

# Chapter 9

## Brassinosteroid Sensing and Signaling in Plants



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### 9.1 Brassinosteroids and the Core Brassinosteroid Signaling Pathway

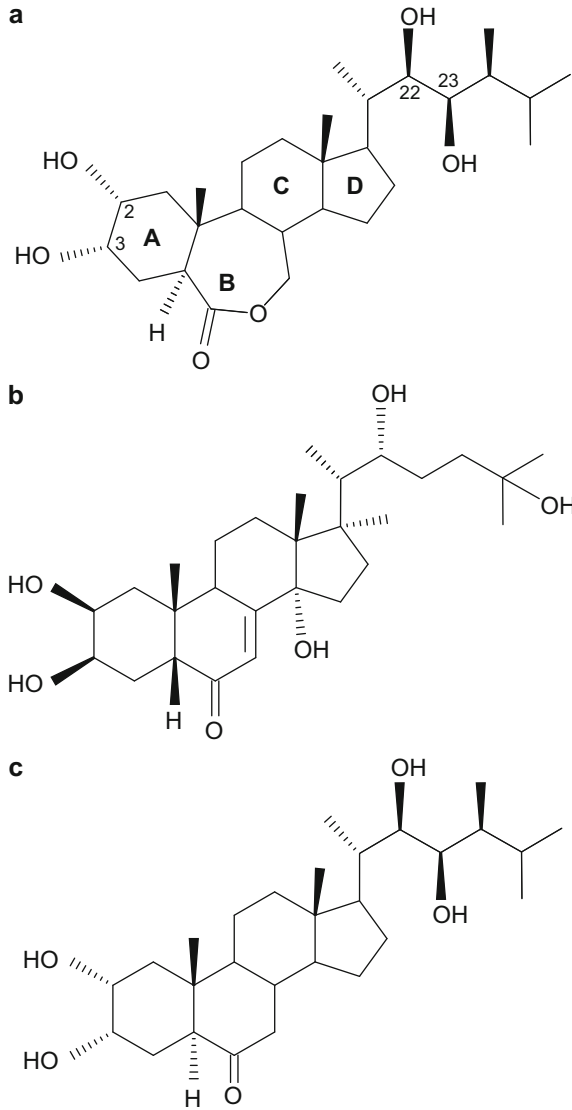
In 1979, a growth-promoting hormone was isolated from rape pollen (Grove et al. 1979). Its crystal structure revealed a hydroxyprolinated steroid featuring a B-ring lactone with structural similarities to the insect molting hormone ecdysone (Fig. 9.1a, b). It was named brassinolide and since its discovery dozens of related brassinosteroids, which can promote cell elongation, division, and differentiation, have been reported from different plant species (Bajguz 2007). Using synthetic brassinolide, brassinosteroid-deficient and -insensitive mutants were identified in forward genetic screens in the model plant *Arabidopsis*. In this way, the core biosynthetic pathway for the hormone and many components of the brassinosteroid signal transduction cascade were defined. One of the first genes to be cloned was BRI1 (Clouse et al. 1996; Li and Chory 1997), a plasma-membrane-localized receptor kinase with an extracellular leucine-rich repeat (LRR) domain, a single membrane spanning helix and a cytoplasmic kinase domain (Li and Chory 1997) (Fig. 9.2). BRI1 is one of ~200 LRR receptor kinases found in the *Arabidopsis* genome (Shiu and Bleecker 2001). BRI1 acts as receptor for brassinosteroids and binds the steroid hormone using its LRR-domain (Wang et al. 2001; Kinoshita et al. 2005).

BRI1 can interact with the BRI1 ASSOCIATED KINASE 1 (BAK1), which is a positive regulator of brassinosteroid signaling (Li et al. 2002; Nam and Li 2002) (Fig. 9.2). BAK1 is part of the SERK family of co-receptor kinases (Brandt and

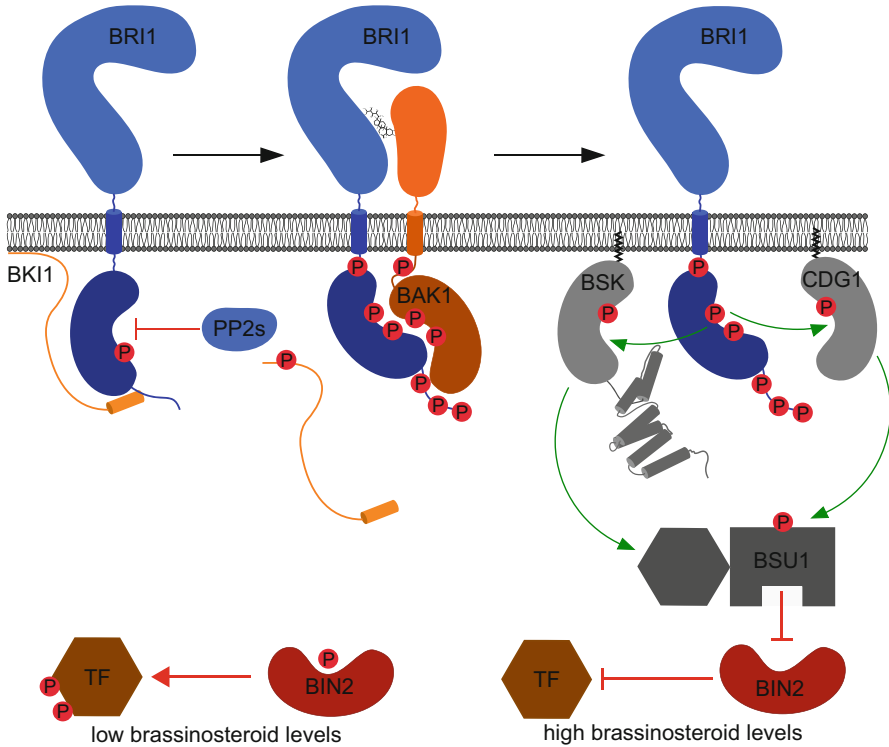
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**Fig. 9.1** Chemical structure of different steroid molecules. (a) Brassinolide, (b) ecdysone, and (c) castasterone



