residues form the hydrophobic core of the domain (hence the term leucine-rich repeat) (**Figure 5*a***). Individual LRRs stack onto each other to build up large assemblies; in bacterial and animal proteins, they stack parallel to a common axis, giving rise to a horseshoe-shaped structure (64). Plant LRR domains contain a variation of the LRR consensus sequence, which creates a second β-strand oriented perpendicular to the canonical β-strand, forming the inner surface of the solenoid (**Figure 5*a***). This additional β-strand forces plant LRRs to stack out of plane, giving rise to a twisted or superhelical assembly, which has important functional implications (see below).

The twisted nature of plant LRR domains was first observed in the crystal structure of the plant polygalacturonase-inhibiting protein (PGIP), which has ten LRRs (31) (**Figure 5*b***). Based on the PGIP structure, van der Hoorn et al. (125) proposed that the ectodomains of plant LRR-RKs may form superhelical assemblies. This was experimentally confirmed in crystal structures of the LRR-RK BRI1, in which 25 individual LRRs fold into a right-handed superhelix, completing more than one full turn (49, 104) (**Figure 5*c***). The hydrophobic ends of LRR assemblies are not

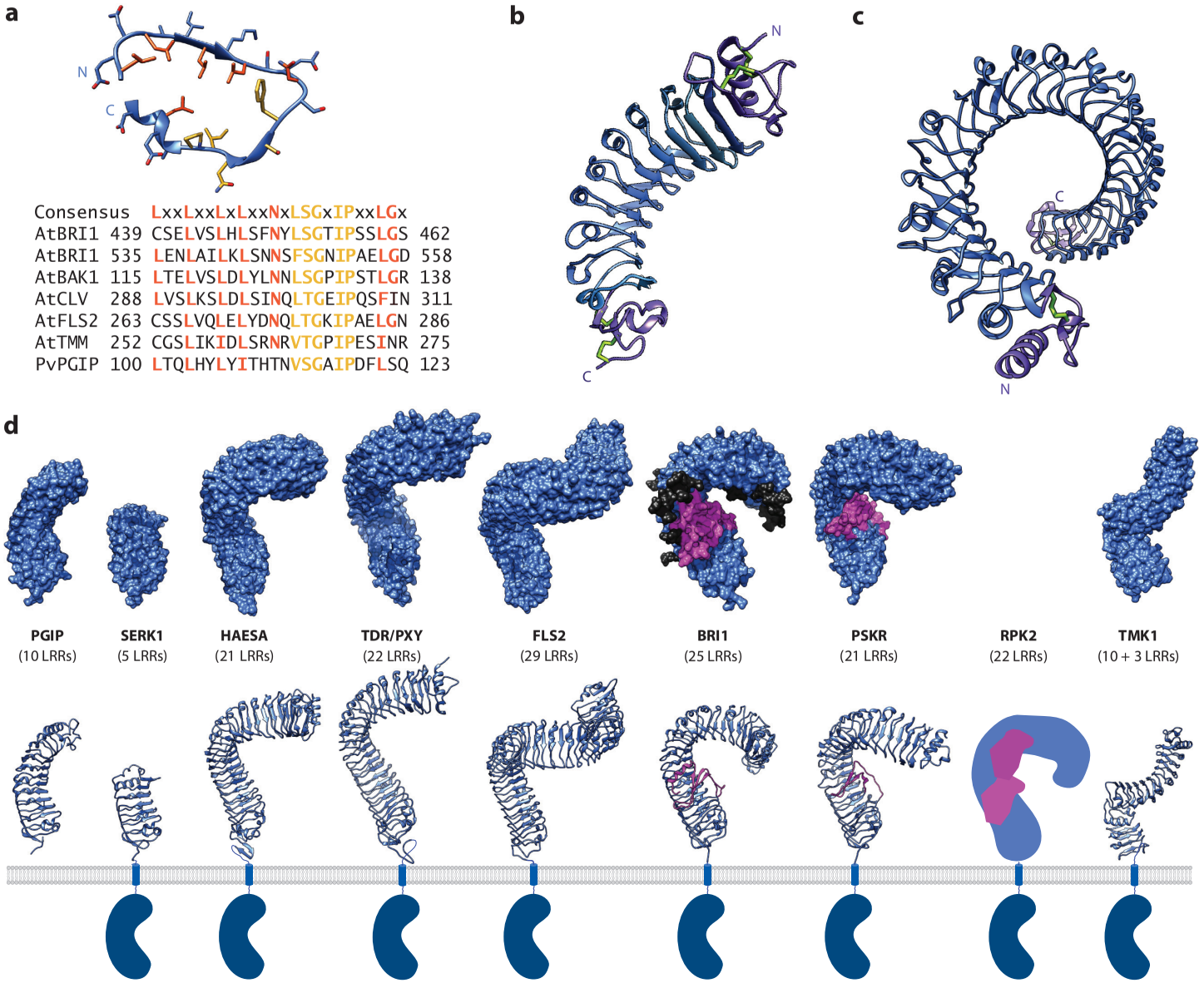
exposed to the solvent but are masked by hydrophilic N- and C-terminal capping domains, which often are stabilized by disulfide bonds (see **Figure 5*b*,*c***).

Several other LRR-RK ectodomains have now been structurally investigated, allowing us to structurally categorize them (**Figure 5*d***). One simple way to sort LRR-RKs is by size, because the number of individual LRRs in the ectodomains of different LRR-RKs varies greatly. All ligand-binding LRR-RKs characterized thus far (HAESA, TDR/PXY, FLS2, BRI1, and PSKR) have large LRR ectodomains consisting of 21–29 LRRs (49, 100, 104, 114, 120, 122, 129, 144) (**Figure 5*d***). By contrast, crystal structures of the LRR-RKs *Arabidopsis thaliana* SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 (AtSERK1), AtSERK3 [also termed *Arabidopsis thaliana* BRI1-ASSOCIATED RECEPTOR KINASE (AtBAK1)], and OsSERK2 confirmed the presence of small ectodomains composed of five LRRs, as predicted from their sequence. SERK-family LRR-RKs have been functionally and structurally characterized as non-ligand-binding co-receptor kinases (84, 101, 119) (**Figure 5*d***). These findings have led to speculation that large LRR-RKs are ligand-binding receptors, and small LRR-RKs act as co-receptors required for the activation of LRR-RK signaling complexes (101, 109). However, only a tiny fraction of the ~200 LRR-RKs have been characterized in molecular detail, and thus this rule of thumb may need to be modified or extended: Recently, LRR-RLPs with few LRRs in their ectodomain, such as the TOO MANY MOUTHS (TMM) protein, have been implicated in direct EPF peptide ligand binding (for comparison, the size of the TMM ectodomain should match the one shown for PGIP in **Figure 5*d***) (70). By contrast, no direct interaction with CLE peptides has been observed for the large LRR-RK RPK2 and the RLP CLV2, both of which are involved in maintenance of plant stem cell populations (106, 108). From a structural point of view, there is no reason that small LRR proteins should not be able to sense specific ligands. Indeed, the small variable lymphocyte receptors, which are LRR-containing proteins that control the recognition of antigens in the adaptive immune system of jawless vertebrates, have evolved to bind complex ligands using as few as five LRRs (41). Also, there is no structural reason that LRR-RKs and LRR-RLPs with many repeats should all be ligand-binding modules. In fact, they could also act as co-receptor kinases, general protein-protein interaction modules, or structural proteins.

