Review

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The growth–defense pivot: crisis management in plants mediated by LRR-RK surface receptors

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Plants must adapt to their environment and require mechanisms for sensing their surroundings and responding appropriately. An expanded family of more than 200 leucine-rich repeat (LRR) receptor kinases (LRR-RKs) transduces fluctuating and often contradictory signals from the environment into changes in nuclear gene expression. Two LRR-RKs, BRASSINOSTEROID INSENSITIVE 1 (BRI1), a steroid receptor, and FLAGELLIN SENSITIVE 2 (FLS2), an innate immune receptor that recognizes bacterial flagellin, act cooperatively to partition necessary growth–defense trade-offs. BRI1 and FLS2 share common signaling components and slightly different activation mechanisms. BRI1 and FLS2 are paradigms for understanding the signaling mechanisms of LRR-containing receptors in plants.

Making sound decisions: to defend and grow

Plants are stuck in an environment from which they cannot escape [1]. Thus, discrimination of prolonged signals from a constant barrage of background noise, as well as proper integration of these inputs into endogenous growth programs, is fundamental to the success and survival of the 350 000 species of flowering plant that currently inhabit Earth [1].

Plants process complex information from their local environment [2–5]. They can anticipate dawn and the seasons, remember winter to flower at the right time of year, and communicate with each other and with other species through unique, volatile metabolites made through sophisticated biochemical pathways [2,5–7]. Because they are sessile, plants forage for light and nutrients through differential growth [5,8]. Thus, plants invest carefully calibrated resources into their growth programs to satisfy their needs [9]. However, plants are constantly integrating signals from other living organisms that are on or near their bodies, thus sorting pathogens and pests from mutualists and symbionts [10–15]. Because they cannot run away from attack, plants have evolved a sophisticated two-tiered immune system that controls the production of a diverse arsenal of small molecules to fend off predators, defend themselves against pathogens, attract pollinators, and mediate communication within and between species [13,14,16–18]. Responding to living organisms like pathogens requires temporary modulation of investments made in growth [9]. When plants sense the presence of pathogens, they activate a series of energetically costly defense programs that eventually culminate in the cessation of pathogen proliferation [9,17]. Plants make regulatory decisions by processing information that originates at the single-cell level and distributing secondary messengers locally and distally [17]. Thus, integration of environmental signals often requires management of growth–defense trade-offs [9].

Over the past 2 decades, many individual pathways that regulate growth and plant immune and defense responses have been elucidated [1,3,19,20]. However, we do not understand how this information is integrated within the plant – either spatially or temporally – to optimize growth rate and body plan for life in a particular environment. This review is motivated by recent structural and biochemical studies that implicate a role for LRR-RKs in the battle between growth and defense. We provide an overview of how the shared molecular components of these two distinct pathways act finely to balance growth–defense trade-offs in plants.

Unique receptor kinases involved in growth, development, and defense

Plants and animals exploit extracellular LRRs (eLRRs) for the perception of either self- or non-self-derived signals at

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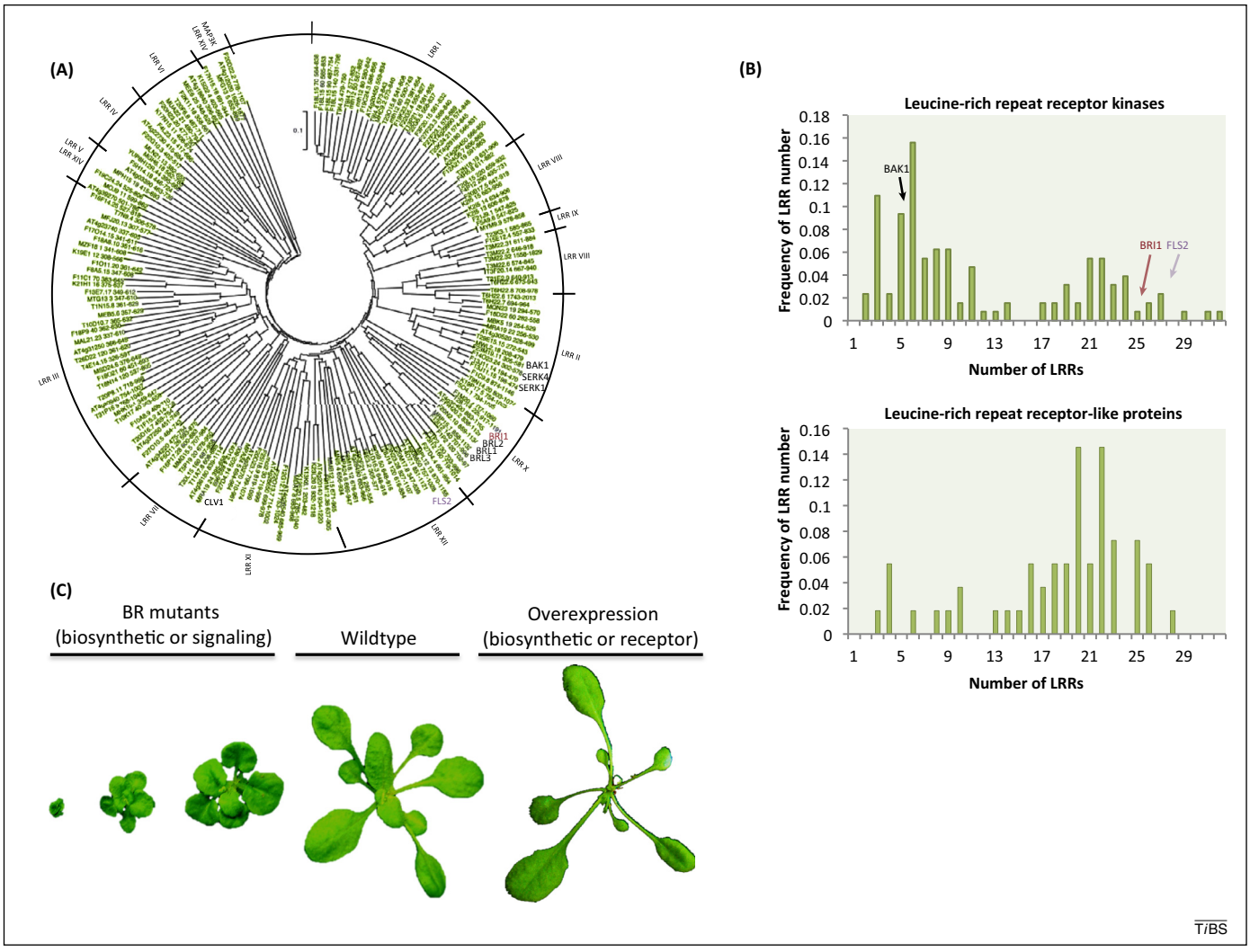