**Fig. 9.2** Schematic view of the brassinosteroid signaling cascade. In the absence of brassinosteroids, BRI1 is kept inactive by the inhibitor protein BKI1 and by PP2 family phosphatases. Upon ligand binding, the co-receptor BAK1 is recruited, inhibitory BKI1 is released, and the cytoplasmic kinase domains of BRI1 and BAK1 can auto- and trans-phosphorylate each other. This enables BRI1 to trans-phosphorylate membrane-attached cytoplasmic kinases (BSKs and CDG1), which activates the BSU1 phosphatase. BSU then dephosphorylates the GSK3 kinase BIN2. Thus, when brassinosteroid signaling is active, BIN2 cannot phosphorylate the transcription factors (TFs) BES1 and BZR1, thereby activating them. Green arrows indicate activating phosphorylations and red arrows inhibitory phosphorylation events

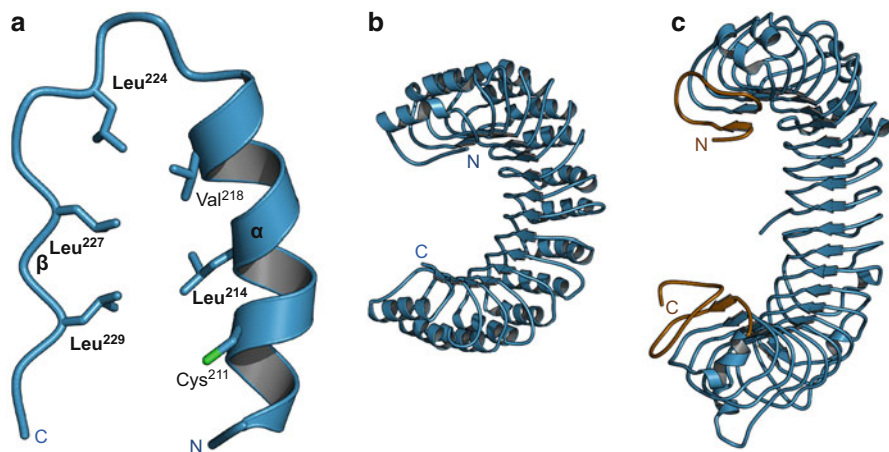
Hothorn 2016). Active BRI1 can trans-phosphorylate different cytoplasmic kinases, which are attached to the plasma-membrane via covalent lipid anchors: the BRI1 SIGNALING KINASES (BSKs) (Tang et al. 2008) and CONSTITUTIVE DIFFERENTIAL GROWTH (CDG1) (Kim et al. 2011) (Fig. 9.2). These cytoplasmic kinases can activate the down-stream phosphatase BRASSINOSTEROID INSENSITIVE SUPPRESSOR 1 (BSU1) (Mora-García et al. 2004; Kim et al. 2011), which in turn dephosphorylates and inactivates the GSK3-type kinase BRASSINOSTEROID INSENSITIVE 2 (BIN2) (Li et al. 2001; Li and Nam 2002; Mora-García et al. 2004; Kim et al. 2011) (Fig. 9.2). When brassinosteroid levels are low and BRI1 is inactive, BIN2 efficiently phosphorylates the transcription factors BRASSINAZOLE RESISTANT 1 (BZR1) (Wang et al. 2002) and BRI1-EMS-SUPPRESSOR 1 (BES1) (Yin et al. 2002), keeping them in an inactive state

bound to 14-3-3 proteins (Gampala et al. 2007) (Fig. 9.2). When brassinosteroids are sensed by BRI1, BIN2 is inactivated, leading to unphosphorylated BZR1/BES1, which accumulate in the nucleus and bind to their target promoters (Yin et al. 2002; Zhao et al. 2002; Vert and Chory 2006). Over the past few years, a combination of structural biology, quantitative biochemistry, and plant genetics has provided detailed mechanistic insights into the early steps of brassinosteroid signal transduction, namely, how the hormone is specifically sensed by its receptor BRI1 and how ligand binding at the cell surface leads to activation of the cytoplasmic signaling cascade.

## 9.2 The Structure of the BRI1 Extracellular Domain

Different brassinosteroid-insensitive mutant alleles map to the *brl1* locus (Clouse et al. 1996; Li and Chory 1997). Protein engineering (He et al. 2000) and ligand-binding assays using radiolabeled brassinosteroids (Wang et al. 2001; Kinoshita et al. 2005) revealed that BRI1 acts as a receptor for brassinosteroids. The hormone is directly sensed by the extracellular LRR domain of BRI1, which is exposed at the cell surface and to the lumen of endosomes (Friedrichsen et al. 2000; Geldner et al. 2007).

