Tyrosine phosphorylation of the BRI1 receptor kinase emerges as a component of brassinosteroid signaling in *Arabidopsis*

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Brassinosteroids (BRs) are essential growth-promoting hormones that regulate many aspects of plant growth and development. Two leucine-rich repeat receptor-like kinases (LRR-RLKs) are involved in BR perception and signal transduction: brassinosteroid insensitive 1 (BRI1), which is the BR receptor, and its coreceptor BRI1-associated kinase 1 (BAK1). Both proteins are classified as serine/threonine protein kinases, but here we report that recombinant cytoplasmic domains of BRI1 and BAK1 also autophosphorylate on tyrosine residues and thus are dual-specificity kinases. With BRI1, Tyr-831 and Tyr-956 are identified as autophosphorylation sites in vitro and in vivo. Interestingly, Tyr-956 in kinase subdomain V is essential for activity, because the Y956F mutant is catalytically inactive and thus this site cannot be simply manipulated by mutagenesis. In contrast, Tyr-831 in the juxtamembrane domain is not essential for kinase activity but plays an important role in BR signaling in vivo, because expression of BRI1(Y831F)-Flag in transgenic *bri1-5* plants results in plants with larger leaves (but altered leaf shape) and early flowering relative to plants expressing wild-type BRI1-Flag. Acidic substitutions of Tyr-831 restored normal leaf size (but not shape) and normal flowering time. This is an example where a specific tyrosine residue has been shown to play an important role in vivo in plant receptor kinase function. Interestingly, 6 additional LRR-RLKs (of the 23 tested) were also found to autophosphorylate on tyrosine in addition to serine and threonine, suggesting that tyrosine signaling should be considered with other plant receptor kinases as well.

It is well known that two leucine-rich repeat receptor-like kinases (LRR-RLKs) are involved in brassinosteroid (BR) perception and signal transduction: the receptor brassinosteroid insensitive 1 (BRI1) (1, 2) and its coreceptor, BRI1-associated kinase 1 (BAK1) (3, 4). Both of these receptor kinases have an extracellular domain, a single-pass transmembrane domain, and an intracellular cytoplasmic domain (CD) consisting of a juxtamembrane (JM) region, serine/threonine kinase domain (KD), and short carboxyl-terminal (CT) polypeptide (5–8). It is known that BRs bind to the extracellular domain of BRI1 (1) and thereby activate its KD and promote association with the coreceptor, BAK1 (3, 4, 6). This process results in release of BKI1, a recently described inhibitor protein (9), and phosphorylation of both BRI1 and BAK1 on multiple serine and threonine residues either as a result of autophosphorylation and/or transphosphorylation by their cytoplasmic KDs (6, 7, 10).

Autophosphorylation has been extensively studied in vitro by using recombinant CDs of the receptor kinases. Early studies demonstrated that when BRI1-CD was incubated with [γ -³²P]-ATP, phosphorylation of threonine and serine residues was readily detected (5–7, 11). More recently, staining with Pro-Q Diamond phosphoprotein stain and immunoblotting with antiphosphothreonine antibodies demonstrated that the CDs of BRI1 and BAK1 are highly autophosphorylated as isolated from *Escherichia coli*, and many of these sites have now been identified by mass spectrometry (6). During the course of our studies, we recently made the surprising observation that recombinant

BRI1-CD and BAK1-CD were also recognized by antiphosphotyrosine antibodies, suggesting that they may be dual-specificity kinases rather than serine/threonine kinases as classified (5–7, 11). The occurrence of tyrosine phosphorylation was confirmed for BRI1 both in vitro and in vivo, and we also identified two sites of tyrosine autophosphorylation. One of these sites (Tyr-831) is not essential for kinase catalytic activity but when manipulated by site-directed mutagenesis is demonstrated to play a key role in BR signaling in vivo.