Figure 1. A comparison of leucine-rich repeat (LRR) receptor kinase (LRR-RK) domains and LRR numbers. **(A)** The kinase domains of LRR-RKs in the *Arabidopsis* genome were aligned by HMMER software [92], a neighbor-joining phylogenetic tree was created for the kinase-domain alignment using MEGA [93], and the phylogenetic tree was tested with bootstrapping. LRR-RKs discussed in this review are labeled accordingly. The kinase subfamilies are indicated along the outer edge of the alignment. **(B)** LRRs for each LRR-RK from the kinase alignment were identified using HMMER for domain analysis with a low stringency for the LRR domain from the Pfam database PF00560 [92]. For comparison, the LRRs from receptor-like proteins (LRR-RLPs) were also identified using the Pfam database. The total number of LRRs for each LRR-RK and LRR-RLP were calculated to give the distribution of the numbers of LRRs seen in these proteins. The frequency of each number of LRRs was determined by dividing each value by the total number of LRR-RKs and LRR-RLPs analyzed and then plotting the result. The validity of this method was verified using known LRR numbers based on crystal structures and analyzing several LRR-RKs by hand. LRR-RKs discussed in the review are labeled accordingly. **(C)** Mutations in pathways that are controlled by LRR-RKs can have obvious growth and development phenotypes. Disruption of endogenous brassinosteroid (BR) signaling through loss-of-function mutations in steroid biosynthesis or signaling translates into severe dwarfism. Opposite effects can be achieved by gain-of-function mutations that elevate signaling through increased biosynthesis or receptor overexpression.

the cell surface [21,22]. Forward genetic studies aimed at uncovering mutations in genes controlling plant growth, development, and defense converged on a role for LRR kinases in all of these processes [21,23,24]. More than 200 LRR-RKs have since been identified in the genome of *Arabidopsis* and these are likely to reflect an adaptive strategy in which plants use sophisticated extracellular sensors to determine the current and anticipated state of their growth environment (Figure 1) [25,26].

LRR-RKs are typically characterized by a series of eLRRs, a single transmembrane pass, and, in most cases, a functional intracellular kinase domain. The kinase domains of LRR-RKs can be further divided into RD and non-RD families, based on the presence or absence of an arginine located before a catalytic aspartate residue [27]. Non-RD kinases lack the strong autophosphorylation activities of RD kinases and display lower enzymatic activities [28]. The differences in activities possibly indicate that these two classes of kinase are subject to distinct regulatory mechanisms [27]. The eLRRs control the assembly of signaling-competent LRR-RK complexes by conferring ligand specificity and recruiting appropriate signaling partners [21,22]. A comparison of the LRR-RK kinase domains shows a close relationship between the proteins, which may support the model of a common protein kinase ancestor (Figure 1A) [26]. However, there are clear differences in the number of LRRs in each LRR-RK (Figure 1B). Analysis of LRR numbers demonstrates a bimodal distribution that separates the LRR-RKs into two groups with median numbers of LRRs of six and 22, respectively (Figure 1B). Interestingly, proteins with closely related kinase domains also tend to have similar numbers of LRRs. For comparison, LRR receptor-like proteins (LRR-RLPs), which lack a kinase domain, do not show a

bimodal distribution and instead have a higher average number of LRRs (Figure 1B). It appears that there is a functional connection between these two groups of LRR-RK, representing receptors and coreceptors, respectively (discussed below). Importantly, LRR-RLPs do not show this distribution because they might function differently, perhaps through direct ligand interaction.

LRR-RKs are core modulators of plant growth

Of the approximately eight phytohormones that control plant growth, brassinosteroids (BRs), which are polyhydroxylated steroid hormones that play essential roles in nearly all phases of plant development, are the only class that utilizes a cell surface receptor [1,3,29–32]. Receptors for the other plant hormones are mainly distributed in the cytoplasm, nucleus, or endoplasmic reticulum (ER) [1,3,33]. Because of the striking and easy-to-score phenotypes of BR biosynthetic and response mutants (Figure 1C), the BR pathway is the best understood LRR-RK signal transduction pathway in plants [31,34]. The organizational principles of BR signaling diverge radically from the paradigms of animal steroid signaling, which involve the members of a nuclear receptor superfamily (Box 1).

BRs are perceived by three LRR-kinases: BRI1, BRL1, and BRL3 (Figure 1A) [35]. BRI1 is ubiquitously expressed and accounts for most of the steroid-binding activity [36,37]. By contrast, BRL1 and BRL3 have very restricted expression in a subset of vascular and root cells where they subtly regulate shoot and root development [35,36]. Loss-of-function mutations in BR biosynthetic or signaling genes cause severe plant dwarfism, primarily due to lack of cellular expansion [31,34].

Most BR signaling components are now known and a pathway linking steroid recognition at the cell surface to changes in gene expression has been proposed (Figure 2) [34,38,39]. On treatment of seedlings with brassinolide (BL), the most potent BR, BRI1 phosphorylates the negative regulator BRASSINOSTEROID KINASE INHIBITOR 1 (BKI1) on tyrosine 211, causing its displacement from the membrane to the cytosol where it is inactive [40]. The LRR-RK coreceptor BRI1-ASSOCIATED KINASE 1 (BAK1) can then freely associate with BRI1 to form a complex that elevates the signaling output of the pathway through a series of reciprocal transphosphorylation events [41]. In parallel, activated BRI1 phosphorylates the serine/threonine kinase BR SIGNALING KINASE 1 (BSK1) [42,43]. BSK1 is a receptor-like cytoplasmic kinase (RLCK) that positively regulates the BR pathway by relaying BRI1 signals to downstream signaling components. Downstream from BSK1 is BRASSINOSTEROID INSENSITIVE 2 (BIN2), which was identified from a gain-of-function mutation (*bin2-1D*) that resulted in a dwarf mutant that is indistinguishable from *bri1* loss-of-function mutants (Figure 1C) [44]. BIN2, a glycogen synthase kinase 3 (GSK3)-type kinase, negatively regulates the pathway by phosphorylating the downstream transcription factors BRASSINAZOLE RESISTANT 1 (BZR1) and BRI1–EMS-suppressor 1 (BES1) at multiple sites (Figure 2A) [39,45]. On treatment with BRs, BIN2 is dephosphorylated at Tyr200 by the protein phosphatase 1 (PP1)-like phosphatase BSU1, leading to its inactivation [46,47]. Further dephosphorylation of BES1 and BZR1 by protein phosphatase 2A (PP2A) allows them to multimerize as either homodimers or heterodimers on the promoters of target genes (Figure 2B) [48]. The current model for BR signaling suggests that the signal is amplified via a linear phosphorylation/dephosphorylation cascade leading to the transcriptional regulation of hundreds of genes and, ultimately, coordinated cell expansion [49,50].