Individual leucine-rich repeats are 20–30 amino-acid long sequence stretches rich in leucine residues and forming an  $\alpha/\beta$  hairpin structure (Kajava 1998) (Fig. 9.3a). Several repeats stack together to form a solenoid, which in the case of bacterial and human LRR proteins, such as ribonuclease inhibitor and Toll-like receptor 3, is reminiscent of a horseshoe (Kobe and Deisenhofer 1993; Choe et al. 2005)



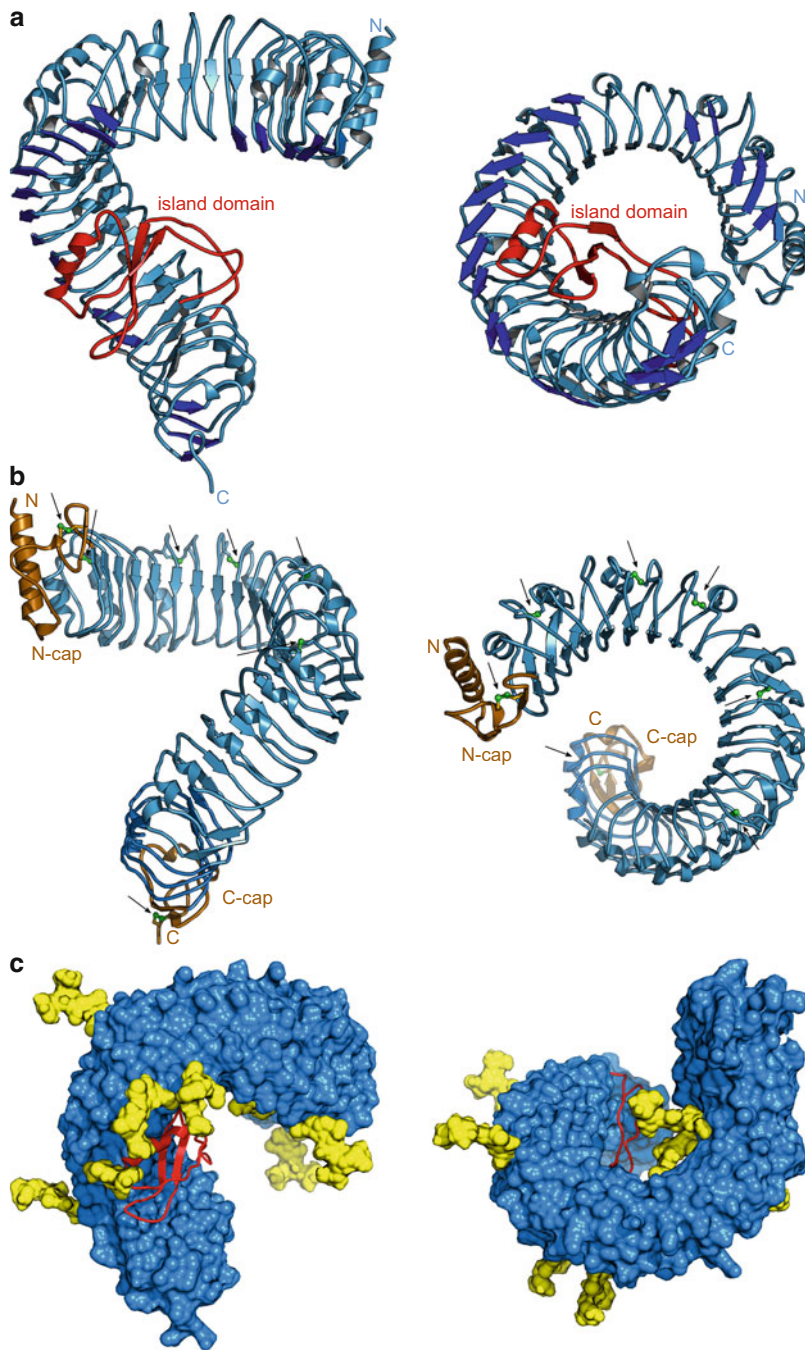
**Fig. 9.3** Structural features of animal and bacterial LRR domains. (a) Ribbon diagram of a canonical LRR (ribonuclease inhibitor residues 207–231, PDB-ID 1DFJ) featuring an  $\alpha$ -helix, a  $\beta$ -sheet, and conserved leucine residues forming a hydrophobic core (in bonds representation), (b) ribbon diagram of the horseshoe-shaped bacterial ribonuclease inhibitor (PDB-ID 1DFJ), and (c) human Toll-like receptor 3 (PDB-ID 1ZIW) LRR domains. The N- and C-terminal capping domains are shown in brown

(Fig. 9.3b, c). The inner surface of the horseshoe is formed by a parallel  $\beta$ -sheet, the outer surface is composed of an array of helices, and the hydrophobic core is formed by the conserved leucine residues (Fig. 9.3b, c). At their N- and C-termini, the hydrophobic LRRs are shielded by hydrophilic capping domains (Fig. 9.3c).