Results

The key observation that served as the foundation of the present study was that recombinant BRI1-CD and BAK1-CD cross-reacted strongly with antiphosphotyrosine antibodies (Fig. 1A). This finding was surprising because both BRI1 and BAK1 are classified as serine/threonine protein kinases (12, 13) and have been shown to autophosphorylate only on serine and threonine residues (5–7, 11). Hence, it was critical to confirm the specificity of the antiphosphotyrosine antibodies, which we did in several different ways. First, addition of phosphotyrosine-conjugated BSA (pY-BSA) to the immunodetection buffer resulted in the disappearance of immunoblot bands indicating specificity of detection (Fig. 1A). Second, mBRI1, a kinase-inactive mutant of BRI1 resulting from a K911E substitution (6), was not autophosphorylated and did not cross-react with the antiphosphotyrosine antibodies, indicating that phosphorylated residues on BRI1 were specifically being detected (Fig. 1B). Third, pretreatment of BRI1-CD with a recombinant protein tyrosine phosphatase, PTP1B (14), eliminated the recognition of BRI1 by the antiphosphotyrosine antibodies without substantially affecting the staining with Pro-Q Diamond (Fig. 1C) or recognition by antiphosphothreonine antibodies (data not shown). Similar results to those shown in Fig. 1 for BRI1-CD were also obtained with recombinant BAK1-CD (data not shown). Collectively, these results indicated that the antiphosphotyrosine antibodies were specifically recognizing phosphotyrosine residues in the receptor kinases.

One possible explanation for the lack of detection of phosphotyrosine residues in BRI1 and BAK1 after [³²P]ATP autophosphorylation in vitro is that the stoichiometry of tyrosine autophosphorylation is extremely low and may be detectable only with very sensitive immunoblotting protocols. However, the

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Fig. 1. BRI1 is phosphorylated on tyrosine residues in vitro and in vivo. (A) Detection of phosphotyrosine residues in recombinant BRI1 and BAK1 CDs by immunoblotting with antiphosphotyrosine (pY) antibodies. Preincubation of the antibodies with pY-BSA eliminated the immunoblot signal indicating specificity of the detection. Protein abundance was visualized by Coomassie Brilliant Blue (CBB) staining. (B) The kinase-inactive K911E mutant, mBRI1 (6), does not autophosphorylate as indicated by the lack of immunoblot signal with antiphosphotyrosine (pY) antibodies, antiphosphothreonine (pT) antibodies, or staining with Pro-Q Diamond phosphoprotein stain. (C) Treatment of the recombinant BRI1-CD with recombinant PTP1B dephosphorylates tyrosine residues without substantially reducing phosphorylation on serine/threonine residues, as indicated by staining with Pro-Q Diamond phosphoprotein stain. (D) Phosphorylation of BRI1-Flag on tyrosine residues in vivo. Nontransgenic wild-type plants served as a control to demonstrate specificity of the immunoprecipitation of BRI1-Flag from transgenic plants, and mBRI1-Flag served as a control to demonstrate specificity of the interaction with the antiphosphotyrosine antibodies.

stoichiometry of tyrosine phosphorylation on recombinant BRI1-CD was in fact quite high, because $\approx 50\%$ of the BRI1-Flag protein could be immunoprecipitated with immobilized antiphosphotyrosine antibodies (Fig. S1). Thus, the failure to detect phosphotyrosine by [^{32}P]-phosphoamino acid analysis may be because the recombinant protein is already highly autophosphorylated on tyrosine residues as isolated from *E. coli*. Analysis of recombinant BRI1-CD tryptic peptides by liquid chromatography-tandem mass spectrometry (LC/MS/MS) was consistent with tyrosine phosphorylation of residue 831 (Fig. S2). We showed that S838 of BRI1-CD (which is on the same tryptic peptide as Y831) is heavily phosphorylated (6, 10) and may have suppressed the ionization efficiency for the phosphorylated Y831 peptide, thus complicating data-dependent LC/MS/MS identification of this residue.

Importantly, BRI1 was also found to be phosphorylated on tyrosine residues in vivo. As shown in Fig. 1D, immunoprecipitated BRI1-Flag protein from transgenic *Arabidopsis* plants clearly cross-reacted with antiphosphotyrosine antibodies, establishing that tyrosine phosphorylation of BRI1 occurs in vivo. The failure to immunoprecipitate protein from wild-type plants with the anti-Flag antibodies and the failure to detect a phosphotyrosine signal in immunoprecipitated mBRI1-Flag served as critical controls to validate the detection of phosphotyrosine in BRI1-Flag in vivo (Fig. 1D).