Box 1. A few distinguishing organizational principles of plant steroid signaling pathways

- Plants perceive steroid hormones at the cell surface using a membrane-bound LRR-receptor serine/threonine/tyrosine kinase, as opposed to the familiar nuclear receptors utilized by metazoans [94,95].
- The key signaling output is dephosphorylation of the BES1/BZR1 transcription factors, which homo-or heterodimerize to activate or repress target genes [48].
- With the exception of the receptor BRI1, each BR signaling component is encoded by a gene family of three to 12 members, most with partially redundant functions (*BIN2*, ten-gene family [96]; *BSU1*, four-gene family [47]; *BES1/BZR1*, six genes [97]; *BKI1*, seven genes [40]; *BSK1*, 12 genes [42]; and *BAK1*, five genes [65]). Redundancy may be built into plant signaling systems to generate fine-tuned and cell type-specific responses. However, knowledge concerning the extent of the functional overlap between, and specific expression patterns of, gene-family members is rather limited.
- Response modulation by negative-feedback mechanisms (through *BKI1* and repression of biosynthetic gene expression) ensures that the response circuit is shut down when the initial signal drops below a threshold.

LRR-RKs at the front line of plant defenses

LRR-RKs also play a critical role in plant immunity [17,51]. The plant response to invading pathogens involves the detection of microbe-associated molecular patterns (MAMPs) by high-affinity pattern-recognition receptors (PRRs) that survey the extracellular space [17,51]. The detection of non-self molecules by PRRs drives a hierarchical and stereotypic defense response that ultimately results in the cessation of microbial growth and signaling to both nearby uninfected cells and systemic tissues [17,51]. Acute PRR signaling results in the accumulation of reactive oxygen intermediates (ROIs), ion channel activation, activation of defense-related mitogen-activated protein kinases (MAPKs), and transcriptional reprogramming [17,51]. Collectively, these responses constitute early MAMP-triggered immunity (MTI) and often occur over seconds, minutes, or hours [17,51]. Longer-term MTI responses, which occur over hours to days, include hormone biosynthesis, production of compounds with microbicidal and/or microbiostatic activities, and cell wall reinforcement through callose deposition [17,51]. Unlike BRs, which act, from the receptor to specific transcription factors, through an essentially linear signaling pathway, MTI appears to act through a ramified signaling network and the outputs noted above are often not linearly connected (Figures 2 and 3).

In plants, the bacterial flagellum is sensed by FLS2 [52]. Plants that are unable to sense a 22-amino acid epitope