The crystal structure of the *Arabidopsis* BRI1 LRR domain unexpectedly revealed a right-handed superhelix composed of 25 individual LRRs with an inner diameter of  $\sim 30$  Å and an out diameter of  $\sim 60$  Å, and not the canonical horseshoe structure (Hothorn et al. 2011; She et al. 2011) (Fig. 9.4a). The helix completes one entire turn, with a rise of about  $\sim 70$  Å. Crystal structures of different plant LRR domains now reveal that many if not all plant LRRs form helical or highly twisted assemblies (See Chap. 3). The twist is generated by the presence of a non-canonical, second  $\beta$ -sheet, which is oriented perpendicular to the central  $\beta$ -sheet lining the inner side of the LRR solenoid (Fig. 9.4a). This second  $\beta$ -sheet in plant LRR proteins is caused by the Lt/sGxIP consensus sequence of the plant-specific LRR subfamily (Kajava 1998). The LRRs of BRI1 are flanked by N- and C-terminal capping domains, which are stabilized by disulfide bonds (Fig. 9.4b). In the weak *bri1-5* mutant allele, Cys69 is mutated to a tyrosine residue, disrupting a disulfide bond in the N-terminal capping domain of BRI1 (Noguchi et al. 1999). This structural alteration of the N-terminal cap de-stabilizes the receptor and affects its secretion to the plasma membrane (Hong et al. 2008). Five additional disulfide bonds map to the LRR core itself, covalently linking consecutive LRR segments (Fig. 9.4b) (Hothorn et al. 2011). In addition, the LRR ectodomains of BRI1 and many but not all plant receptor kinases carry N-glycosylation sites. In the BRI1 structure, large surface areas of the LRR domain are thus masked by carbohydrate (Fig. 9.4c). These carbohydrate structures may be involved in the structural stabilization of the LRR domain but may also control protein–protein interactions (Hothorn et al. 2011).

### 9.3 The BRI1 Island Domain and the Steroid-Binding Site

While most of the 25 individual LRR motifs in BRI1 are connected via short loops, a large insertion is present between LRRs 21 and 22 (Hothorn et al. 2011; She et al. 2011) (Figs. 9.4a and 9.5a). This 70 amino-acid stretch forms a small “island” domain that folds back onto the LRR core, making extensive polar and apolar interactions with LRRs 13–25 (Fig. 9.5a, b). The island domain contains a central anti-parallel  $\beta$ -sheet sandwiched between the BRI1 LRR domain and a small helix and stabilized by a disulfide bond (Fig. 9.5a). Structures of the BRI1 ectodomain crystallized in the presence of brassinolide, a potent brassinosteroid in *Arabidopsis*, reveal one steroid molecule located in a binding pocket formed by both the LRR core (LRRs 21–25) and by the island domain (Hothorn et al. 2011; She et al. 2011) (Fig. 9.5b, c). Comparing the steroid-bound structure with the structures of “apo” BRI1 indicates that the island domain is rather mobile and flexible in the absence of ligand. Steroid binding induces a conformational rearrangement and fixing of the island domain, making BRI1 competent to engage in protein–protein interactions (Hothorn et al. 2011).



**Fig. 9.4** The BRI1 extracellular domain folds into a spiral-shaped solenoid. **(a)** A plant-specific 2nd  $\beta$ -sheet forces the BRI1 LRR domain (PDB-ID 3RIZ, in light-blue) into a spiral shape. The *island domain* (in red) inserts between LRR 21 and 22 and maps to the inside of the helix. The plant specific  $\beta$ -sheet is highlighted in dark blue. Side view on the left, top view on the right. **(b)** The

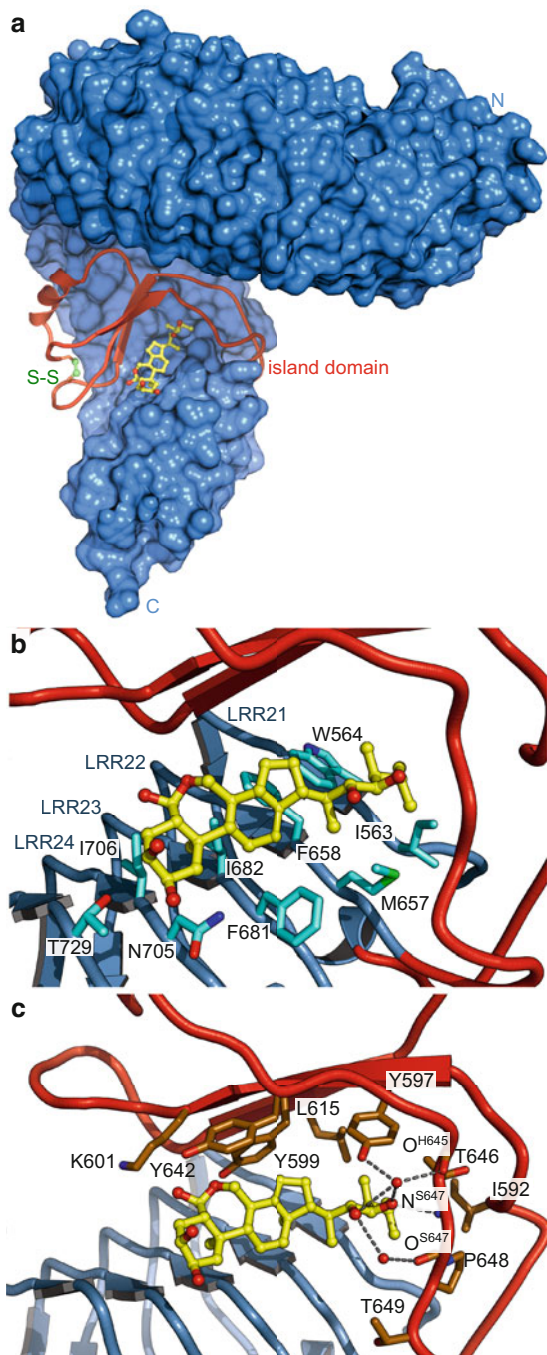
The steroid-binding site provides a  $\sim 550 \text{ \AA}^2$  hormone-receptor interface formed by BRI1 LRRs 23–25. This hydrophobic surface is in contact with the A–D rings of brassinolide (Fig. 9.5b) (Hothorn et al. 2011; She et al. 2011). The alkyl chain of the hormone is bound in a small pocket that is formed by residues originating from the LRRs 21 and 22 and from the island domain (Fig. 9.5c). Polar main-chain and side-chain interactions with the 22, 23-diol moiety of brassinolide originate from the island domain (Fig. 9.5c). There are few specific interactions between BRI1 and the B-ring lactone of brassinolide, rationalizing why brassinosteroids carrying modifications of their B-ring, such as castasterone (Fig. 9.1), can be sensed by the receptor (Wang et al. 2001; Back and Pharis 2003; Hothorn et al. 2011) (Fig. 9.5b, c). However, large parts of the steroid hormone, including the crucial  $2\alpha,3\alpha$ -diol moiety are not in contact with BRI1 (Back and Pharis 2003; Hothorn et al. 2011; She et al. 2011). A similar mode of brassinolide binding has been reported for the BRI1 homologue BRL1 (She et al. 2013).

