Published in final edited form as:

Curr Opin Plant Biol. 2016 October ; 33: 92–100. doi:10.1016/j.pbi.2016.06.014.

Brassinosteroid signaling and BRI1 dynamics went underground

Yvon Jaillais^{#,1} and Grégory Vert^{#,2}

¹Laboratoire Reproduction et Développement des Plantes, Univ Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, F-69342, Lyon, France

²Institute for Integrative Biology of the Cell (I2BC), CNRS/CEA/Univ. Paris Sud, Université Paris-Saclay, 91198 Gif-sur-Yvette, France

Abstract

Brassinosteroids (BRs) are a group of steroid molecules perceived at the cell surface and that act as plant hormones. Since their discovery as crucial growth substances, BRs were mainly studied for their action in above ground organs and the BR signaling pathway was largely uncovered in the context of hypocotyl elongation. However, for the past two years, most of the exciting findings on BR signaling have been made using roots as a model. The Arabidopsis root is a system of choice for cell biology and allowed detailed characterization of BR perception at the cell membrane. In addition, a series of elegant articles dissected how BRs act in tissue specific manners to control root growth and development.

Introduction

Brassinosteroids (BRs) are perceived by combinatorial pairs of Receptor-Like Kinases (RLK) at the plasma membrane (PM). Binding of the ligand to the extracellular domain of BR-INSENSITIVE-1 (BRI1), BRI1-LIKE-1 (BRL1) and BRL3 triggers dimerization with BRI1-ASSOCIATED-KINASE-1 (BAK1) (or close family members from the SOMATIC-EMBRYOGENESIS-RECEPTOR-KINASE (SERK) family), which activates the *trans*-phosphorylation of their kinase domains. Activation of receptor complexes leads to a phosphorylation/de-phosphorylation cascade that inactivates GSK3 kinases from the BR-INSENSITIVE-2 (BIN2) family. This allows the accumulation of BRASSINAZOLE-RESISTANT-1 (BZR1) and BR-INSENSITIVE-EMS-SUPPRESSOR-1 (BES1) transcription factors in the nucleus where they bind to the promoter of thousands of genes and regulate their transcription (see recent reviews for more details on the molecular mechanism of BR signaling [1,2]). Although much progress has been made over the past two decades on the canonical BR signaling pathway, several key questions in the BR field have started to be addressed only recently. These include how BRs are dynamically perceived by their receptor complexes at the cell surface, and how specific developmental or environmental contexts trigger different genomic responses to control growth and development. Interestingly, most of the recent advances in the BR field arose from work performed using roots. Indeed, the root has become the go-to model to study subcellular

[#]corresponding authors: yvon.jaillais@ens-lyon.fr; Gregory.Vert@i2bc.paris-saclay.fr.

signaling mechanisms and here we highlight some recent findings made with this model notably on the regulation of the BR receptor complex. We also review the emerging roles of BR signaling in root development, focusing on its effects on 1) tissue differentiation and 2) the control of the root meristem size.

BRI1, a model RLK to study signaling dynamics

Building of a BR receptor complex at the cell surface

After intensive endoplasmic reticulum (ER)-mediated quality control of BRI1 folding and degradation of misfolded BRI1 by the ER-associated degradation machinery [3,4], BRI1 finds its way through the Golgi and the *trans*-Golgi Network/early endosome (TGN/EE) before cycling between the cell surface and brefeldin A (BFA)-sensitive endosomes (Figure 1) [5,6]. Efficient exocytosis and endosomal recycling of BRI1 to the cell surface was recently shown to require the vacuolar ATPase subunit DE-ETIOLATED3 (DET3) for proper acidification of TGN/EE compartments [7]. When BRI1 reaches the PM, BRI1 hetero-dimerizes with its co-receptor BAK1 in a ligand-dependent manner and initiates BR signaling, as elegantly established by a decade of genetic, biochemical, cell biological and structural studies (Figure 2) [1,8–13].