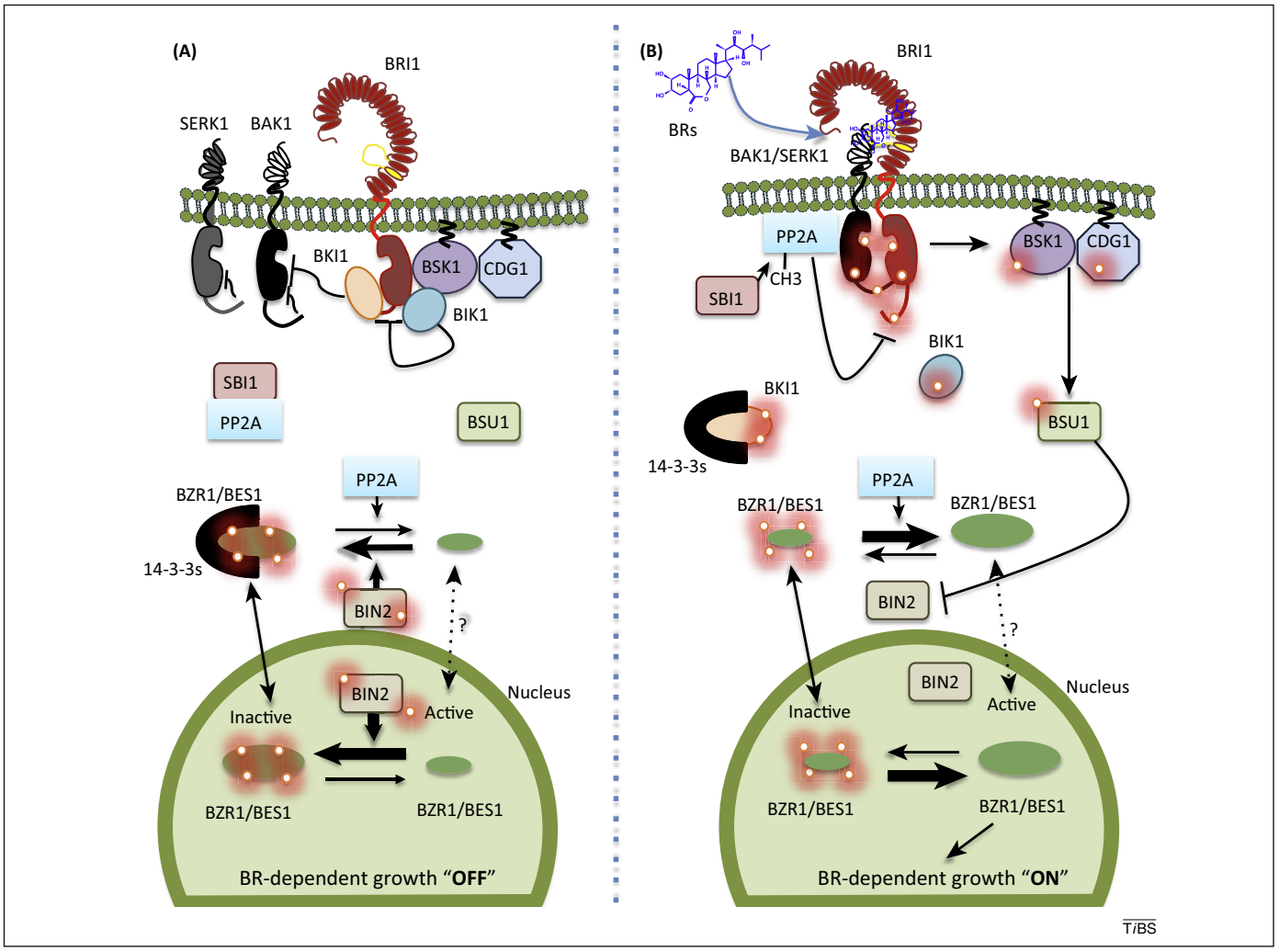

Figure 2. A model of brassinosteroid (BR) signaling from the cell surface to the nucleus. **(A)** In the absence of BR, signal-competent BRASSINOSTEROID INSENSITIVE 1 (BRI1) is regulated by *cis* and *trans* mechanisms. The kinase domains of BRI1-ASSOCIATED KINASE 1 (BAK1) and BRI1 are both inhibited by their own C-terminal tail [98]. BRI1 activity is further inhibited by association with BRI1 KINASE INHIBITOR 1 (BKI1), a plasma membrane-associated phosphoprotein. In this configuration, BRASSINOSTEROID INSENSITIVE 2 (BIN2) exists in an active and phosphorylated form. As a negative regulator of BR signaling, cytoplasmic and nuclear localized BIN2 inactivates BRASSINAZOLE RESISTANT 1 (BZR1) and BRI1–EMS-suppressor 1 (BES1), two plant-specific transcription factors. BIN2 has a dual action on BZR1/BES1: first, by phosphorylating them in the cytoplasm, BIN2 promotes their cytoplasmic retention by phosphopeptide-binding 14-3-3 proteins [99]; and second, by phosphorylating them in the nucleus, BIN2 blocks their DNA-binding and transcriptional activities. **(B)** Binding of BR to BRI1 triggers tyrosine phosphorylation of BKI1 and its subsequent dissociation from the plasma membrane, thereby allowing the recruitment of BAK1/somatic embryogenesis receptor kinase (SERK) 3 or 1. Meanwhile, released cytosolic BKI1 interacts with and thus titrates 14-3-3 proteins away from BZR1 [99,100], promoting the accumulation of BZR1/BES1 in the nucleus in their active forms. In the absence of ligand, BAK1/SERK1 are autoinhibited by their C-terminal tails; this inhibition is relieved by binding to BRI1 [98]. After a series of reciprocal transphosphorylation events on serine/threonine and tyrosine residues, signal-competent BRI1–BAK1 hetero-oligomers phosphorylate the receptor-like cytoplasmic kinases (RLCKs) BR SIGNALING KINASE 1 (BSK1) and Constitutive Differential Growth 1 (CDG1), which in turn activate bri1 SUPPRESSOR 1 (BSU1); BSU1 dephosphorylates and inactivates BIN2. Inactivation of BIN2, coupled with the activities of PROTEIN PHOSPHATASE 2A (PP2A), allows the accumulation of BZR1/BES1 in their dephosphorylated active forms. BZR1/BES1 can multimerize either on their own or with other transcription factors to bind target promoters to either repress or activate the expression of hundreds of genes to optimize BR-regulated growth (Figure 5). Once BR signals have been transduced, the leucine carboxyl methyltransferase SUPPRESSOR OF bri1 (SBI1) promotes PP2A association with the plasma membrane by methylating it [101]. Once at the membrane, PP2A triggers specific termination of BR signaling via BRI1 dephosphorylation and subsequent degradation [101]. The brassinolide (BL)-docking platform is depicted in yellow. BRI1 constitutively cycles between the plasma membrane and early endosomal compartments and can signal from either location (not shown here; [102]). BRI1 may also exist as preformed homo-oligomers at the cell surface [103]. For simplicity, we show BRI1 as a monomer. Phosphorylation events are indicated by the small white circles surrounded by a fluorescent red halo. The question marks highlight knowledge gaps in the signaling pathway.

derived from bacterial flagella [flagellin 22 (flg22)] are more susceptible to bacterial infection [53]. Molecular players acting with, or downstream of, FLS2 are emerging at a fast pace (Figure 3). However, FLS2 signaling remains under dissection (Figure 3). The recent discovery that NADPH oxidase respiratory burst oxidase homolog D (RbohD) interacts physically with FLS2 within minutes of flg22 treatment, driving a calcium increase and subsequent flg22-induced ROI production, represents a major milestone in closing this gap [54,55]. Future studies aimed at understanding how the FLS2 signaling complex is organized in light of specific phosphorylation events that drive signaling will significantly advance our knowledge of the ramified structure of this complex signaling network.

LRR-RK signaling pathways are core modulators of growth–defense trade-offs

Sustained MTI, achieved by nonphysiological flg22 treatments, can result in a chronic response known as seedling