#### 9.4 Receptor Activation of BRI1 Requires Shape-Complementary Co-receptor Kinases

Based on the sequence similarities between the LRR ectodomains of BRI1 and animal Toll-like receptors, ligand-induced homo-dimerization had been proposed to mediate BRI1 receptor activation (Wang et al. 2005b). Indeed, homo-oligomers of BRI1 have been observed in planta, however they appear to be constitutive rather than induced by brassinosteroid binding (Wang et al. 2005b). The purified BRI1 ectodomain behaves as a monomer in solution, and in contrast to Toll-like receptors (Leonard et al. 2008; Liu et al. 2008) shows no tendency to oligomerize in the presence of the steroid ligand (Hothorn et al. 2011; She et al. 2011; Bojar et al. 2014). This finding, together with the observation that the steroid-binding site maps to the inner face of the BRI1 helix, suggested that BRI1 activation requires interaction with a shape-complementary helper protein (Hothorn et al. 2011). A BRI1 co-receptor candidate, BAK1, had already been identified using forward genetics and protein–protein interaction screens (Li et al. 2002; Nam and Li 2002). BAK1 loss-of-function mutants display mild brassinosteroid-insensitive phenotypes (Li and Nam 2002; Li et al. 2002). It was then noted that BAK1 acts redundantly with other SERK family LRR receptor kinases (Nam and Li 2002; Karlova et al. 2006; He et al. 2007). Importantly, *serk1 serk3 (bak1) serk4* triple knock-out mutants phenocopy *bril* null mutants, suggesting that BRI1 and SERKs are both essential to sense brassinosteroids at the plasma membrane (Gou et al. 2012).



**Fig. 9.4** (continued) BRI1 LRR domain is shielded by N- and C-terminal capping domains (in brown) and stabilized by disulfide bridges (in green, highlighted by arrows). The island domain has been omitted for clarity. (c) Multiple glycosylations mask large areas of the receptor surface (LRR domain in blue and carbohydrates in yellow, in surface representation, island domain in red and as ribbon diagram)



**Fig. 9.5** Brassinosteroids bind to LRR domain of BRI1. **(a)** Binding of a brassinolide molecule to the LRR (in blue, surface representation) and *island domain* (in red, ribbon diagram) of BRI1 (PDB-ID 3RIZ). Brassinolide is shown in yellow (in bonds representation). **(b)** A hydrophobic surface formed by LRRs 23–25 interacts with the A-D rings of the steroid (colors as in **a**, BRI1 as