Another simple classification scheme is based on whether plant LRR-RK ectodomains contain a so-called island domain. Island domains represent long loop structures inserted between two consecutive LRR repeats. They may fold into domain-like structures that harbor secondary structure elements, as observed in crystal structures of the LRR-RKs BRI1, PSKR, and RPK2 (49, 104, 114, 129). RPK2 contains two island domains that map to the N-terminal and central portions of its LRR domain, but their function is unknown (63, 106, 114). By contrast, most of the characterized peptide ligand LRR-RKs, such as HAESA, TDR/PXY, FLS2, and PEPR, do not contain island-domain structures (89, 100, 120, 122, 144). This suggests that plants may have evolved island-domain-containing LRR ectodomains to facilitate the specific binding of relatively small ligands. Interestingly, animal immune receptors sense complex small-molecule ligands not by using island domains, but rather via independent protein modules such as the MD-2 protein, which binds its lipopolysaccharide ligand before being recognized by the LRR domain of TLR4 (96).

The LRR domain of TMK1 features a non-LRR domain, which disrupts the regular repeat structure, creating a sharp kink in the overall assembly (75) (**Figure 5*d***). Although TMK1 has been implicated in the recognition of the auxin-loaded ABP1 protein (auxin being a tryptophan-derived plant growth hormone), it is unclear whether the unusual ectodomain structure of TMK1 represents a specific adaptation for the recognition of a large protein ligand (19, 137).

The ectodomains of plant LRR-RKs vary greatly in size and shape, and it is difficult to predict the function of LRR-RKs based on their sequence or ectodomain structure. A better functional


a
N
C
Consensus LxxLxxLxLxxNxLSGxIPxxLGx
AtBRI1 439 CSELVSLHLSFNYLSGTIPSSLGS 462
AtBRI1 535 LENLAILKLSNNSFSGNIPAELGD 558
AtBAK1 115 LTELVSLDLYLNNLSGPIPSTLGR 138
AtCLV 288 LVSLKSLDLSINQLTGEIPQSFIN 311
AtFLS2 263 CSSLVQLELYDNQLTGKIPAELGN 286
AtTMM 252 CGSLIKIDLSRNRVTGPIPESINR 275
PvPGIP 100 LTQLHYLYITHTNVSGAIPDFLSQ 123
b
N
C
c
C
N
d
PGIP
(10 LRRs)
SERK1
(5 LRRs)
HAESA
(21 LRRs)
TDR/PXY
(22 LRRs)
FLS2
(29 LRRs)
BRI1
(25 LRRs)
PSKR
(21 LRRs)
RPK2
(22 LRRs)
TMK1
(10 + 3 LRRs)

classification of plant LRR-RKs will become possible only once the cellular and biochemical roles of many orphan plant LRR-RKs and LRR-RLPs have been investigated in mechanistic detail.

LIGAND RECOGNITION BY ISLAND-DOMAIN-CONTAINING LEUCINE-RICH-REPEAT RECEPTOR KINASES

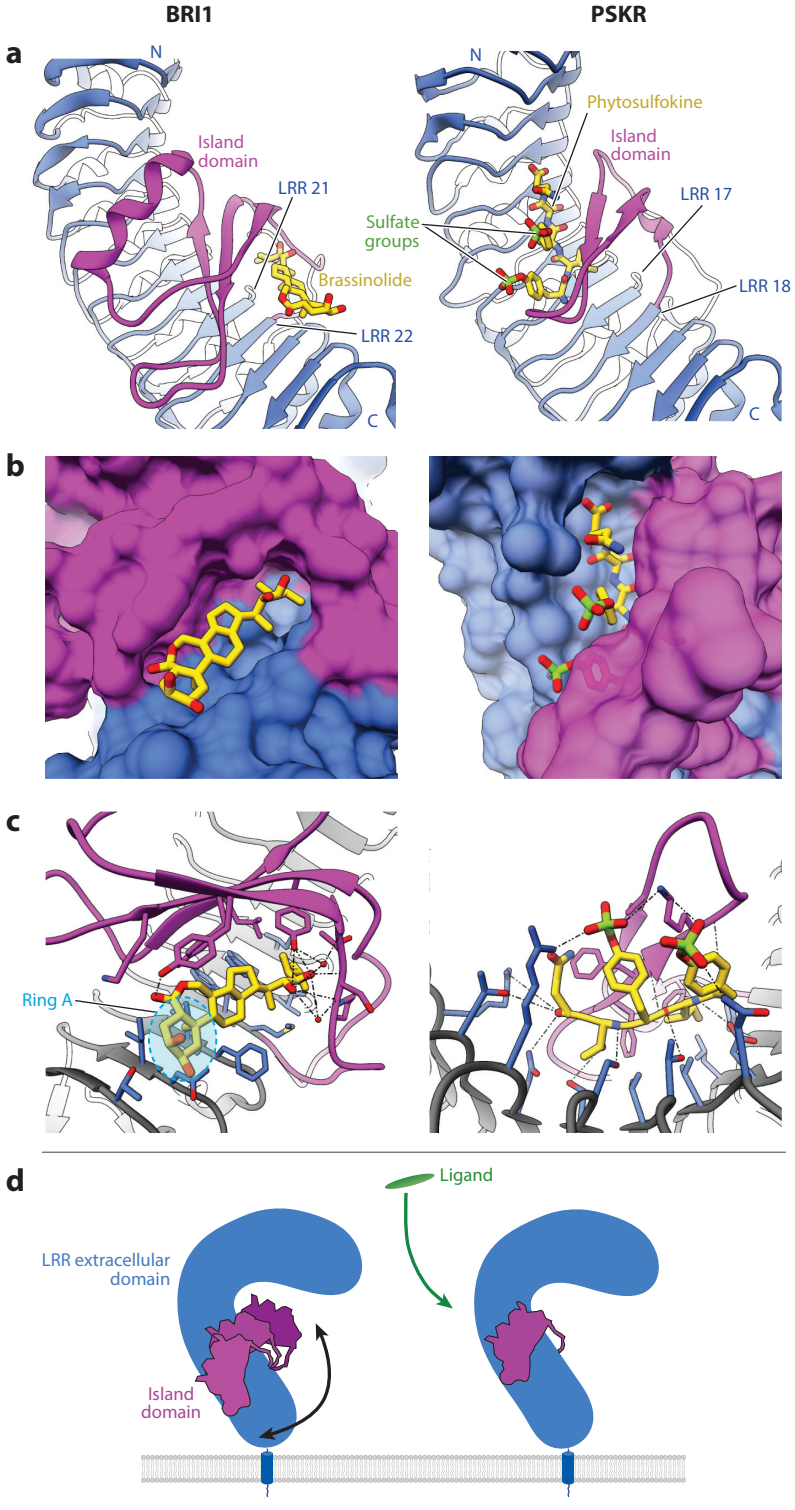
The first crystal structures of a plant LRR-RK, for the brassinosteroid receptor BRI1, revealed the presence of an island domain approximately 70 amino acids long inserted between LRRs 21 and 22 (49, 104), which is also conserved in the LRR-RKs BRI1-LIKE 1–3 (BRL1–3) (17, 105, 150). The BRI1 island domain (see **Figure 6**) folds into an antiparallel β-sheet connected via long loops and a small helix (49). Together, the island domain and the BRI1 LRR core (LRRs 21–25; see **Figure 6**) form the binding pocket for the steroid hormone (**Figure 6*b***). The alkyl chain of brassinolide inserts deeply into the pocket (**Figure 6*b***). The A–D rings of the steroid bind to a hydrophobic face formed by the LRR core but leave the A ring with its two hydroxyls exposed to the solvent (see **Figure 6*c***). Overall, the receptor establishes mainly hydrophobic interactions with the ligand, with both the island domain and the LRR core contributing important residues (49). In contrast to the binding mode of NAG oligomers to LysM domains, few backbone interactions are observed in the BRI1-brassinolide complex structure (**Figures 3*c*** and **6*c***).