Autophosphorylation of tyrosine residues in vitro was also observed in recombinant cytoplasmic domains of several other receptor kinases. Of the 23 LRR-RLKs that were tested, 11 were clearly autophosphorylated (as evidenced by strong Pro-Q Diamond phosphoprotein staining) and of these 11, 9 contained phosphothreonine and 6 contained phosphotyrosine by immunoblotting (Fig. S3). Therefore, tyrosine phosphorylation may be of broad significance in plant receptor kinase signaling.

To investigate the role of specific tyrosine residues of BRI1, we produced site-directed mutants of BRI1-CD by individually replacing each of the 10 tyrosine residues with phenylalanine. The corresponding recombinant mutant proteins were compared with active wild-type BRI1-CD and the kinase-inactive

mutant, mBRI1-CD, for ability to catalyze autophosphorylation and transphosphorylation reactions. Substitution of tyrosine at positions 956, 1052, 1057, and 1072 with phenylalanine strongly inhibited autophosphorylation as indicated by almost complete elimination of Pro-Q Diamond staining and cross-reactivity with antiphosphothreonine and antiphosphotyrosine antibodies (Fig. 2A and B). As expected, these substitutions also strongly inhibited kinase activity on a previously identified optimal peptide substrate for BRI1-CD (5) (Fig. 2C). Because these 4 tyrosine residues are essential for BRI1 kinase activity, the site-directed mutagenesis approach cannot be used to determine whether these residues are potential autophosphorylation sites. In contrast, substitution of the tyrosine residues at positions 898, 945, 961, 1058, and 1070 with phenylalanine did not reduce the phosphotyrosine immunoblot signal relative to wild-type BRI1 (Fig. 2B), and all retained considerable peptide kinase activity (Fig. 2C), suggesting that these tyrosine residues are not essential for activity and are also not major sites of tyrosine autophosphorylation. The only site-directed mutant that had reduced tyrosine autophosphorylation but retained significant autophosphorylation activity on serine and threonine residues and peptide kinase activity was the Y831F mutant (Fig. 2A and B). These results suggested that Tyr-831 is an autophosphorylation site. Interestingly, the inhibition of peptide kinase activity observed in the Flag-BRI1(Y831F) site-directed mutant was substantially ameliorated when Tyr-831 was substituted with an aspartate residue (Fig. S4), which is consistent with the notion that Tyr-831 is a major site of tyrosine autophosphorylation and that autophosphorylation stimulates kinase activity. However, because the BRI1-Y831F mutant still had $\approx 40\%$ of the phosphotyrosine immunoblot signal of the wild-type protein, there is at least 1 additional tyrosine autophosphorylation site among the residues that when mutated resulted in the kinase-inactive phenotype.

As an additional approach to localize sites of tyrosine autophosphorylation, we used the Flag-BRI1-CD construct to produce a set of deletion mutants lacking either the JM domain, the CT domain, or both. All constructs had the KD, which contains 9 of the 10 tyrosine residues, with a Flag-epitope tag at

Fig. 2. Identification of tyrosine residues on BRI1 that affect autophosphorylation and transphosphorylation of a synthetic peptide substrate. (A) Effect of site-directed mutagenesis of tyrosine residues in the BRI1-CD on autophosphorylation of the recombinant protein. (B) Densitometry analysis of antiphosphotyrosine immunoblots similar to those shown in A. (C) Tyrosine mutagenesis inhibits transphosphorylation of the SP11 (5) synthetic peptide.

the N terminus (Fig. 3A). All of the recombinant proteins had significant autophosphorylation activity, as evidenced by Pro-Q Diamond staining (Fig. 3B). Interestingly, autophosphorylation of the constructs lacking the JM domain (BRI1-KC and BRI1-K) was primarily on serine residues as there was little or no evidence for phosphothreonine or phosphotyrosine by immunoblotting (Fig. 3B); only the constructs with the JM domain (BRI1-JKC and BRI1-JK) had robust autophosphorylation on both threonine and tyrosine residues. Furthermore, the constructs containing the JM domain also had the highest rates of peptide kinase activity (Fig. 3C), suggesting that the JM domain plays a greater role as an activator of kinase activity (≈ 5 -fold activation) than the CT domain plays as a negative regulator ($\approx 20\%$ inhibition). These results are consistent with the *in vivo* result of Wang *et al.* (7) that the JM domain is essential for a functional BRI1 receptor, and with our previous *in vitro* results showing that specific JM residues played a critical role in peptide substrate phosphorylation by BRI1-KD (6).