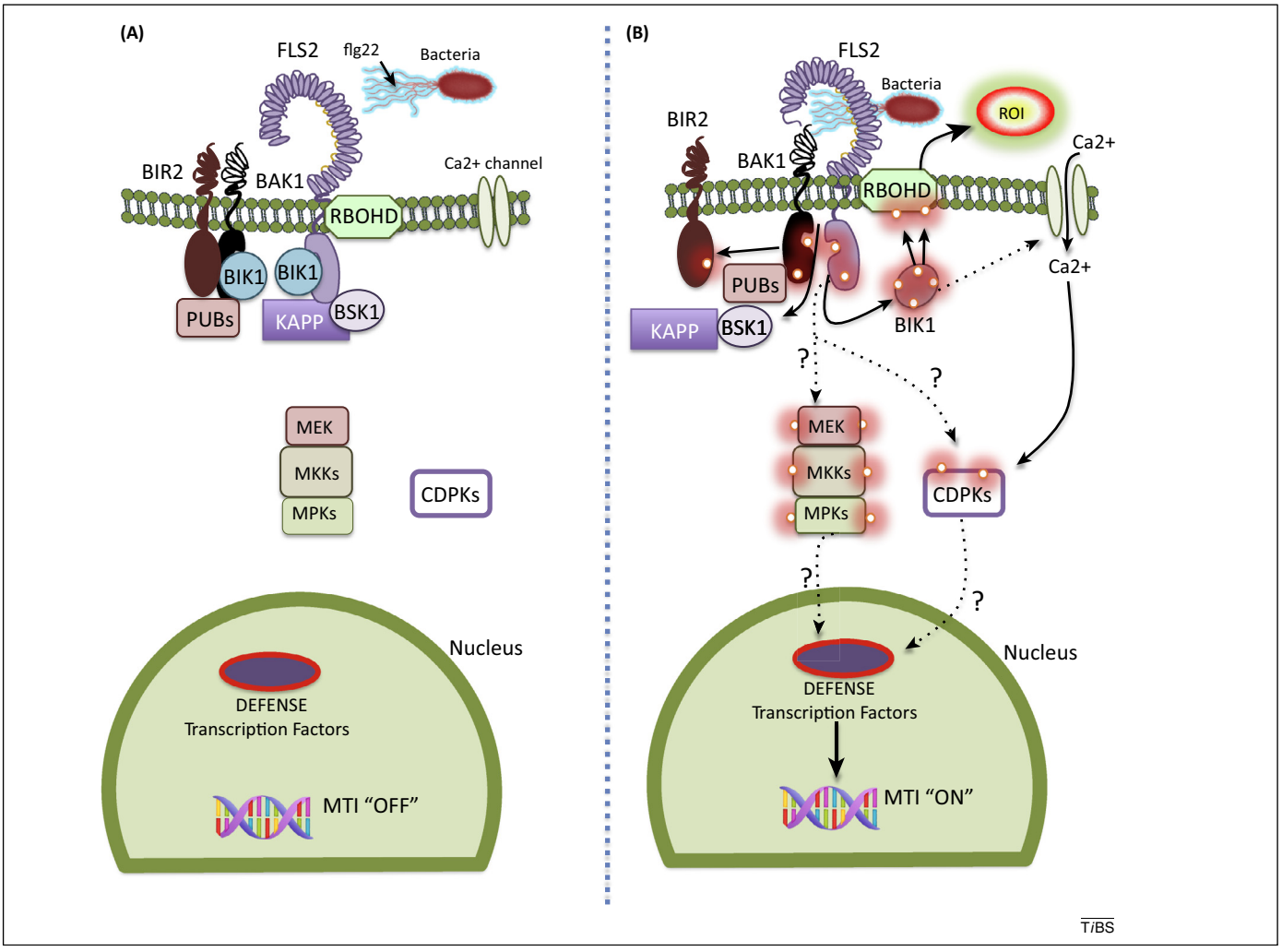

Figure 3. A model of FLAGELLIN SENSITIVE 2 (FLS2)-controlled defense in *Arabidopsis*. **(A)** In the absence of ligand, the signaling potential of FLS2 is kept in check by its association with kinase-associated protein phosphatase (KAPP) [104]. In this signal-competent state, FLS2 associates with at least BOTRYTIS INDUCED KINASE 1 (BIK1), BR SIGNALING KINASE 1 (BSK1), and respiratory burst oxidase homolog D (RbohD). BIK1 is a member of the family of AvrPphB SUSCEPTIBLE1 (PBS1)-like proteins (PBLs). Inactive FLS2 cycles on and off the plasma membrane via Brefeldin A-sensitive vesicle transport [105]. PBLs belong to a small subfamily of acylated receptor-like cytoplasmic kinases (RLCKs). BRI1-ASSOCIATED KINASE 1 (BAK1) also associates with BIK1 in the absence of flagellin 22 (flg22) and is sequestered away from FLS2 by BAK1-interacting receptor kinase 2 (BIR2). BSK1 (Figure 2) also associates with FLS2 to regulate positively flg22-dependent signaling. **(B)** As in brassinosteroid (BR) signaling, flg22 detection triggers the very rapid formation of a FLS2–BAK1 hetero-oligomer. flg22 binding allows the release of BAK1 from BIR2 and the dissociation or inactivation of KAPP phosphatases. The FLS2–BAK1 oligomer undergoes a series of transphosphorylation events and phosphorylates BIK1 at both tyrosine and serine/threonine residues. BIK1 autophosphorylates on multiple tyrosine residues, transphosphorylates FLS2 and BAK1, and then dissociates from the complex. BIK1 targets at least the NADPH oxidase RBOHD for phosphorylation, activating an extracellular superoxide burst and intracellular calcium increase. Downstream of receptor activation, mitogen-activated protein kinases (MPKs), mitogen-activated protein kinase kinases (MEK/MKKs), and calcium-dependent protein kinases (CDPKs) are activated by an unknown mechanism and may contribute to induction of flg22-responsive genes. Once flg22 signals have been transduced, BSK1 partly dissociates from the receptor complex and BAK1 phosphorylates closely related U-box E3 ubiquitin ligase proteins (PUBs), which in turn ubiquitylate FLS2 to regulate its abundance at the cell surface [104]. The flg22-docking platform is depicted in yellow on the concave side of the FLS2 leucine-rich repeats (LRRs). Like BRI1, FLS2 can exist as a homo-oligomer. For clarity we depict FLS2 as a monomer. Phosphorylation events are indicated by the small white circles surrounded by a fluorescent red halo. The question marks associated with the broken arrows highlight important knowledge gaps in the signaling pathway.

growth inhibition (SGI) [56]. SGI has been interpreted to represent transient growth–defense-trade-offs occurring on flg22 perception and can also be triggered by other MAMPs [57–59]. BAK1 acts as a coreceptor for both BRI1 and FLS2 (Figures 2 and 3) and other PRRs [28,56,60–63]. Recent studies demonstrated that the FLS2/BAK1 receptor pair remains functional when their respective cytosolic kinase domains are reversed by swapping, suggesting that receptor–coreceptor formation acts simply as a molecular switch for receptor activation [64].

BAK1 is a positive regulator of both BR-mediated cell expansion and growth and flg22-triggered defenses. *bak1*-null mutants are incompletely insensitive to flg22 and BR, suggesting some redundancy at this level of the signaling hierarchy. The other members of the somatic embryogenesis receptor kinase (SERK) family, of which BAK1 is the third (SERK3), are obvious candidates for mediators of this redundancy [65]. In addition to BAK1, BRI1 also associates with SERK1 and SERK4/BAK1-like 1 (BKK1) (Figure 2) [61,65]. SERK1 and SERK4/BKK1 can, to some extent, compensate for the loss of BAK1 in BR signaling [65]. BAK1 and SERK4 also exhibit overlapping functions in MTI through their functional association with FLS2 (Figure 3) [62], although most of the activity is due to BAK1. BAK1/SERKs, therefore, seem to act as 'bilateral' regulators of cellular expansion and MTI.