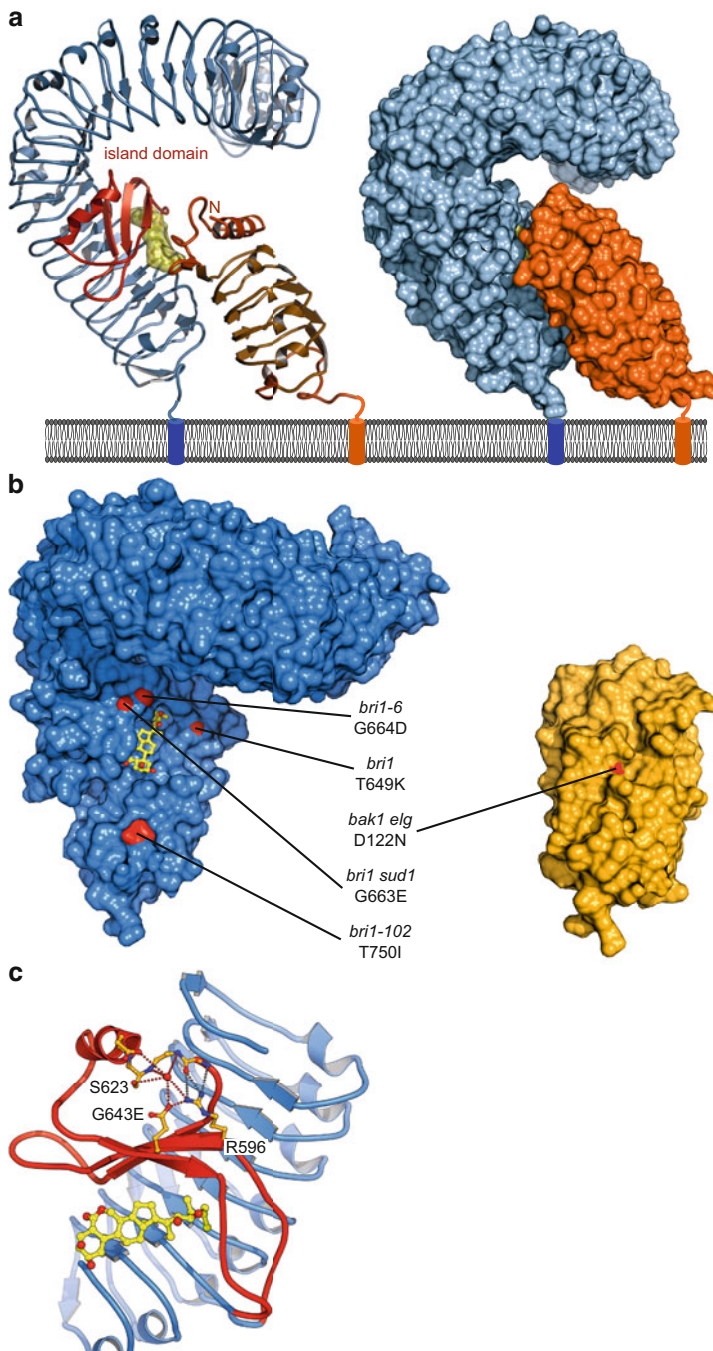


SERK proteins share their overall architecture with BRI1. The crystal structure of SERK1 revealed a short LRR ectodomain with 5 repeats (Santiago et al. 2013). In vitro, the LRR domains of SERK1 or BAK1 form tight heterodimers with the BRI1 ectodomain, but only in the presence of brassinosteroids (Santiago et al. 2013; Sun et al. 2013; Bojar et al. 2014). Crystal structures of BRI1-brassinolide-SERK1 (Santiago et al. 2013) and BRI1-brassinolide-BAK1 (Sun et al. 2013) complexes revealed that SERK co-receptors directly bind to the BRI1 LRR domain, to the island domain, and importantly to the brassinolide itself (Fig. 9.6a). The SERK1/BAK1 N-terminal capping domain completes the steroid-binding site, with the hormone acting as a “molecular glue,” which promotes the association between receptor and co-receptor (Fig. 9.6a) (Santiago et al. 2013; Sun et al. 2013).

The effects of several genetic loss- and gain-of-function alleles in BRI1 and in BAK1 can be rationalized in light of the complex structures: Mutation of Gly644 in BRI1 into aspartate causes the loss-of-function phenotype of *bri1-6* plants (Fig. 9.6b) (Noguchi et al. 1999). The affected glycine residue is located in the center of the BRI1 island domain and may be important for both brassinosteroid binding and for proper recruitment of the co-receptor (Hothorn et al. 2011; Santiago et al. 2013; Hohmann et al. 2018). The neighboring Gly643 in the island domain is a glutamate residue in the BRI1 gain-of-function mutant *sud1* (Belkhadir et al. 2012). The BRI1 *sud1* mutant protein has been crystallized, and its structure revealed that Glu643 stabilizes the island domain. This possibly promotes the binding of steroid ligands as well as recruitment of the co-receptor (Fig. 9.6c) (Santiago et al. 2013). The semi-dwarf mutant *093AR* in barley maps to the *bri1* locus, replacing Thr573 with a lysine residue (Gruszka et al. 2011). The corresponding Thr649 in *Arabidopsis* BRI1 again maps to the island domain of the receptor, highlighting its important role in steroid sensing and co-receptor recruitment (Fig. 9.6b) (Hothorn et al. 2011; Santiago et al. 2013). Thr750, which is located in LRR 25 outside the steroid-binding pocket, is found mutated into an isoleucine in the strong loss-of-function mutant *bri1-102* (Fig. 9.6b) (Friedrichsen et al. 2000). Consistently, this mutant does not interfere with steroid sensing itself, but rather inhibits binding of the SERK co-receptor (Wang et al. 2001; Santiago et al. 2013). The *elg* (elongated) mutant of BAK1 causes a gain-of-function brassinosteroid signaling phenotype (Jaillais et al. 2011a). Asn122 in the LRR ectodomain of BAK1 is replaced by an asparagine residue in *elg* mutant plants, again supporting an essential role for SERK co-receptors in brassinosteroid sensing and signaling (Fig. 9.6b) (Jaillais et al. 2011a). The mutation has been recapitulated in rice SERK2 and the mutant protein has been crystallized (McAndrew et al. 2014). In this structure, Asn122 disrupts the formation of a nearby salt-bridge, but it remains to be understood how this structural