Sequence- and modification-specific antibodies were developed to determine whether individual tyrosine residues in BRI1 were indeed autophosphorylation sites. As shown in Fig. 4A, recombinant BRI1-CD cross-reacted with antibodies directed against the sequences surrounding phosphotyrosine-831, phosphotyrosine-956, and phosphotyrosine-1072, suggesting that all three residues are sites of tyrosine autophosphorylation *in vitro*. The specificity of the modification-specific antibodies was indicated by: (i) the lack of cross-reaction with the kinase-inactive mutant, mBRI1, (ii) almost complete removal of the immunoblot signal by pretreatment of wild-type BRI1 with PTP1B (Fig. 4A), and (iii) specific inhibition of the immunoblot signals by only the appropriate phosphopeptide (Fig. S5). Furthermore, the BRI1 site-directed mutants also reacted with the antibodies in the expected manner. The Y956F and Y1072F mutants were kinase inactive as expected (Fig. 2) and accordingly did not cross-react with any of the phosphospecific antibodies (Fig. 4A). However, the Y831F mutant was kinase active as expected (Fig. 2) and cross-reacted with the anti-pY956 and anti-pY1072 antibodies but not the anti-pY831 antibodies. Importantly, full-length BRI1-Flag was phosphorylated on tyrosine residues *in vivo* (Figs. 1D and 4B) and the modification-specific antibodies identified Tyr-831 and Tyr-956 as *in vivo* sites of tyrosine phosphorylation (Fig. 4B). The failure to immunoprecipitate

protein from wild-type plants using anti-Flag antibodies and the failure to detect a specific phosphotyrosine signal in immunoprecipitated mBRI1-Flag, a kinase-inactive mutant, served as critical controls to validate the detection of site-specific tyrosine phosphorylation in BRI1-Flag *in vivo*. Likewise, the lack of cross-reaction of BRI1(Y831F)-Flag with the anti-pY831 antibodies confirmed the sequence specificity of these antibodies when applied to native full-length proteins. Interestingly, Tyr-1072 was not autophosphorylated *in vivo* as it was *in vitro*, and thus, autophosphorylation of this residue is apparently not required for BRI1 kinase activity. Rather it may be the hydroxyl function at residue 1072 that is needed for kinase function. However, the most important point to note is that Tyr-831 and Tyr-956 on BRI1 are clearly established as *in vivo* phosphorylation sites.

To determine whether tyrosine phosphorylation is required for BRI1 function *in vivo*, we transformed the weak *bri1-5* mutant, which is an intermediate dwarf, with wild-type BRI1-Flag or site-directed mutants where Tyr-831 or Tyr-1052 was manipulated. The dwarf phenotype of the *bri1-5* mutant (15) was rescued by expression of wild-type BRI1-Flag (Fig. 5A), as demonstrated (6, 7), but only slightly by the BRI1(Y1052F)-Flag construct, which is consistent with the kinase-inactive phenotype of the corresponding recombinant protein (Fig. 2). However, whereas expression of BRI1(Y831F)-Flag clearly rescued the dwarf phenotype of the *bri1-5* mutant, the aberrant leaf shape (round leaves) of the *bri1-5* mutant (15) was not completely rescued. This result is visually apparent in the plants grown for 30 days under short days (Fig. 5A) and in plants grown for 32 days under long days (Fig. 5B). Quantitative analysis confirmed that whereas *bri1-5* leaves were small and nearly round, expression of the wild-type BRI1-Flag construct resulted in production of larger and elongated leaves as expected (Fig. 5C). In the case of the Y1052F site-directed mutant there was only a slight rescue of leaf size, whereas the Y831F site-directed mutant produced leaves that were even larger than wild-type BRI1 but the normal elongated leaf shape was only partially restored (Fig. 5C). Both of the acidic substitutions, BRI1(Y831D) and BRI1(Y831E), also rescued the dwarf phenotype but the elongated leaf shape was also not completely restored by the acidic substitutions. Interestingly, leaf size in the acidic substitutions was not in-