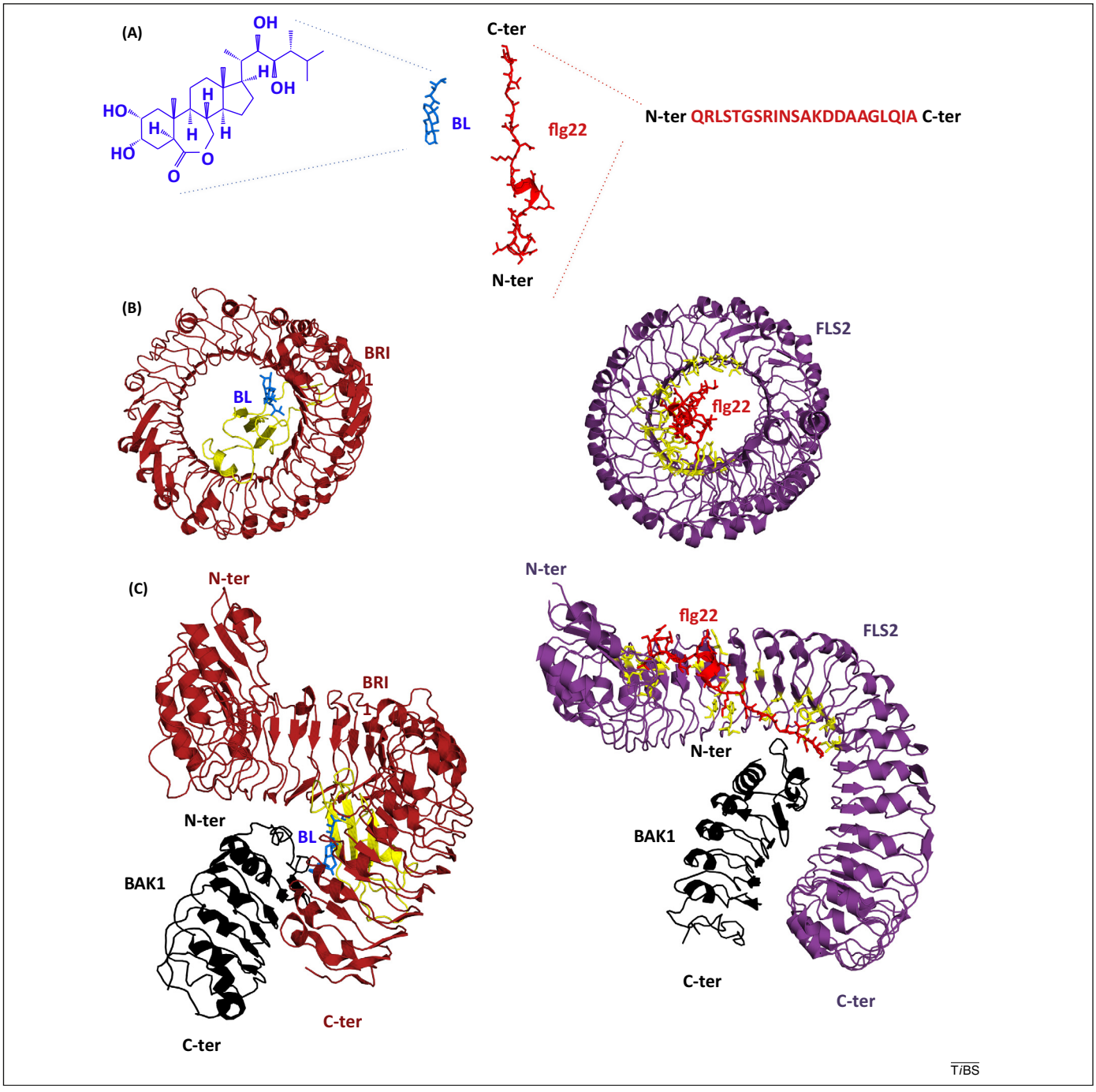

Figure 4. Atomic structures of ligand, receptor, and coreceptor leucine-rich repeat (LRR) complexes. **(A)** Comparison of brassinolide (BL) (in blue) and flagellin 22 (flg22) (in red) atomic structures to scale. **(B)** Top-down view of BRASSINOSTEROID INSENSITIVE 1 (BRI1) (in red) and FLAGELLIN SENSITIVE 2 (FLS2) (in purple) extracellular domains (ECDs) in complex with their respective ligands. BL (in blue) interacts with a 70-amino acid loop-out island domain (in yellow) between LRR 20 and 21 and lies on the surface of LRR 21–25 (of a total of 25 LRRs). The flg22-binding platform (in red) is distributed across 14 LRRs (in yellow) of FLS2 (28 LRRs in total). flg22 establishes direct links with amino acids that project their lateral chains from the concave side of the FLS2 LRR solenoid (LRR 3–16; in yellow). The central part of flg22 interacts with the lateral chains of residues derived from FLS2 LRR 2–6. FLS2 specifically recognizes the C- and N-terminal segment of flg22. **(C)** The N-terminal capping domain of BRI1-ASSOCIATED KINASE 1 (BAK1) (in black) is important for recognition of BL (in blue) and folds on top of the BRI1 steroid-binding pocket (in yellow) where it establishes contacts with BRI1 LRR 25 and with the hormone itself. Bulky amino acids distributed throughout the BAK1 ECD also establish an interaction with the BRI1 C-terminal capping domain. In complex with FLS2, the N terminus of BAK1 associates through two separate interfaces involving LRR 18–20 and 23–26 of FLS2. Mutations of key amino acids in either greatly reduce the binding of BAK1 to FLS2.

Atomic structures pave the way for a refined understanding of LRR-RK signaling

The atomic structures of the extracellular domains (ECDs) of the BRI1–BAK1, BRI1–SERK1, and FLS2–BAK1 receptor complexes were solved with their cognate ligands (Figure 4) [66–70]. These spectacular structures helped to rationalize a decade of forward genetic and molecular studies and will drive functional studies aimed at understanding the mechanisms underlying ligand-dependent LRR-RK activation. The plant eLRRs evolved different ligand-binding domains to recognize ligands, such as the plant steroid BL and the 22-amino acid flagellin peptide (Figure 4A) [67,69,70]. Although the extended flg22 peptide establishes many direct contacts along 14 LRRs on the concave side of the FLS2 solenoid, BL

interacts with a loop-out island domain between LRRs 21 and 22 as well as LRRs 23–25 (Figure 4B). The overall shape and curvature of the plant-specific twisted horseshoe-like structures formed by BRI1 and FLS2 ECDs differ in only minor ways because of the rigidity of the LRR fold (Figure 4B) [67,69,70]. The important consensus emerging from these data is that the binding of flg22 or BL to the ECDs of FLS2 or BRI1, respectively, reveals distinct ligand interfaces that are subsequently recognized by the LRRs of BAK1 (Figure 4C). Thus, the assembly of ternary LRR signaling-competent complexes (e.g., BRI1–BL–BAK1, FLS2–flg22–BAK1) seems to follow a 1:1:1 stoichiometry controlled by a specific sequence of limited local events driven by only minor structural rearrangements (Figure 4C) [67,69,70]. Understanding the adaptive value of BAK1 recruitment to several LRR-RK–ligand interfaces will be key to understanding fully cell surface signaling in plants.