A smaller island domain of ∼35 amino acids is present in the LRR-RK PSKR, which binds a disulfated pentapeptide (81, 82). In the ligand-bound PSKR structure, the island domain folds into a small antiparallel β-sheet similar to the one described for BRI1 (129) (**Figure 6*a***). However, the PSKR island domain does not provide a full binding pocket for its ligand, but rather covers part of phytosulfokine using a distal loop structure (**Figure 6*a*,*b***). In contrast to brassinosteroid binding by BRI1, the phytosulfokine peptide establishes mainly hydrophilic interactions with its receptor, forming an intense hydrogen bond network that involves both side-chain and main-chain atoms (**Figure 6*c***). In the PSKR structure, the two sulfate moieties of the ligand make only a few contacts with two basic residues from the LRR core and with a central lysine from the island domain (**Figure 6*c***). However, the desulfated peptide binds the receptor with much weaker affinity compared with the bioactive form (129).

Comparison of the ligand-bound BRI1 and PSKR complex structures with their corresponding apo forms strongly suggests that LRR-RK island domains are flexible and largely disordered in

Figure 5

The ectodomains of leucine-rich-repeat (LRR) receptor kinases (RKs), which vary in size and shape but are composed of highly similar repeat units. (*a*) Ribbon diagram of a plant-type LRR (*top*) along with a structure-guided sequence alignment of individual repeat units from different plant RKs (*bottom*). The hydrophobic interior of an LRR features a large number of conserved leucines and other hydrophobic residues (shown in *orange*). The plant-unique motif allows for the formation of a superhelix (shown in *yellow*). The sequence alignment at the bottom highlights plant LRR sequence fingerprints. (*b*) Ribbon diagram of PGIP [Protein Data Bank (PDB) ID 1OGQ; **http://www.rcsb.org**]. The LRRs are shown in blue, the N- and C-terminal capping domains are shown in purple, and the stabilizing disulfide bonds are shown in green in a bonds representation. (*c*) Ribbon diagram of BRI1 (PDB ID 3RIZ), shown in a top view with the island domain omitted, using the same colors as in panel *b*. As this protein has 25 LRRs, it folds into a spiral-shaped assembly. (*d*) Structural features of different plant LRR-RKs. Shown in the bottom row are ribbon diagrams, drawn to scale, of PGIP, SERK1 (PDB ID 4LSC), HAESA (PDB ID 5IXO), TDR/PXY (PDB ID 5GIJ), FLS2 (PDB ID 4MN8), BRI1, PSKR (PDB ID 4Z61), RPK2 (structure not deposited in PDB, shown here as a schematic view), and TMK1 (PDB ID 4HQ1). BRI1 and PSKR each contain one island domain (shown in *magenta*); RPK2 contains two island domains. In TMK1, the stacking of the LRR repeats is interrupted by a non-LRR insertion, giving rise to a kinked structure with two solenoids. Shown below the ribbon diagrams are schematic views of the transmembrane helices (*blue cylinders*) and cytoplasmic kinase domains (*dark blue kidney shapes*), also drawn to approximate scale; shown above the ribbon diagrams are surface views of the respective extracellular domains. All plant LRR extracellular domains are glycosylated in vivo; as an example, the glycan structures in the BRI1 domain are highlighted in black.


BRI1
PSKR
a
N
Island domain
LRR 21
Brassinolide
LRR 22
C
N
Phytosulfokine
Island domain
LRR 17
Sulfate groups
LRR 18
C
b
c
Ring A
d
Ligand
LRR extracellular domain
Island domain

the absence of a ligand (49, 129). Upon ligand binding, the island domains become structured and their position becomes fixed with respect to the LRR core (**Figure 6*d***). Thus, ligand binding to BRI1 and PSKR changes the surface properties of their ectodomains, whereas the stable LRR core itself does not undergo conformational changes. This finding led to the speculation that ligand binding to island-domain-containing ectodomains creates a docking platform for a helper protein, which is required for receptor activation (see below) (49).

LIGAND RECOGNITION IN PEPTIDE-BINDING LEUCINE-RICH-REPEAT RECEPTOR KINASES

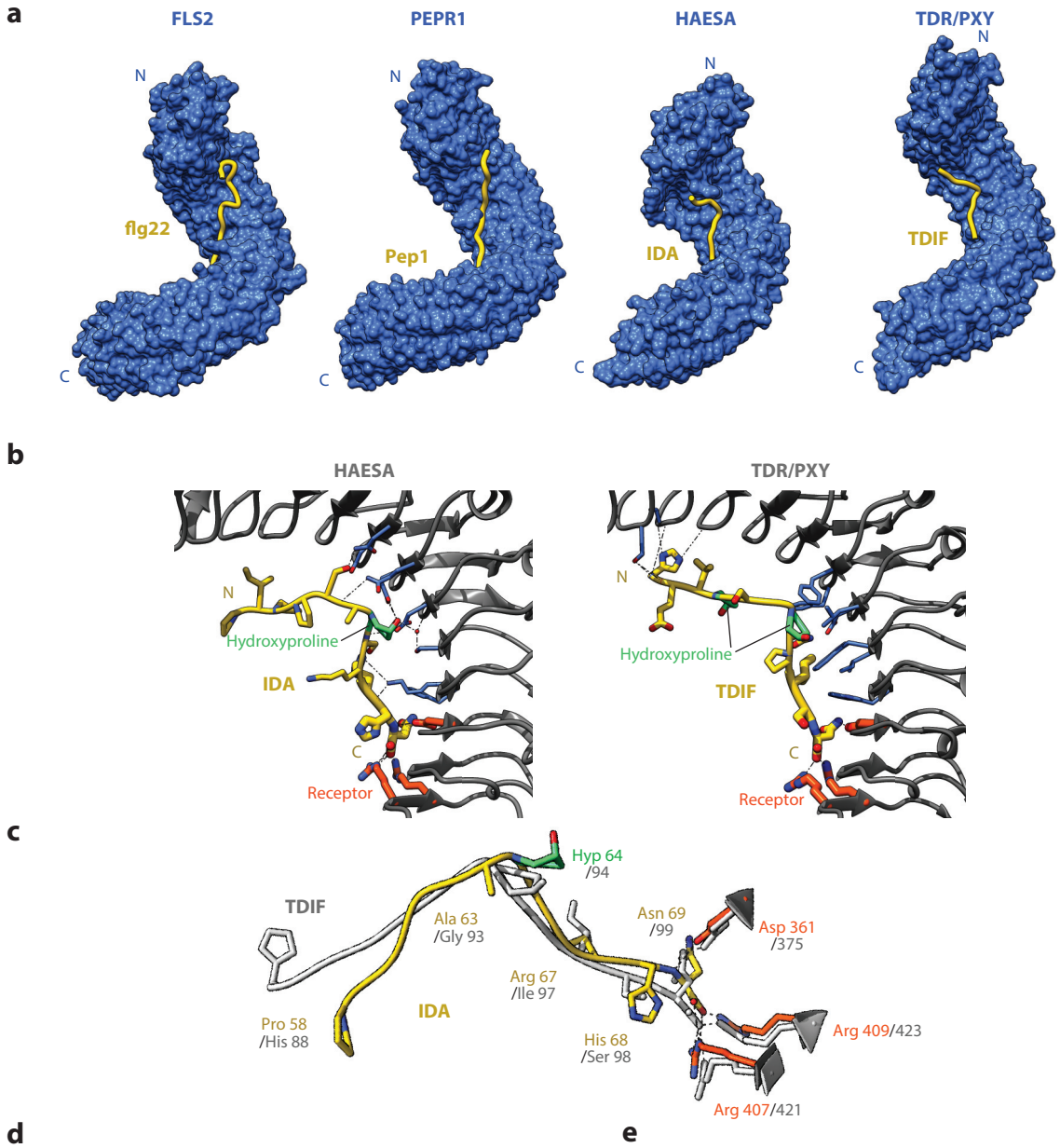
The first structure for a peptide-binding plant LRR-RK was reported for the plant pattern recognition receptor FLS2 (120), which senses a conserved epitope of bacterial flagellin, the 22-amino-acid peptide flg22 (22, 37). FLS2 features a large ectodomain with 29 individual repeats and no island domain (**Figures 5*c*** and **7*a***). The receptor recognizes the synthetic flg22 peptide, which binds in a fully extended conformation along the inner side of the LRR spiral (**Figure 7*a***). LRRs 3–18 contribute to the formation of the flg22 binding groove in FLS2. The peptide makes extensive side-chain and main-chain contacts with the receptor. The flg22 N terminus is rather hydrophilic, and its C terminus is composed mainly of small hydrophobic residues (120).