The formation of BRI1/BAK1 complex is regulated antagonistically from outside and inside of the cell. The BR ligand acts like a molecular glue that helps bringing together the extracellular domains of BRI1 and BAK1 in the cell wall (Figure 2) [10,12,13], although a certain proportion of preformed BRI1/BAK1 heterodimers may exist in the resting state [14]. The BRI1/BAK1 association is also regulated in the cytosol by BRI1-KINASE-INHIBITOR1 (BKI1), a cell autonomous negative regulator of BRI1 [15,16]. BKI1 is a non-structured protein that acts via two conserved linear motifs: a C-terminal peptide that binds the BRI1 kinase domain and a membrane hook that targets this protein to the PM (Figure 2) [15]. The BKI1 C-terminal tail is a peptide of 20 residues that is necessary and sufficient to bind to the BRI1 kinase domain [15]. In vitro, this C-terminal tail peptide inhibits the co-immunoprecipitation of BAK1 by BRI1 [15]. Although this remains to be directly shown, it is possible that BKI1 and BAK1 compete for interaction on the same binding-interface at the surface of BRI1 kinase [1,15]. Interestingly, ligand binding to the extracellular domain of BRI1 triggers the release of BKI1 from the PM [16], which presumably allows a tight interaction between BRI1 and BAK1 kinases and thereby activates downstream BR signaling [1]. BKI1 is targeted to the PM via its membrane hook, which consists of a repetition of doublets of dibasic residues (lysine and/or arginine) [15]. Following ligand perception, BRI1 rapidly phosphorylates BKI1 on a conserved tyrosine residue within its membrane hook. This triggers BKI1 release from the PM and allows BR signaling to take place (Figure 2) [15].

Removing BRI1 from the cell surface and vacuolar targeting

The mechanisms driving BRI1 internalization from the cell surface into endosomes are diverse and their relative spatial and temporal contribution to BRI1 endocytosis is still unclear due to our lack of knowledge on the plant endocytic machinery and to the limited resolution of the experimental approaches routinely employed. Several lines of evidence

however point to the requirement of clathrin-mediated endocytosis (CME) for BRI1 internalization (Figure 1). Genetic or pharmacological interference with clathrin cage formation or with the ADAPTOR-PROTEIN-2 (AP-2) endocytic adaptor builds up the PM pool of BRI1, blocks the uptake of fluorescently-labeled ligand, abolishes the sensitivity to BFA, and consequently leads to activation of the BR pathway [8,11,17]. The plant-specific TPLATE-MUNISCIN-LIKE (TML) protein, member of the TPLATE adaptor complex (TPC) and related to the metazoan F-BAR-DOMAIN-CONTAINING-FER/CIP4-HOMOLOGY-DOMAIN-ONLY (FCHo) CME nucleator, also appears important to mediate BRI1 endocytosis [18]. However, since TML and AP-2 have overlapping but also distinct functions [18], it will be important to decipher whether BRI1 is internalized in a common TML/AP-2-dependent manner and/or using the two separate pathways. Clathrin-independent endocytosis (CIE) recently emerged as an alternative to CME for retrieving BRI1 from the cell surface (Figure 1). High-resolution imaging shows significant overlap and co-diffusion of BRI1 with the AtFlot1 flotillin protein involved in PM microdomain formation [17]. Consistently, drugs impairing sterol-based microdomains or genetic interference with AtFlot1 affect the pace of BRI1 endocytosis and lead to increased BRI1 PM pools. Interestingly, BRs enhance the recruitment of BRI1 into PM microdomains and the proportion of BRI1 being endocytosed by flotillin-dependent endocytosis (Figure 1) [17]. The existence of two internalization pathways further complexifies our understanding of BRI1 dynamics and the roles of factors contributing to BRI1 endocytosis, such as the ARF-GEFs GNOM and GNOM-LIKE-1 [11], will have to be reinvestigated in the context of CME and CIE.

The molecular switch dictating the fate of BRI1 at the PM recently emerged with the findings that BRI1 carries polyubiquitin chains on several intracellular lysine residues (Figure 1) [19]. These chains are assembled using ubiquitin moieties linked by their lysine 63 and trigger proteasome-independent BRI1 degradation [19], similar to what has been shown for the ubiquitin-mediated endocytosis of the archetypal EPIDERMAL-GROWTH-FACTOR-RECEPTOR (EGFR) in mammals [20]. Expression of a non-ubiquitinatable BRI1, mutated for all ubiquitinated lysine residues, leads to increased BRI1 PM pools and to BR hypersensitivity, further establishing the cell surface as the primary site for BR signaling initiation. Tracking single endocytic events by Total Internal Reflection Fluorescence (TIRF) high-resolution imaging revealed that global loss of BRI1 ubiquitination does not abolish internalization but only slows it down, supporting the existence of ubiquitin-independent routes for BRI1 trafficking [19].

Overall, the diverse adaptors and pathways driving BRI1 dynamics may be differentially used depending on extracellular BR levels or cell types to alter the rate of endocytosis, to direct BRI1 towards a recycling or degradative fate to sustain or attenuate BR signaling, or to target BRI1 to different downstream partners offering variation around the BR theme. This is reminiscent of the multiple mechanisms driving the internalization of EGFR [20,21]. The current view of EGFR signaling/trafficking suggests that CME drives sustained EGFR signaling with little role on degradation across a wide range of EGF concentrations, while EGFR CIE would occur at high EGF levels to target activated EGFR for lysosomal turnover in a ubiquitin-dependent manner [21–23].