Although the BAK1/SERKs cannot display ligand-binding activities on their own, the structures establish BAK1 and SERK1 as 'true' coreceptors that interact directly with both flg22 and BL (Figure 4C) [67,69,70]. Unlike the reported structures for various animal Toll-like receptor ectodomains, no homodimeric complexes were found in the crystallographic cell units of these plant eLRRs [71]. Thus, homo-oligomerization may not be required for their activation. Nevertheless, some caution has to be taken when comparing structural data and proposed models based on *in vivo* data. For example, substituting a key residue involved in flg22 binding caused FLS2 to lose the ability to interact with flg22 and BAK1 but retain the ability to transduce flg22 signals in a transient protoplast overexpression reporter system [70]. Furthermore, substituting key residues that create the FLS2-binding platform caused BAK1 to lose the ability to interact with FLS2 and to be phosphorylated on flg22 treatment, but nevertheless retain flg22-driven activation of downstream MAPK signaling [70]. These discrepancies reveal that the specific biochemical assays used do not faithfully reflect events occurring in wild type cells and/or that crystallographic knowledge is insufficient to understand precisely the highly sophisticated activation mechanisms of LRR-RKs. Detailed *in vivo* functional studies at native transgenic expression levels in appropriate genetic backgrounds are necessary to fully validate the proposed crystallographic models.

The structures essentially validate and reinforce the previous proposal of a 'double-lock' mechanism whereby BRI1 and FLS2 activation were predicated on the high-affinity recruitment of the LRR domain of BAK1 to the respective ligand-bound LRRs [72]. The slight alteration in structure of the kinase domain of BRI1 in its inhibited and activated forms indicates, to some extent, that BL drives conformational juxtaposition of the intracellular kinase domains to favor transphosphorylation and subsequent activation of signaling [73].

Is BAK1 a rate-limiting modulator of growth–defense trade-offs?

In native conditions, detectable amounts of BAK1 are found in 'off-ligand' FLS2 and BRI1 immunoprecipitates (IPs), albeit at much lower levels than in the presence of the respective ligands [62,72,74]. Sustained BR signaling can inhibit FLS2-dependent responses in a unidirectional way, giving rise to the idea that BRI1–BAK1 complex formation during heightened BR signaling could make BAK1 limiting for FLS2 function [60,75]. Measurement of FLS2–BAK1 complex formation in elevated BR signaling conditions showed no detectable decrease in FLS2–BAK1 association [75,76], consistent with a model in which an optimal range of endogenous BR concentrations is required for balanced growth and MTI, presumably in a BAK1-independent fashion.

However, elevated BRI1 levels (OxBRI1; see Figure 1C) antagonize flg22-mediated signaling in a BAK1 dose-dependent manner, indicating that the coreceptor could be rate limiting when MTI and BR signaling occur concurrently [60]. Because increased interaction between BAK1 and FLS2 remains detectable on flg22 treatment in OxBRI1 plants, elevated levels of BRI1 might interfere with BAK1-mediated phosphorylation of FLS2 [76]. Elevated BRI1 levels perhaps alter the competency of BAK1 for its activity in FLS2 signaling by 'phosphocoding' it in a manner that prevents appropriate BAK1-dependent phosphorylation of FLS2. Support for this idea can be derived from recent work showing that the phosphorylation and release of BOTRYTIS-INDUCED KINASE1 (BIK1) from, and recruitment of BAK1 to, FLS2 after flg22 exposure is retained even when a specific FLS2 phosphorylation site (Ser938) required for full signaling is mutated [77]. Thus, proper association between BAK1 and FLS2, likely triggering a highly specific phosphocode, is probably needed to drive appropriate MTI signaling [28]. Future studies aimed at dissecting how the sequential phosphorylation and dissociation of BIK1 from its FLS2 complex is modulated by different doses of BRI1, BAK1, flg22, and BL will be instrumental in understanding whether BAK1-dependent and BAK1-independent mechanisms are used to regulate the BR–MTI nexus at the cell surface.

Kinase dead receptors keep LRR-RK signaling pathways in check

The newly discovered BAK1-interacting RK2 (BIR2) negatively regulates BAK1-mediated flg22 responses (Figure 3) [78]. The structure of the kinase domain of BIR2 shows that it is an enzymatically dead kinase with an occluded nucleotide-binding site [79]. BIR2 interacts constitutively with BAK1, thereby preventing its interaction with FLS2 [78]. flg22 signals lead to BAK1 dissociation from BIR2, enabling enhanced BAK1 recruitment to FLS2 [78]. BR-regulated responses are not affected in *bir2* mutant plants, suggesting that BIR2 does not sequester BAK1 for amplified BR signaling in the presence of BL or that this function is redundant (there are four closely related BIR proteins in the *Arabidopsis* reference genome [78]). However, single BL and flg22 treatments can independently trigger the release of BAK1 from BIR2 and this release is dramatically enhanced when plants are treated concomitantly with both ligands [78]. Thus, in addition to its regulatory role in MAMP signaling, BIR2 could compensate for the decreased availability of BAK1 when both BR and flg22 signaling need to occur in parallel. We envision that BIR2 acts as a 'BAK1 reservoir' to modulate BAK1 distribution to at least FLS2 and BRI1. Future mechanistic studies need to determine how BRs and flg22 regulate the release of BAK1 from BIR2.

Common RLCKs act independently in distinct LRR-RK signaling pathways

Consistent with potential crosstalk between different LRR-RK signaling systems, the membrane-bound RLCKs BIK1 and BSK1 associate with both BRI1 and FLS2 in the absence of ligand (Figures 2 and 3) [80–82]. BIK1 is differentially phosphorylated by BRI1 and FLS2 to derepress downstream activation [80,81]. Similarly BSK1, a key player in BR signaling as noted above, also associates with, and positively regulates, FLS2 signaling (Figures 2 and 3) [43,82]. Whether BIK1 and BSK1 dosage is important for the sustained function of FLS2 or BRI1 pathways remains an open question. Thus, although three direct physical intersection points linking BRs and flg22 signaling have been identified (BAK1, BSK1, and BIK1), the exact mechanisms by which BRs modulate MAMP signaling at the receptor complex are unclear (Figure 5). Differential phosphorylation of the molecular players acting concomitantly with FLS2 and BRI1 could explain the inhibitory effects of BRs on MTI, but this supposition requires in-depth clarification [83]. The next challenge will then be to determine the structures of complete LRR-RK signaling complexes comprising the full-length receptors, ligands, and downstream RLCKs. This ambitious goal will require the reconstitution of structurally and functionally intact receptors in a lipid bilayer for subsequent crystallography or cryo-electron microscopy (EM) analyses [84].