The 23-amino-acid peptide AtPep1 is an endogenous danger signal produced in response to cellular damage caused by, for example, pathogenic attacks (52). AtPep1 is sensed by the LRR-RKs PEPR1 and -2 (66, 138, 139). PEPR1 consists of 27 canonical LRRs and lacks an island domain (122). AtPep1 binds in a completely extended conformation to the inner surface of the PEPR1 ectodomain, covering LRRs 4–18 (122) (**Figure 7*a***). The mode of ligand binding and the orientation of AtPep1 reflect those of flg22, as described above (**Figure 7*a*,*b***). However, the C terminus of AtPep1 is drastically different from flg22. Although the C-terminal alanine of flg22 appears to be completely disordered in the FLS2-flg22 complex structure (120), asparagine 23 and the terminal carboxyl of AtPep1 establish hydrogen bonds with a strictly conserved arginine residue in LRR 18 of the PEPR1 ectodomain (122). This arginine is part of a specific pocket that accommodates the AtPep1 C terminus, and deletion of the invariant C-terminal asparagine abolishes binding of AtPep1 to its receptor (122) (**Figure 7*d*,*e***).

Many plant peptide hormones control aspects of growth and development rather than immune responses (80). The small peptide INFLORESCENCE DEFICIENT IN ABSCISSION (IDA),

Figure 6

Structures of the leucine-rich-repeat (LRR) receptor kinases (RKs) BRI1 (*left*) and PSKR (*right*). Both of these RKs bind their ligands using their LRR cores and specialized island domains. (*a*) Brassinolide (shown in *yellow* in a bonds representation) binds to the extracellular domain of BRI1 [shown as a ribbon diagram; Protein Data Bank (PDB) ID 3RJ0; **http://www.rcsb.org**] in a pocket formed by the LRR core (shown in *blue*) and the island domain (shown in *magenta*). The pentapeptide hormone phytosulfokine (shown in *yellow*, with sulfur atoms shown in *green*) binds to the LRR core domain of PSKR (shown in *blue*; PDB ID 4Z64) and to the island domain of the receptor (shown in *magenta*). (*b*) Surface views of the respective complexes reveal the presence of a steroid-binding pocket in BRI1, whereas the island domain of PSKR covers only parts of phytosulfokine. The colors correspond with those in panel *a*. (*c*) The BRI1 island domain and LRR core have many hydrophobic and polar interactions with brassinolide. Only ring A, with its two hydroxyls, remains solvent exposed (highlighted in *cyan*). Phytosulfokine binds its receptor, PSKR, using mainly polar interactions. (*d*) Structures of apo-BRI1 and apo-PSKR reveal that their island domains are highly flexible in the absence of a ligand. Upon ligand binding, the island domain becomes ordered and, together with the ligand, creates a protein docking platform. In this schematic, the ligand is shown in green, the LRR extracellular domains are shown in blue, and the island domains are shown in magenta.



d

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AtPEPR1 440 VDFIGNKLTG -- 30X -- GHCKTIRRFI 489
AtHAESA 360 VDLSYNRFSG -- 30X -- GKCKSLTRVR 409
AtHSL2  368 FDVSTNRFSG -- 30X -- GDCHSLNYIR 417
AtPXY   374 MDVSNNSFTG -- 30X -- TRCESLWRFR 423
AtBAM1  364 VDLSSNKLTG -- 30X -- GKCESLTRIR 413
AtCLV1  366 LDVSDNHLTG -- 30X -- GKCKSLTKIR 415
AtRGFR3 411 IDLSYNSLSG -- 30X -- GNCTNLYRLR 460
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e

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PEP1        70 ATKVKAKQRGKEKVSSGRPGQHN 92
PEP3        74 EIKARGKNKTKPTPSSGKGGKHN 96
IDA         58 -----------PIPPSAPSKRHN 69
IDL1        67 -----------LVPPSGPSMRHN 78
IDL2        72 -----------PVPASGPSRKHN 83
TDIF/CLE41  88 -----------HEVPSGPNPISN 99
CLE9       109 -----------RLVPSGPNPLHN 120
CLV3        70 -----------RTVPSGPNPLHN 82
RGF1       104 ----------DYSNPGGHHPRHN 116
RGF2        97 ----------DYWKPRHHPPKNN 109
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for example, controls abscission processes, such as the shedding of floral leaves in *Arabidopsis*, in concert with the LRR-RK HAESA and its homolog HSL2 (15, 59, 126). Like other peptide hormones (80), IDA is processed from a longer propeptide into a bioactive dodecamer (100, 117). The HAESA ectodomain contains 21 individual LRRs and has no island domain. The receptor provides a binding groove for IDA that covers LRRs 2–14, running along the inner side of the superhelix (100) (**Figure 7*a***). IDA contains a central proline residue that must be posttranslationally modified for activity (16). This hydroxyproline modification in IDA is read out by the HAESA LRR domain, which establishes a hydrogen-bonding network with the residue's hydroxyl group (**Figure 7*b***). The interactions between the IDA hydroxyproline and HAESA are critical for binding (100).

As described above for AtPep1, IDA also contains a C-terminal asparagine residue, which together with the free C terminus of the peptide makes extensive contacts with two arginines and an aspartate residue of HAESA (**Figure 7*b,c***). Interestingly, this residue is conserved among many different plant peptide hormone families, as are the arginine and aspartate residues in their cognate receptors (100) (**Figure 7*d,e***). Among them is the LRR-RK TDR/PXY, whose peptide ligand TRACHEARY ELEMENTS DIFFERENTIATION INHIBITORY FACTOR (TDIF)/CLE41 is also an active hydroxyprolinated dodecamer with a C-terminal asparagine (**Figure 7*e***). TDIF binds its receptor in a way that is highly similar to the one described for HAESA-IDA (100, 144) (**Figure 7*a–c***); despite their low overall sequence homology and their very different functions in plant development, HAESA and TDR/PXY contain ectodomains of very similar shape and size that bind their peptide ligands using the same binding surface (**Figure 7*a***). Consistently, the corresponding binding surface in the LRR-RK BAM1 maps to its CLE9 peptide-binding site (110).

Both HAESA and TDR/PXY recognize the C-terminal asparagine in IDA and TDIF using an invariant arrangement of polar interactions provided by conserved arginine and aspartate residues originating from the LRR cores (**Figure 7*b–d***). The same mode of binding is used by the LRR-RKs PEPR1 and RGFR3, which bind AtPep1 and RGF1 peptides, respectively, with a C-terminal asparagine (115, 122) (**Figure 7*a,d,e***). Notable differences between IDA and TDIF binding are (*a*) that the central hydroxyproline in TDIF does not engage in polar interactions with TDR/PXY, instead binding to a rather hydrophobic pocket that provides no hydrogen-bonding partners, and (*b*) that TDIF features a flexible glycine residue preceding the central hydroxyproline, which induces a more pronounced kink in the peptide (144) (**Figure 7*b,c***). The corresponding residue in IDA is an alanine (100) (**Figure 7*c***).