Fig. 3. The JM domain is a positive regulator of BRI1 kinase activity and is required for tyrosine phosphorylation. (A) Schematic representation of the Flag-BRI1-CD and the deletion mutants used in these experiments. J, K, and C refer to the JM, kinase, and C-terminal domains, respectively, and the hatch marks identify the location of the 10 tyrosine residues in BRI1-CD. The shaded portion of the KD depicts the activation loop. (B) Autophosphorylation of the recombinant proteins shown in A as measured with Pro-Q Diamond stain and immunoblotting with antiphosphotyrosine (pY) or antiphosphothreonine (pT) antibodies. (C) Transphosphorylation activity of the recombinant full-length and truncated proteins using the SP11 synthetic peptide as substrate.

creased above that observed with the wild-type BRI1-Flag construct (Fig. 5C).

Another important observation was that the *bril*-5:BRI1(Y831F)-Flag plants flowered earlier than the other

genotypes (Fig. 5B). In Fig. 5A, plants were grown under short days and the BRI1(Y831F)-Flag transgenic plants were just beginning to bolt whereas the other plants were not, while under long days (Fig. 5B) BRI1(Y831F)-Flag plants clearly flowered earlier than wild-type BRI1. Thus, the early flowering phenotype was independent of photoperiod. As quantified in Fig. 5C, the *bri1-5*:BRI1(Y831F)-Flag plants flowered 7–10 days earlier than the *bri1-5* mutant control or *bri1-5* expressing wild-type BRI1-Flag or the Y1052F mutant. Importantly, the acidic substitutions at Tyr-831 restored the normal flowering time observed for wild-type BRI1-Flag plants. These results are consistent with the notion that there is a link between BR signaling and flowering (16) and suggests that phosphorylation of Tyr-831 may play some role in this linkage.

Discussion

We have shown that BRI1 is autophosphorylated on tyrosine residues, and we have demonstrated tyrosine phosphorylation of a plant receptor kinase *in vivo*. As noted in the Introduction, this finding is surprising because BRI1 has only been shown in previous studies to autophosphorylate on serine and threonine residues. None of the recognized consensus motifs for tyrosine kinases or dual-specificity kinases are contained in BRI1 ([Table S1](#)) or the other plant receptor kinases shown in the present study to have serine, threonine and tyrosine kinase activity (data not shown). Identifying the KD residues that control dual specificity of catalytic activity will be an important topic for future studies. It is relevant to note that two previous studies identified plant receptor kinases with tyrosine kinase activity *in vitro*: PRK1, a pollen-expressed receptor kinase that was shown to autophosphorylate on serine and tyrosine residues *in vitro* (17), and AtSERK1, which is transiently expressed during embryogenesis and was reported to phosphorylate myelin basic protein and casein on serine, threonine, and tyrosine residues *in vitro* (18). These results suggested that PRK1 and AtSERK1 are dual-specificity kinases; however, the occurrence of phosphotyrosine *in vivo* was not established nor was the significance of tyrosine phosphorylation elucidated, as was done in the present study with BRI1.

We also document the ability of several other receptor kinases to autophosphorylate on tyrosine residues. In addition to BRI1 and BAK1, many recognized receptor kinases, including BKK1 (19), HAESA (20), and the light-repressible receptor kinase (At3g21340) (21), all appear to be dual-specificity kinases (Fig. S3). Of particular interest is BAK1, which is a multifunctional

Fig. 4. Identification of Tyr-831, Tyr-956, and Tyr-1072 as phosphorylation sites on BRI1 using modification-specific antibodies. (A) Immunoblot analysis of recombinant Flag-BRI1-CD expressed in *E. coli*. The kinase-inactive mBRI1, wild-type BRI1 (treated or not with PTP1B), and the site-directed mutants Y831F, Y956F, and Y1072F were affinity purified and analyzed by immunoblotting (IB) with the indicated antibodies. (B) Immunoblot analysis of full-length BRI1-YAG constructs expressed in transgenic *Arabidopsis* plants. Protein was immunoprecipitated (IP) from nontransgenic wild-type plants (Ws-2) and transgenic plants using immobilized anti-Flag antibodies, and the purified protein was analyzed by immunoblot analysis as in A. Seedlings were grown in liquid culture and treated with 10^{-7} M brassinolide for 2 h before harvest.

the *bri1-5* mutant background were as described (6). The same BRI1-Flag construct was used to generate the site-directed mutants, Y831F, Y831D, Y831E, and Y1052F, and each construct was transformed individually into the *bri1-5* mutant as described (6).