**Fig. 9.5** (continued) ribbon diagram, relevant residues as well as brassinolide in bonds representation). (c) A pocket formed by the island domain and LRRs 21 and 22 binds the steroid’s alkyl chain. The island domain interacts strongly with the 22,23-diol moiety of brassinolide (colors as in **b**, important island domain residues in bonds representation, dotted lines represent interactions, red spheres depict important water molecules)



**Fig. 9.6** Receptor activation of BRI1 involves a shape-complementary co-receptor. **(a)** Overview of the ternary BRI1-brassinolide-SERK1 complex (PDB-ID 4LSX, left ribbon diagrams, right panel surface views). The BRI1 LRR domain is shown in blue, the *island domain* in red, the SERK1 LRR domain in orange, and brassinolide highlighted in yellow. **(b)** Known gain- and loss-of-function

change affects brassinosteroid signaling (Jaillais et al. 2011a; McAndrew et al. 2014). Taken together, several genetic alleles in BRI1 and in SERKs highlight the importance of their LRR ectodomains for steroid hormone sensing and brassinosteroid receptor activation.

## 9.5 The Kinase Domains of BRI1 and SERKs Can Activate Each Other in the Cytosol

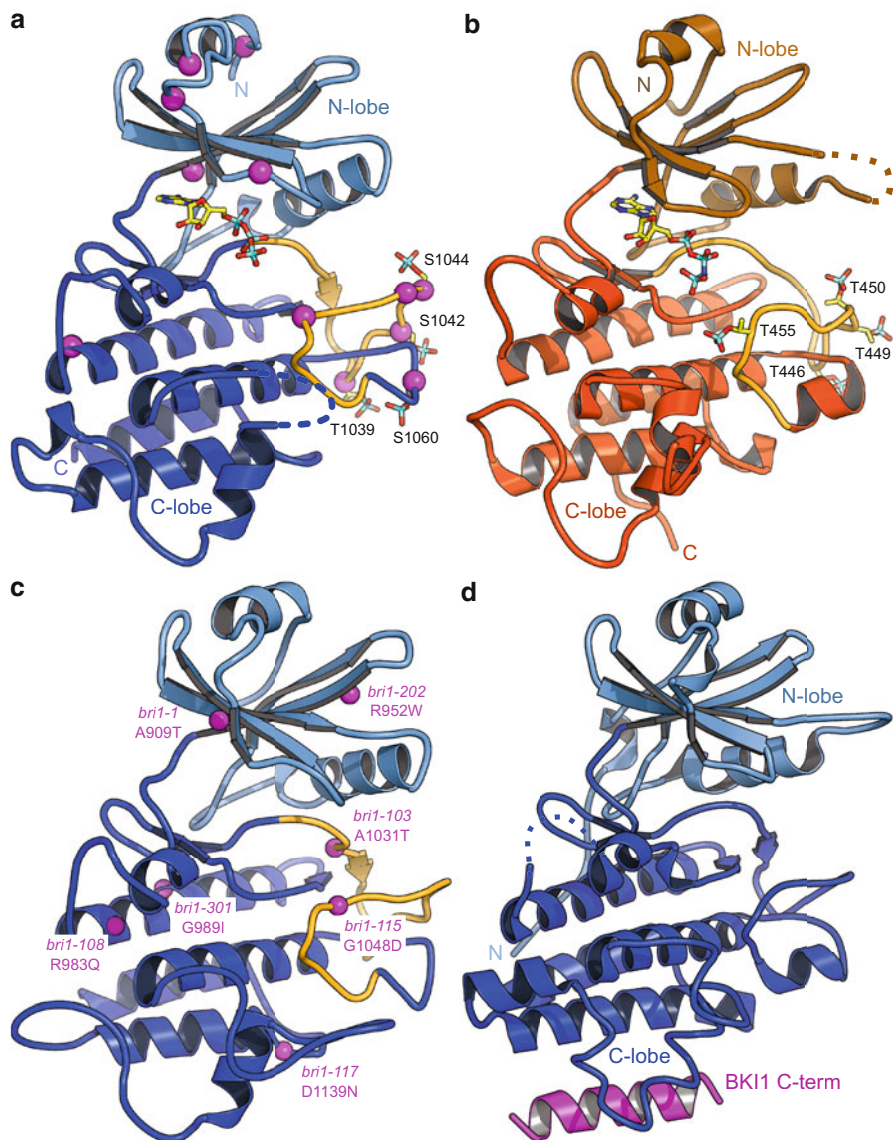
Formation of a BRI1-brassinosteroid-SERK signaling complex at the cell surface is not sufficient to trigger the cytoplasmic side of the brassinosteroid signaling pathway (Fig. 9.2). It was noted early on that the kinase domains of receptor and co-receptor are being phosphorylated in response to brassinosteroid sensing (Wang et al. 2005b; Wang et al. 2008) and that the isolated cytoplasmic domains of BRI1 and BAK1 can interact in vitro (Li et al. 2002; Nam and Li 2002).