Figure 7

The conserved mode of peptide ligand binding used by plant leucine-rich-repeat (LRR) receptor kinases (RKs). (*a*) Surface views of the structures of known peptide-binding LRR-RKs. Shown are FLS2-flg22 [Protein Data Bank (PDB) ID 4MN8; **http://www.rcsb.org**], PEPR1-Pep1 (PDB ID 5GR8), HAESA-IDA (PDB ID 5IXQ), and TDR/PXY-TDIF (PDB ID 5GIJ). Receptor extracellular domains are shown in blue, and peptides are shown as yellow C_α traces. (*b*) Peptide binding of IDA to HAESA (*left*) and of TDIF to its receptor, TDR/PXY (*right*). A conserved aspartate and two arginines in the receptor (shown in *orange*) coordinate the peptides' C termini. The LRR backbone is shown in dark gray as a ribbon diagram, with selected side chains shown in blue and orange in bonds representations. Peptides are shown in yellow and the conserved hydroxyproline is shown in green, both in bonds representations. (*c*) Structural superposition of the ligand-bound HAESA and TDR/PXY LRR ectodomains [r.m.s.d. (root-mean-square deviation) of ~1.0 Å, comparing 311 corresponding C_α atoms]. IDA (shown in *yellow*) and TDIF (shown in *gray*) exhibit a highly conserved mode of binding. In both structures, the C termini of the peptides are bound in specific pockets formed by invariant arginine and aspartate residues. (*d,e*) Sequence alignments of different peptide-binding LRR-RKs (panel *d*) and plant peptide hormones (panel *e*). These alignments reveal conserved sequence fingerprints (shown in *orange*) that are involved in the recognition of the C-terminal asparagine residue that is conserved in many plant peptide hormones. Proline residues that may be hydroxyprolines in planta are shown in green.

The invariant C termini in different plant peptide hormones and the presence of conserved sequence fingerprints in their corresponding receptors allow us to speculate that peptide hormone binding by plant LRR-RKs follows certain principles. First, plant peptide hormones bind their receptor in a fully extended conformation along the inner surface of the LRR superhelix (with the exception of phytosulfokine, which binds to an island domain, as discussed above). Second, the orientation of the ligand follows the orientation of the ectodomain, with the N terminus of the peptide pointing towards the N terminus of the receptor and vice versa. Third, LRR-RK ectodomains act as molecular rulers that measure the correct size of the bioactive peptide by making specific interactions with its mature C terminus and to a lesser extent with its N terminus (this is less pronounced in flg22 binding to FLS2). And fourth, posttranslational modifications are critical determinants of high-affinity binding for many plant peptide hormones. This suggests that the signaling capacity of plant peptide hormones in vivo is defined not only by their expression, secretion, and diffusion, but also by their processing and posttranslational modification (80). Interestingly, the sequence features in LRR-RKs and plant peptides outlined above have been exploited for the discovery of novel receptor-ligand pairs (109, 113).

LEUCINE-RICH-REPEAT RECEPTOR KINASE ACTIVATION BY SHAPE-COMPLEMENTARY CO-RECEPTOR KINASES

The architectural similarities between plant LRR-RKs and animal TLRs have led to speculation that ligand-induced homo-oligomerization results in the activation of plant LRR-RKs. Indeed, homo-oligomers of plant RKs have been observed in vivo, but they appear to be constitutive rather than induced by ligand binding, and thus they are unlikely to play a role in receptor activation (48, 118, 131). The crystal structure of the plant LRR-RK BRI1 revealed a spiral-shaped ectodomain that is largely masked by carbohydrates and provides a ligand-binding site for brassinosteroids at the inner surface of the spiral (49, 104). Together, the architecture of the BRI1 ectodomain and biochemical experiments suggested that receptor homodimerization does not represent the activation mechanism for BRI1 (49, 104). The ligand-bound BRI1 ectodomain structure revealed (*a*) that brassinosteroid binding induces ordering of the island domain but no other conformational changes within the LRR domain (**Figure 6*d***), (*b*) that the ligand-binding pocket and a larger surface area below are not masked by carbohydrates (**Figure 5*d***), and (*c*) that several genetic gain- and loss-of-function alleles map to this surface area of BRI1 (49). Based on these findings, Hothorn et al. (49) hypothesized that a shape-complementary helper protein might interact with BRI1 upon brassinosteroid binding, activating the receptor.

One candidate helper protein was SERK3/BAK1, a plant LRR-RK with a small ectodomain. SERK3/BAK1 was initially described as a genetic component of the brassinosteroid signaling pathway (72, 91), as a BRI1-interacting protein (72, 91, 130), and as a BRI1 phosphorylation target (130). In addition, it interacts with FLS2 in a ligand-dependent manner (23). SERK3/BAK1 belongs to the small SERK family of LRR-RKs, which comprises five members in *Arabidopsis* (10). These genes were initially identified based on their expression during somatic embryogenesis (102). Gou et al. (38) provided strong genetic support that SERK RKs play an essential and redundant role in brassinosteroid signal transduction.

A crystal structure of isolated SERK1 revealed a short ectodomain with five LRRs (101). SERK1 forms a heterodimer with the BRI1 ectodomain only in the presence of brassinosteroids, supporting a ligand-induced heterodimerization activation mechanism (101). A ternary BRI1-brassinolide-SERK1 complex structure revealed that the SERK1 ectodomain binds to the BRI1 superhelix, establishing direct contacts with the island domain; the BRI1 LRR core; and, importantly, brassinolide itself (**Figure 8*a*,*b***). The ligand-binding site is complete only once the

co-receptor has been recruited by the receptor, with the hormone acting as a molecular glue that promotes the association of the receptor and co-receptor (101). In the complex structure, BRI1 and SERK1 are oriented in such a way that their C termini—which in the context of the full-length receptors connect to the transmembrane helices—are facing in the same direction and are in close proximity (7, 101) (**Figure 8*a***). A complex of BRI1 with SERK3/BAK1 revealed that different SERK proteins have very similar interactions with the receptor (119). A large part of the inner surface area of the SERK1/3 ectodomain is in contact with the receptor and the ligand in the complex structures, with residues originating from the SERK1/3 N-terminal capping domains and their respective LRR cores (101, 119) (**Figure 8*c***).

In the phytosulfokine receptor PSKR, binding of the short peptide ligand anchors the otherwise highly mobile island domain onto the receptor's LRR core (129). PSKR and SERKs have overlapping signaling functions (42, 82, 102), and structural and biochemical experiments have suggested that SERKs also act as co-receptor kinases for PSKR (129) (**Figure 8*a*,*b***). In the structure of the PSKR-phytosulfokine-SERK1 complex, the co-receptor binding site and its mode of binding are highly similar to those observed in the BRI1-brassinolide-SERK1 complex (**Figure 8*a*,*b***). The striking difference is that the SERK1 N-terminal capping domain contacts only the PSKR1 island domain and not the phytosulfokine itself, an interaction that has been described as an allosteric receptor activation mechanism (129) (**Figure 8*b***).