Preparation of Microsomal Membranes for Immunoprecipitation and Immunoblot Analysis. *A. thaliana* plants were grown in shaking liquid culture (30), and microsomal membranes were isolated as described (6). Membranes were solubilized with 1% (vol/vol) Triton X-100, clarified by centrifugation, and diluted so that protein concentration was adjusted to 0.5–1.0 mg/mL, and Triton X-100 was reduced to 0.1%.

BRI1-Flag was then immunoprecipitated with prewashed anti-Flag M₂ affinity gel (Sigma–Aldrich) and analyzed by immunoblotting after transfer to PVDF membranes using anti-Flag antibody (1:5,000 dilution), antiphosphotyrosine antibody (1:500), antiphosphothreonine antibody (1:500), or custom modification antibodies (anti-pY831, anti-pY956, or anti-pY1072 antibodies). The custom antibodies were produced against the following sequences: pY831, KKEAELEMPYAEHG; pY956, ERLLVpYEFMK; and pY1072, KGD-VYSpYGVV. The antibodies were produced by GenScript and sequentially affinity purified by using the nonphosphorylated and phosphotyrosine-containing antigen peptides. Immunoblots were scanned with an Odyssey Infrared Imaging System (LI-COR Bioscience) for visualization. As indicated, blots were also stained with ProQ Diamond phosphoprotein stain and scanned by using a Typhoon Molecular Dynamics phosphor/fluorescence imager.

Site-Directed Mutagenesis of Flag-BRI1-CD. The described Flag-BRI1-CD construct (5) was used as the template for site-directed mutagenesis with the QuikChange XL Site-Directed Mutagenesis Kit (Stratagene). Ten individual constructs were generated with the following substitutions: Y831F, Y898F, Y945F, Y956F, Y961F, Y1052F, Y1057F, Y1058F, Y1070F, and Y1072F. All constructs were sequenced in both directions to verify specific mutations and lack of additional mutations.

Recombinant Protein Purification and In Vitro Peptide Kinase Assay. Flag-BRI1-CD and its mutations were expressed in BL21(DE3)pLysS cells and purified by using anti-Flag M₂ affinity gel (Sigma–Aldrich). After elution from the beads, the protein solution was dialyzed against a 1,000× volume of buffer containing 20 mM Mops, pH 7.5, and 1 mM DTT. Peptide substrate phosphorylation assays were performed as described (5) using the SP11 peptide (sequence: GRJRRIASVEJJK, where J is norleucine; produced by Bethyl Laboratories).