The cytoplasmic domains of BRI1 and BAK1 are each composed of a catalytic kinase core, an N-terminal juxta-membrane region, and a C-terminal tail (Fig. 9.2). Current working models suggest that in the absence of brassinosteroids, the kinase domains of BRI1 and of SERKs are kept in a basal state and are under negative regulation by protein phosphatases (Wang et al. 2016). In its basal state, the kinase domain of BRI1 may also bind the BRI1 inhibitor protein BKI1 (Wang and Chory 2006; Jaillais et al. 2011b). Upon brassinosteroid sensing, the extracellular LRR domains of BRI1 and an SERK interact at the cell surface (Jaillais et al. 2011a; Santiago et al. 2013; Sun et al. 2013). The resulting, ligand-induced heterodimeric signaling complex brings the trans-membrane helices of the receptor and co-receptor in close proximity (Fig. 9.6a) (Santiago et al. 2013; Sun et al. 2013). This, in turn, allows the kinase domains of BRI1 and SERKs to interact (Wang et al. 2008). BRI1 and SERKs then trans-phosphorylate and activate each other, rendering the BRI1 kinase domain competent to phosphorylate its substrates, including BSKs, CDG1, and BKI1 (Fig. 9.2) (Wang and Chory 2006; Tang et al. 2008; Jaillais et al. 2011b; Kim et al. 2011).

While the mechanistic details of BRI1 receptor activation remain to be resolved, crystal structures of the isolated BRI1 and BAK1 kinase domains have been reported (Yan et al. 2012; Bojar et al. 2014). These structures revealed that plant receptor kinases share significant homology with the animal IRAK family of kinases (Fig. 9.7a, b). Both plant receptor kinases (including BRI1 and BAK1) and animal IRAK kinases are dual-specificity kinases, able to auto- and trans-phosphorylate on



**Fig. 9.6** (continued) alleles map to the receptor – co-receptor interaction surface (BRI1 LRR domain in blue, in surface representation, island domain omitted, brassinolide in yellow and in bonds representation, SERK1 on the right in yellow). (c) The glutamate residue 643 in BRI1<sup>sud1</sup> stabilizes the island domain (PDB-ID 4LSA, ribbon diagram, colors as in a, interacting residues in orange and in bonds representation, red sphere depicts a water molecule)



**Fig. 9.7** Structures of the BRI1 and BAK1 kinase domains. **(a)** A ribbon diagram of the BRI1 kinase domain reveals a canonical kinase fold with *N-lobe* (light blue), *C-lobe* (dark blue), and activation loop (yellow). ATP is bound to the active site (in bonds representation), phosphorylation sites are highlighted with magenta spheres (PDB-ID 5LPY). **(b)** Crystal structure of the BAK1 kinase domain (features as in **a**, but *N-lobe* in brown and *C-lobe* in orange, PDB-ID 3UIM). **(c)** Known loss-of-function alleles (highlighted with spheres in magenta) map to the BRI1 kinase domain (structure as in **a**, nucleotide omitted). **(d)** Binding of the C-terminal helix of BKI1 to the C-lobe of the BRI1 kinase domain competes with BAK1 interaction or substrate binding (BK11 in magenta as ribbon diagram, BRI1 kinase as in **a**, PDB-ID 4OH4)

Ser/Thr and on Tyr residues (Wang et al. 2006; Oh et al. 2009; Jaillais et al. 2011b). The BRI1 and BAK1 kinase domain structures feature the canonical N- and C-terminal kinase lobes, with parts of the juxtamembrane segments and the C-terminal tails being disordered (Fig. 9.7a, b) (Yan et al. 2012; Bojar et al. 2014). The BRI1 activation loop, which determines which substrates can bind, contains structural motifs normally found in either Ser/Thr or tyrosine kinases (Bojar et al. 2014). How BRI1 can both phosphorylate Ser/Thr and tyrosine substrates is poorly understood. Both BRI1 and BAK1 carry a complex pattern of phosphorylation sites, some of which are recapitulated in their crystal structures (Fig. 9.7a, b) (Oh et al. 2000; Wang et al. 2005a, 2005b, 2008; Oh et al. 2009; Bajwa et al. 2013). A large number of weak and strong *bri1* loss-of-function mutations map to the BRI1 kinase domain, likely interfering with the activity of the enzyme (Fig. 9.7c) (Clouse et al. 1996; Li and Chory 1997; Friedrichsen et al. 2000; Xu et al. 2008).

The interaction surface between the BRI1 and BAK1 kinase domains remains to be identified, but it is interesting to note that the inhibitor protein BKI1 disrupts the cytosolic interaction between the receptor and its SERK co-receptor (Jaillais et al. 2011b). BKI1 contains a conserved helical motif at its very C-terminus, which binds to the C-lobe of the BRI1 kinase (Fig. 9.7d) (Jaillais et al. 2011b; Wang et al. 2014). This suggests, that, in the absence of the inhibitor BKI1, BRI1 may form an asymmetric hetero-dimer with BAK1 or other SERKs, bringing the two kinase domains in a configuration that allows them to trans-phosphorylate each other (Bojar et al. 2014). BRI1 itself is able to phosphorylate BKI1 on a Tyr residue, triggering the release of the inhibitor protein from the plasma membrane into the cytosol (Fig. 9.2) (Jaillais et al. 2011b).

Taken together, crystallographic, biochemical, and genetic evidence supports a model, in which the plant steroid receptor BRI1 and a co-receptor kinase together sense brassinosteroids. The ligand induced receptor – co-receptor complex formation at the cell surface-brings the cytoplasmic kinase domains of BRI1 and SERK proteins in close proximity, allowing for trans-phosphorylation and subsequent activation of the cytosolic brassinosteroid signaling pathway. The use of spiral-shaped LRR ligand-binding domains and shape-complementary co-receptors represents a plant-unique signaling paradigm.

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