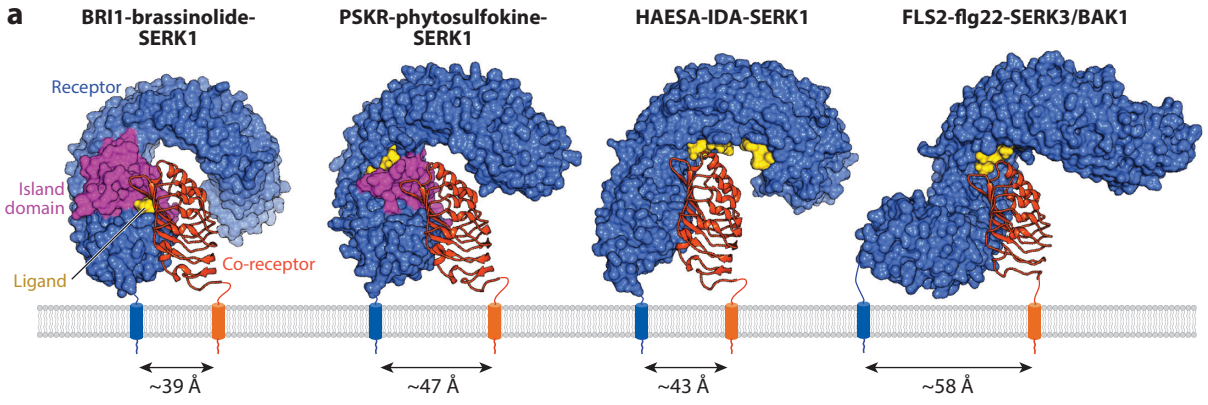
Receptor activation in LRR-RKs that do not contain an island domain can be illustrated using the recently determined HAESA-IDA-SERK1 complex (100). In this high-resolution structure, SERK1 adopts a position similar to those in the complexes described above. In this case, however, the N-terminal capping domain of SERK1 binds on top of the IDA binding groove in HAESA, forming a lid structure that covers the three C-terminal amino acids in IDA (**Figure 8*a*,*b***). These amino acids are conserved among the different IDLs and are also found in other plant peptide hormones, suggesting that these peptides also require co-receptor kinases for receptor activation (100, 115, 122, 144) (**Figure 7*e***). In the case of HAESA, the contribution of the SERK1 ectodomain to IDA binding has been quantitatively assessed: In the absence of the co-receptor, HAESA binds IDA with micromolar affinity, whereas SERK1 shows no detectable binding to the peptide hormone. Upon addition of the SERK1 ectodomain, HAESA binds IDA with a much higher affinity, in the nanomolar range (100). From these experiments, one can deduce that one function of LRR-RK co-receptor kinases is to drastically reduce the off rate of the ligand from the receptor.

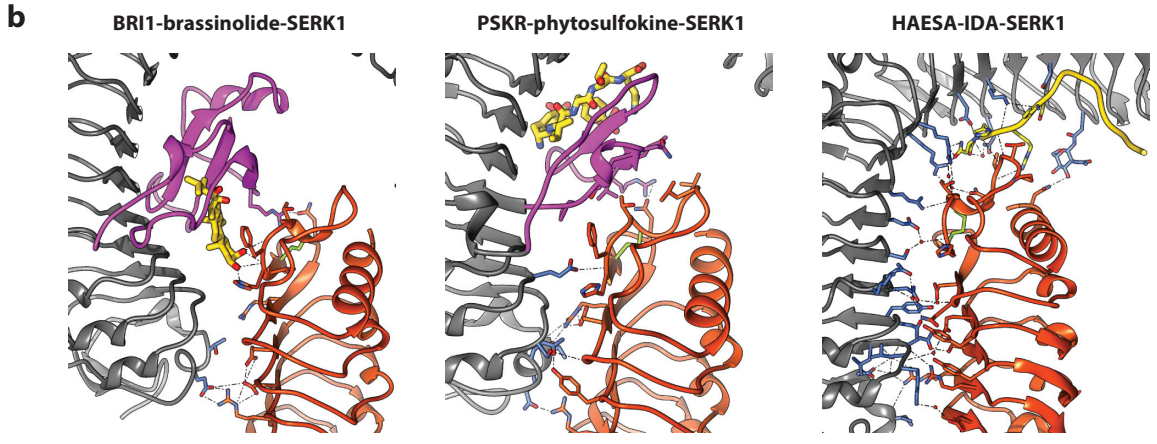
To understand receptor activation at the molecular level, it is important to note that the isolated ectodomains of HAESA and SERK1 do not detectably interact in vitro in the absence of IDA (100). In the presence of IDA, however, SERK1 binds to HAESA with nanomolar affinity, further supporting the molecular-glue hypothesis (100). These effects should be even more pronounced in the context of the plasma membrane, where only lateral diffusion of the receptor and co-receptor may occur and where diffusion may be further restricted by LRR-RK complex organization (12). Consistent with the existing structural and biochemical evidence, SERK proteins have been genetically defined as positive regulators of floral abscission (86, 100). Crystal structures with SERK3/BAK1 have been determined for BRI1 and FLS2 and again revealed a similar position for this SERK-family co-receptor kinase in the ternary complexes (119, 120) (**Figure 8*a***).

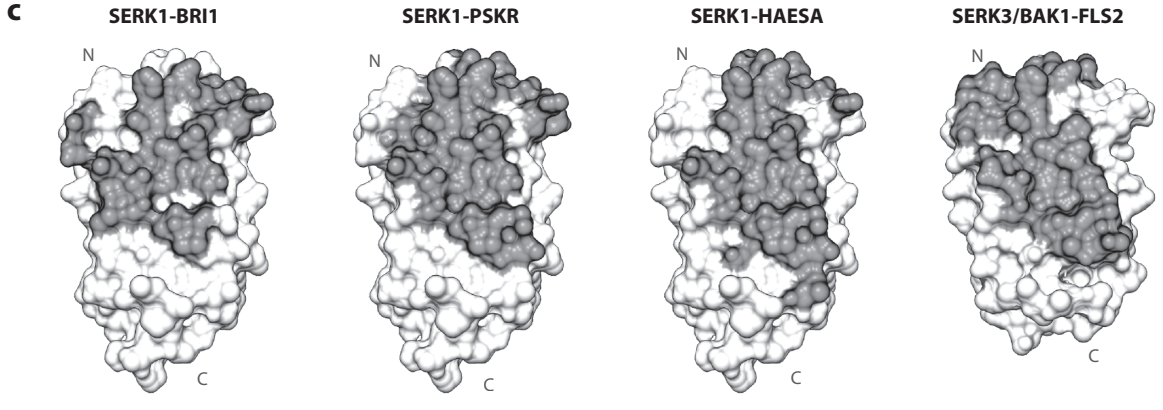
The ligand-binding sites in the known LRR-RKs vary widely in size, corresponding to the different natures of the ligands being recognized (**Figures 6*a*** and **7*a***). The SERK complex structures together rationalize why the ligand-binding sites in all plant LRR-RKs described thus far map at least partly to the C-terminal half of the receptors' ectodomains, a surface area that can be reached by the much smaller co-receptor kinases (**Figure 8*a***).

Over the past few years, SERK proteins have been genetically and/or biochemically defined as co-receptors for very different LRR-RK signaling pathways (1, 10, 20, 23, 38, 49, 60, 72, 85,

86, 91, 99, 100, 115, 122, 129, 145). Why so many functions converge on such a small protein family is not well understood, but structural studies have defined how SERKs can fulfill these different functions: They employ a large set of residues from the inner surface of their ectodomains to interact with different receptors. The binding interfaces that SERKs provide to different







LRR-RKs are highly overlapping but nevertheless are able to form specific interactions with small molecules, peptide ligands, and island domains (**Figure 8*c***).

Taken together, the published studies show that different ligands can promote the tight association of LRR-RK ectodomains with their corresponding co-receptors. The resulting active heterodimeric complex brings the transmembrane helices of receptor and co-receptor in close spatial proximity (**Figure 8*a***). This, in turn, allows the cytoplasmic kinase domains of the receptor and co-receptor to interact.

STRUCTURAL ASPECTS OF RECEPTOR-KINASE-CONTROLLED CYTOPLASMIC SIGNALING EVENTS

Although ligand binding and receptor activation have been structurally investigated for LysM-RKs and LRR-RKs at the level of the cell surface, much less is known about the activation and regulation of the cytoplasmic kinase domains. Crystal structures of the isolated kinase domains of the LRR-RKs BRI1 and SERK3/BAK1 have revealed a conserved kinase domain fold consisting of an N-terminal lobe, a nucleotide-binding site, and a C-terminal lobe (7, 140) (**Figure 9*a*,*c***). In contrast to an earlier report (68), BRI1 does not contain a guanylate cyclase domain or activity (7). Plant RKs are structurally related to the animal Pelle/interleukin-1 receptor-associated kinase 1 (IRAK) family of kinases (7, 140), as previously suggested (111). Plant RKs and IRAKs are dual-specificity kinases that can auto- and transphosphorylate on both serine/threonine and tyrosine residues (7, 55, 94). The BRI1 kinase activation loop is critical for peptide substrate binding and contains structural features reminiscent of both serine/threonine and tyrosine kinases. However, whether and (if so) how the receptor can switch between those different activities are not well understood (7).