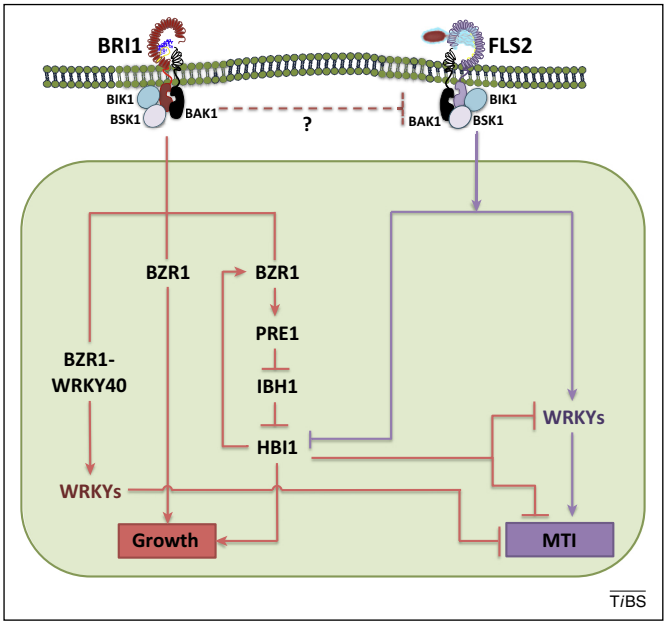

Figure 5. Network representation of brassinosteroid (BR) and flagellin 22 (flg22) signaling crosstalk in the nucleus. Transcriptional regulation is the final level of complexity in the growth–defense nexus between BR signaling and microbe-associated molecular pattern-triggered immunity (MTI). BRASSINAZOLE RESISTANT 1 (BZR1) integrates BR signals to repress MTI. BZR1 forms a complex with W box-binding transcription factor 40 (WRKY40) to upregulate the expression of additional WRKY transcription factors that suppress MTI. HOMOLOG OF BRASSINOSTEROID ENHANCED EXPRESSION 2 INTERACTING WITH ILI1-BINDING bHLH PROTEIN1 (IBH1) (HBI1) is another transcription factor performing dual roles in promoting growth and suppressing defense. HBI1 activates an overlapping set of positive regulators of cell elongation with BZR1 and represses the expression of defense genes including some WRKYs. BZR1 and HBI1 form a positive-feedback loop in which BZR1 activates PACLOBUTRAZOL RESISTANT1 (PRE1) expression to block IBH1-mediated inhibition of HBI1; HBI1 subsequently activates BZR1 by promoting BR biosynthesis and signaling. However, activation of FLAGELLIN SENSITIVE 2 (FLS2)-mediated MTI inhibits the transcription of HBI1, demonstrating antagonistic crosstalk between BR signaling and MTI at the HBI1 node. Signaling events downstream of BRASSINOSTEROID INSENSITIVE 1 (BRI1) and FLS2 are annotated in red and purple, respectively. The rounded green rectangle represents a nucleus.

LRR-RK initiated cross-regulation in the nucleus

BR and MTI signaling pathways intersect downstream of the respective receptors (Figure 5). The demonstration that elevated BR signaling downstream of BIN2 suppresses MTI is a key achievement (Figure 5) [76]. BRs prioritize constitutive growth over induced MTI via BZR1, which elevates the expression of several *WRKY* transcription factors that negatively control early MTI outputs [76]. For this, BZR1 forms a complex with WRKY40, a known negative regulator of immune responses [76]. Thus, BZR1–WRKY40 appears to be an important regulatory decision-making node that integrates BR signals on MTI. Recently, two studies demonstrated that a bHLH protein, *Homolog of brassinosteroid enhanced expression 2 interacting with* ILI1-BINDING bHLH PROTEIN1 (IBH1) (HBI1), is a negative regulator of MTI (Figure 5) [85,86]. *HBI1* expression is enhanced by BR treatment and repressed by flg22-driven MTI; constitutive expression of HBI1 leads to reduced MTI [85,86]. HBI1 is part of a tripartite regulatory module that also contains PACLOBUTRAZOL RESISTANT1 (PRE1) and IBH1 [87]. PRE1, IBH1, and HBI1 form an antagonistic switch that controls BR-dependent transcriptional responses (Figure 5) [87]. In this switch, BZR1 can positively regulate HBI1 function through *PRE1* expression and HBI1 possibly activates BZR1 indirectly, by promoting BR biosynthesis and signaling [87]. Thus, HBI1 acts in concert with BZR1 to balance BR-modulated growth and MTI output, through perhaps partly overlapping transcriptional networks.

Concluding remarks

The current paradigm, derived from analyses of BRI1- and FLS2-mediated signaling, suggests the involvement of further undiscovered LRR-RK signaling pathways regulating plant responses to their environment. Despite the clear interaction between MTI and BR signaling, the causal relevance of MTI modulation by endogenous BR concentrations over developmental time and in specific cell types is open to important investigations. We envision that LRR-RKs will involve complex and diverse ligand-recognition and -activation mechanisms, perhaps more than initially anticipated. The structures of the LRR-RKs Clavata 1 (CLV1) (Figure 1A) and SUPPRESSOR OF BIR1-1 (SOBIR1) and the PRR Elongation Factor Receptor (EFR) in complex with their ligands and coreceptors are eagerly awaited [58,88–90]. EFR activation, which is also BAK1 dependent, is anticipated to be generic and should resemble that of FLS2 and BRI1 [28]. However, CLV1 and SOBIR1 activation, which respectively requires the LRR-RLPs Clavata 2 (CLV2) and *Cladosporium fulvum 4* (Cf-4) for function is likely to uncover new perception and activation paradigms [88,91]. Additional discoveries are expected to further our understanding of the molecular mechanisms required to integrate the time-sensitive perception of environmentally derived signals against the backdrop of ongoing dynamic and coordinated developmental changes.

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