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- Kinoshita T, et al. (2005) Binding of brassinosteroids to the extracellular domain of plant receptor kinase BRI1. *Nature* 433:167–171.
- Wang ZY, Seto H, Fujioka S, Yoshida S, Chory J (2001) BRI1 is a critical component of a plasma-membrane receptor for plant steroids. *Nature* 410:380–383.
- Li J, et al. (2002) BAK1, an *Arabidopsis* LRR receptor-like protein kinase, interacts with BRI1 and modulates brassinosteroid signaling. *Cell* 110:213–222.
- Nam KH, Li J (2002) BRI1/BAK1, a receptor kinase pair mediating brassinosteroid signaling. *Cell* 110:203–212.
- Oh MH, et al. (2000) Recombinant brassinosteroid insensitive 1 receptor-like kinase autophosphorylates on serine and threonine residues and phosphorylates a conserved peptide motif in vitro. *Plant Physiol* 124:751–766.
- Wang X, et al. (2005) Identification and functional analysis of in vivo phosphorylation sites of the *Arabidopsis* brassinosteroid-insensitive1 receptor kinase. *Plant Cell* 17:1685–1703.
- Wang X, et al. (2005) Autoregulation and homodimerization are involved in the activation of the plant steroid receptor BRI1. *Dev Cell* 8:855–865.
- Vert G, Nemhauser JL, Geldner N, Hong F, Chory J (2005) Molecular mechanisms of steroid hormone signaling in plants. *Annu Rev Cell Dev Biol* 21:177–201.
- Wang X, Chory J (2006) Brassinosteroids regulate dissociation of BAK1, a negative regulator of BRI1 signaling, from the plasma membrane. *Science* 313:1118–1122.
- Wang X, et al. (2008) Sequential transphosphorylation of the BRI1/BAK1 receptor kinase complex impacts early events in brassinosteroid signaling. *Dev Cell* 15:220–235.
- Friedrichsen DM, Joazeiro CA, Li J, Hunter T, Chory J (2000) Brassinosteroid-insensitive-1 is a ubiquitously expressed leucine-rich repeat receptor serine/threonine kinase. *Plant Physiol* 123:1247–1256.
- Diévert A, Clark SE (2004) LRR-containing receptors regulating plant development and defense. *Development* 131:251–261.
- Shiu S-H, Bleeker AB (2003) Expansion of the receptor-like kinase/Pelle gene family and receptor-like proteins in *Arabidopsis*. *Plant Physiol* 132:530–543.
- Zhang Z-Y (2002) Protein tyrosine phosphatases: Structure and function, substrate specificity, and inhibitor development. *Annu Rev Pharmacol Toxicol* 42:209–234.
- Noguchi T, et al. (1999) Brassinosteroid-insensitive dwarf mutants of *Arabidopsis* accumulate brassinosteroids. *Plant Physiol* 121:743–752.
- Domagalska MA, et al. (2007) Attenuation of brassinosteroid signaling enhances FLC expression and delays flowering. *Development* 134:1841–1850.
- Mu J-H, Lee H-S, Kao T-H (1994) Characterization of a pollen-expressed receptor-like kinase gene of *Petunia inflata* and the activity of its encoded kinase. *Plant Cell* 6:709–721.
- Shah K, Vervoort J, de Vries SC (2001) Role of threonines in the *Arabidopsis thaliana* somatic embryogenesis receptor kinase 1 activation loop in phosphorylation. *J Biol Chem* 276:41263–41269.
- He K, et al. (2007) BAK1 and BKK1 regulate brassinosteroid-dependent growth and brassinosteroid-independent cell-death pathways. *Curr Biol* 17:1109–1115.
- Jinn TL, Stone JM, Walker JC (2000) HAESA, an *Arabidopsis* leucine-rich repeat receptor kinase, controls floral organ abscission. *Genes Dev* 14:108–117.
- Deeken R, Kaldenhoff R (1997) Light-repressible receptor protein kinase: A novel photo-regulated gene from *Arabidopsis thaliana*. *Planta* 202:479–486.
- Chinchilla D, et al. (2007) A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defense. *Nature* 448:497–500.
- Heese A, et al. (2007) The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proc Natl Acad Sci USA* 104:12217–12222.
- Kemmerling B, et al. (2007) The BRI1-associated kinase 1, BAK1, has a brassinolide-independent role in plant cell-death control. *Curr Biol* 17:1116–1122.
- Shiu SH, Bleeker AB (2001) Receptor-like kinases from *Arabidopsis* form a monophyletic gene family related to animal receptor kinases. *Proc Natl Acad Sci USA* 98:10763–10768.
- Corbit KC, et al. (2003) Activation of Raf-1 signaling by protein kinase C through a mechanism involving Raf kinase inhibitory protein. *J Biol Chem* 278:13061–13068.
- Su W, Huber SC, Crawford NM (1996) Identification in vitro of a posttranslational regulatory site in the hinge 1 region of *Arabidopsis* nitrate reductase. *Plant Cell* 8:519–527.
- Savaldi-Goldstein S, Peto C, Chory J (2007) The epidermis both drives and restricts plant shoot growth. *Nature* 446:199–202.
- Reinhardt B, et al. (2007) Restoration of *DWF4* expression to the leaf margin of a *dwf4* mutant is sufficient to restore leaf shape but not size: The role of the margin in leaf development. *Plant J* 52:1094–1104.
- Clouse SD, Hall AF, Langford M, McMorris TC, Baker ME (1993) Physiological and molecular effects of brassinosteroids on *Arabidopsis thaliana*. *J Plant Growth Regul* 12:61–66.