The BRI1 and SERK3/BAK1 kinase domains can physically interact (7, 72, 91), but the exact configuration of the complex remains to be elucidated. BRI1 and SERK3/BAK1 are autophosphorylated (7, 140), and upon activation they are able to transphosphorylate each other, resulting in complex phosphorylation patterns in their kinase cores, juxtamembrane regions, and C-terminal tails, which may have regulatory roles (93, 130, 131). BRI1 KINASE INHIBITOR 1 (BKI1), a negative regulator of brassinosteroid signaling, contains a C-terminal peptide motif that binds to the C-terminal lobe of the BRI1 kinase (55, 58, 129). The crystal structure of the BRI1-BKI1 complex and the results of in vitro and in vivo biochemical studies suggest that BKI1 inhibits brassinosteroid signaling by competing with the SERK3/BAK1 kinase domain to bind to the BRI1 C-terminal lobe (55, 128) (**Figure 9*e***). This, in turn, suggests that the BRI1 and SERK3/BAK1 kinase modules

Figure 8

SERK proteins as essential co-receptors for leucine-rich-repeat (LRR) receptor kinases (RKs). (*a*) Overview of activated ternary LRR-RK signaling complexes: BRI1-brassinolide-SERK1 [Protein Data Bank (PDB) ID 4LSX; **http://www.rcsb.org**], PSKR-phytosulfokine-SERK1 (PDB ID 4Z64), HAESA-IDA-SERK1 (PDB ID 5IYX), and FLS2-flg22-SERK3/BAK1 (PDB ID 4MN8). Receptors are shown in blue, island domains are shown in magenta, and ligands are shown in yellow in surface views; the SERK1/3 ectodomains are shown in orange as ribbon diagrams. Ligand-triggered complex formation of the extracellular domains brings the membrane helices (*blue* and *orange cylinders*) in proximity. (*b*) Close-up views of the BRI1-brassinolide-SERK1, PSKR-phytosulfokine-SERK1, and HAESA-IDA-SERK1 complexes. (*Left*) SERK1 establishes a zipper-like interface with the BRI1 LRR core and uses its N-terminal capping domain to make direct contacts with the steroid ligand and with the island domain. (*Center*) A direct contact of SERK1 with phytosulfokine is not observed in the PSKR complex structure, where major contacts are made with the island domain of the receptor. (*Right*) In the HAESA-IDA-SERK1 complex, the peptide-binding site is formed by both the receptor and the co-receptor, which also makes extensive contacts with the LRR core of HAESA. The colors correspond with those in panel *a*. (*c*) Surface views of the SERK1/3 ectodomains. Residues involved in complex formation with the LRR-RKs BRI1, PSKR, HAESA, and FLS2 are highlighted in dark gray, revealing few unique and many overlapping interaction sites. The respective receptors are not shown.

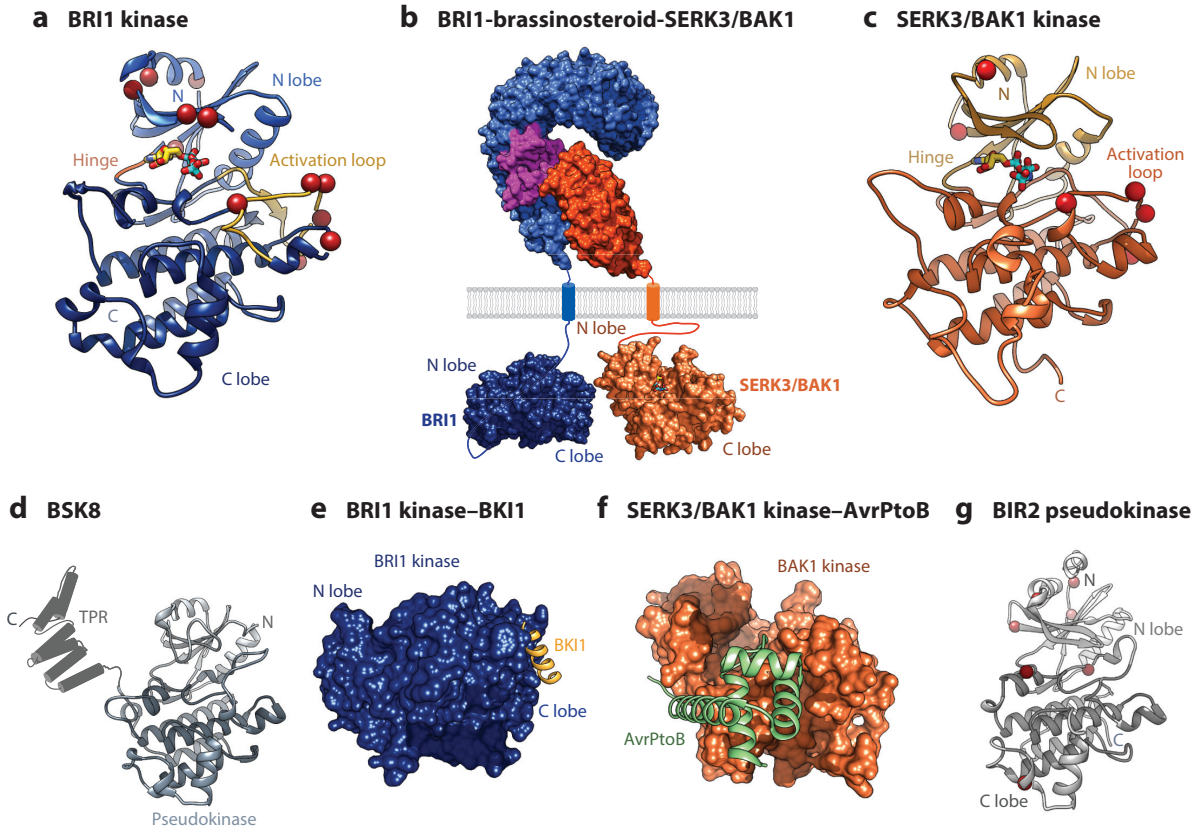


Figure 9

Kinase domains of receptor kinases (RKs). These domains may form asymmetric dimers in the cytosol upon activation and are subject to regulation. (*a*) Ribbon diagram of the active BRI1 kinase domain [shown in *blue*; Protein Data Bank (PDB) ID 5LPY; **http://www.rcsb.org**], showing the typical kinase architecture. The nucleotide bound in the active site is shown in a bonds representation, and known phosphorylation sites are shown as red spheres. (*b*) Model of a leucine-rich-repeat (LRR)–RK signaling complex. Shown is a surface view of the BRI1-brassinolide-SERK3/BAK1 ectodomain complex (PDB ID 4LSX) connected to isolated BRI1 (shown in *blue*) and SERK3/BAK1 (shown in *orange*; PDB ID 3UIM) kinase domains via schematic transmembrane helices (*blue* and *orange cylinders*) and loop segments (*blue* and *orange lines*). Interaction between the N-terminal lobe of one kinase partner and the C-terminal lobe of the other could lead to transphosphorylation and kinase activation. (*c*) Crystal structure of the isolated SERK3/BAK1 kinase domain, showing the same architecture as in panel *a*. (*d*) Structure of the brassinosteroid signaling kinase BSK8, which consists of a pseudokinase domain (shown as a ribbon diagram; PDB ID 4I92) and a tetratricopeptide repeat (TPR) domain (shown as a domain scheme). (*e*) Structure of the BRI1 inhibitor protein BKI1, which binds the C-terminal lobe of the BRI1 kinase domain and may therefore compete with SERK3/BAK1 and/or other substrates for this protein-protein interaction site. BRI1 is shown in a surface view, and BKI1 is shown in yellow as a ribbon diagram (PDB ID 4OH4). (*f*) Structures of the SERK3/BAK1 kinase (shown in *orange* in a surface view) and the bacterial effector protein AvrPtoB (shown in *green* as a ribbon diagram; PDB ID 3TL8), which can inhibit SERK3/BAK1 kinase activity. (*g*) Ribbon diagram of the cytoplasmic pseudokinase domain of the LRR-RK BIR2 (PDB ID 4L68), which physically interacts with the SERK3/BAK1 kinase domain. Known phosphorylation sites are shown as red spheres.

form an asymmetric heterodimer upon dimerization of their respective ectodomains (**Figure 9*b***). In this way, one kinase domain could act as a substrate for the other kinase, as has been described for the animal epidermal growth factor (EGF) receptor (146). Interestingly, the protein inhibitor mitogen-induced gene 6 (MIG6) in the EGF pathway uses a targeting mechanism that is very similar to the one employed by BKI1 (147).

Negative regulators have also been reported for the co-receptor kinase SERK3/BAK1. Among them are the BAK1-INTERACTING RECEPTOR-LIKE KINASE (BIR) proteins. BIR2 interacts with SERK3/BAK1 and negatively regulates plant immune responses (40). The BIR2 kinase domain mediates this interaction by directly binding the SERK3/BAK1 kinase domain (40). A crystal structure of the BIR2 kinase domain revealed a high structural similarity with SERK3/BAK1 (**Figure 9*g***). However, although BIR2 can be efficiently phosphorylated by SERK3/BAK1 in vitro, it lacks several important residues required for kinase activity (6). BIR2 is therefore a pseudokinase, which may negatively regulate SERK3/BAK1 activity by acting as a dummy substrate or by competing for the interaction with other substrates.

Because SERK3/BAK1 is an essential co-receptor for many pattern recognition LRR-RKs, pathogens have evolved effector proteins that can inhibit its function (26). *Pseudomonas syringae* delivers the effector protein AvrPtoB into host cells, where it can bind SERK3/BAK1 with micromolar affinity (21). AvrPtoB inactivates the kinase activity of SERK3/BAK1 by targeting and masking its active site and substrate-binding surfaces (21). Based on the complex structure, AvrPtoB is a competitive inhibitor of SERK3/BAK1, which in vivo may inhibit the activation of SERK3/BAK1-dependent LRR-RKs (36, 103, 136) (**Figure 9*f***).

Although it is now generally accepted that ligand-induced interaction and activation of the cytoplasmic receptor and co-receptor kinase domains lead to the activation of downstream RK signaling pathways, the spatial, temporal, and mechanistic aspects of this process are poorly understood. One of the first substrates of the BRI1 receptor in brassinosteroid signaling is the plasma membrane–associated family of BRI1 SUBSTRATE KINASE (BSK) proteins (116, 123). BSKs contain a kinase domain and a C-terminal tetratricopeptide repeat (TPR) domain (116, 123), a well-known protein-protein interaction module (28). A crystal structure of the AtBSK8 kinase domain revealed that at least some BSKs are pseudokinases (39) (**Figure 9*d***). BRI1-dependent phosphorylation of BSKs appears to release the autoinhibitory TPR domain of the substrate, allowing BSKs to interact with brassinosteroid signaling components in the cytoplasm (143).

Overall, mechanistic knowledge about the cytoplasmic side of RK signaling is limited. A complex interplay between kinases, pseudokinases, and adaptor proteins seems to ensure the propagation of cell surface–perceived signals into the nucleus to regulate gene expression responses.

FUTURE DIRECTIONS

Structural biology has helped shape our mechanistic understanding of plant RK signaling. For two important subfamilies of plant RKs, the LysM-RKs and the LRR-RKs, detailed insights have been obtained into how they specifically sense diverse ligands and how ligand binding at the cell surface activates the cytoplasmic kinase domain of the receptor (via either ligand-induced homodimerization or heterodimerization with a shape-complementary co-receptor kinase). However, many aspects of plant membrane signal transduction remain to be elucidated at the genetic, cellular, and structural levels.

In terms of ligand binding, although detailed information on chitin binding by LysM-RKs is available, the structure of an activated LysM-RK signaling complex remains to be resolved. For LRR-RKs, ligand complexes with small molecules and peptides have been described; it would now be useful to know how plant LRR domains can sense small and large protein ligands, such as ABP1 (137) and the cysteine-rich protein TAPETUM DETERMINANT 1 (TPD1), which is required for pollen development (51, 57, 148, 149). In addition, many plant RKs contain ectodomains that are composed of neither LysM nor LRR modules (111), and how these ectodomains sense endogenous and/or foreign ligands remains to be described at the molecular level. Overall, many

receptor-ligand pairs remain to be discovered, and structural biology and associated quantitative biochemical assays can help to address this issue (100, 109, 115).

Plant RK signal activation mechanisms need to be genetically validated in order to integrate the existing structural models with the physiology of plant membrane signaling cascades. In the case of LRR-RKs, it will be important to determine at the genetic, biochemical, and structural levels whether SERK proteins represent universal co-receptor kinases or if other proteins can also mediate receptor activation (10).

Several additional questions also remain unanswered regarding receptor activation. How do RLPs and membrane-bound receptor-like cytoplasmic kinases (RLCKs) contribute to ligand binding, receptor activation, and downstream signal transduction of plant membrane receptor complexes (1, 14, 56, 70, 90, 133, 141)? How are RK complexes organized at the plasma membrane, and do these structures contribute to ligand sensing, receptor activation, and/or signaling crosstalk with other receptors (65, 113)? What is the temporal and spatial order of phosphorylation events in plant RK signal initiation (61)? And how can plant RKs with similar-looking kinase domains relay specific input signals into very different signaling outputs in the cytosol?

Finally, numerous questions also remain regarding downstream signal transduction. For example, what are the common building blocks of the downstream cytoplasmic signaling cascades, and which building blocks are different? By which mechanisms are signals propagated inside the cell? And what are the roles of pseudokinases and G proteins in plant RK signal transduction (4, 18, 40, 90, 92, 124, 137)?

In the future, a unified genetic and mechanistic description of plant membrane signaling pathways will enable us to better understand how plants control their growth and development and interact with an ever-changing environment.

SUMMARY POINTS

1. Plants have evolved a unique set of membrane receptors that can sense diverse ligands to trigger different signaling cascades controlling many aspects of plant growth, development, and interactions with their environment.
2. Plant membrane receptor kinases contain very different extracellular ligand-binding domains, two of which—the lysin-motif (LysM) and leucine-rich-repeat (LRR) domains—have been characterized in structural detail.
3. LysM-domain-containing receptor kinases bind carbohydrate ligands, which may signal immune responses or symbiotic interactions. Ligand binding by LysM domains is well understood, but several receptor activation mechanisms have been proposed.
4. LRR-domain-containing receptor kinases can bind small-molecule, peptide, and protein ligands in very different ways. However, these receptors have a common activation mechanism that relies on the ligand-induced interaction with a shape-complementary co-receptor.
5. Activation of LRR-type receptor kinases by heterodimerization with a co-receptor kinase may lead to interaction of the kinase modules in the cytosol, which subsequently transphosphorylate and activate each other.
6. Activated receptor kinase complexes can initiate downstream signaling responses via interaction with other signaling proteins, few of which have been structurally characterized.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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