Regardless, the pool of BRI1 destined for degradation must be modified with K63 polyubiquitin chains (Figure 1) [19]. Ubiquitinated BRI1 is likely recognized by the Arabidopsis ENDOSOMAL-SORTING-COMPLEX-REQUIRED-FOR-TRANSPORT (ESCRT) to reach intraluminal vesicles (ILVs) from multivesicular bodies, as evidenced by BRI1 vacuolar targeting defects in the mutant for the ALIX ESCRT-III associated protein (Figure 1) [24]. Failure to ubiquitinate BRI1 or properly sort BRI1 into ILVs results in forced recycling to the cell surface [19,24].

Emerging roles of phosphoinositides in the regulation of the BR receptor complex

Phosphoinositides are well known to regulate receptor kinase signaling in animals [25]. The main phosphoinositide species, which are involved in receptor signaling in animals are PI(4,5)P₂ and PI(3,4,5)P₃ [26]. However, PI(3,4,5)P₃ does not exist in plants and PI(4,5)P₂ is present at very low concentrations in the PM [27–29]. By contrast, PI4P massively accumulates at the PM in plants [30]. Because of these differences, the potential role of PI4P in RLK signaling has largely been overlooked. Recent work highlighted the recruitment of BKI1 to the PM through electrostatic interactions with anionic phospholipids, in particular PI4P (Figure 2) [30]. PI4P accumulation at the PM generates net negative charges and thereby induces a local electrostatic potential that is different from other cellular membranes [30]. This electrostatic field in turn recruits proteins with polybasic linear motifs (such as the BKI1 membrane hook) or cationic domains to the PM. This model for BKI1 membrane recruitment very likely explains mechanistically why tyrosine phosphorylation of BKI1 triggers its dissociation from the PM (Figure 2). Tyrosine phosphorylation impacts the local net positive charge of BKI1 membrane hook, likely inducing an electrostatic switch leading to the repulsion of BKI1 from the membrane [31]. Similar electrostatic switches have been described in mammals for the Ras protein [32], however it remains to be determined if PI4P specifically regulates BR signaling or is a general regulator of RLK activities.

Root development and the emergence of new paradigms in BR signaling

BRs control the final length of the root in complex manners that are sometimes antagonistic (Figure 3) [2]. BRs impact root growth by modulating the elongation of differentiated cells [33], but also by modulating meristem size [34,35]. Indeed, BRs can either promote cell-cycle progression or cell differentiation, which have opposite effects on meristem size and final root length [34,35]. In addition, BRs also promote cell division in the Quiescent Center (QC) and the differentiation of distal stem cells (Figure 3) [34–38]. Expression of BRI1 or a constitutively active BZR1 (*bzr1-1D*) in the epidermis can rescue the short root meristem size of a *bri1* mutant [35,36]. However, only epidermis-specific BRI1 expression can rescue the QC division phenotype of *bri1* non-cell autonomously, while *bzr1-1D* cannot [35,36], suggesting that a signal is transmitted from the epidermis to inner tissues independently of BZR1 [35]. The molecular nature of this elusive signal is currently unknown, but might involve components of the cell wall integrity pathway, which is plugged into BR signaling via direct interaction between the RECEPTOR-LIKE-PROTEIN-44 (RLP44) and BAK1 (Figure 3) [39].

BR signaling and root cell differentiation

BR signaling is important for patterning the root epidermis into root hair cells (trichoblast) and non-root hair cells (atrachoblast) (Figure 4) [40,41]. BIN2 phosphorylates two transcription factors involved in epidermal cell patterning and that are specifically expressed in trichoblast: ENHANCER OF GLABRA3 (EGL3) and TRANSPARENT TESTA GLABRA1 (TTG1) [40]. Phosphorylation of TTG1 by BIN2 inhibits its transcriptional activity. On the other hand, BIN2 phosphorylates EGL3 within a putative Nuclear Localization Sequence (NLS), which inhibits its nuclear accumulation and may facilitate its movement from trichoblast into atrichoblast cells [40]. As such, low BR signaling stimulates trichoblast formation, while active BR signaling promotes atrichoblasts (Figure 4).

Surprisingly, BR signaling seems to have opposite effects on cell elongation in differentiated epidermal cell types (Figure 4): while trichoblast-specific expression of BRI1 promotes cell elongation in all differentiated tissues, its expression in atrichoblasts boosts ethylene production, which in turn inhibits cell elongation through deposition of crystalline cellulose [33]. In addition, BR signaling was shown to control the phloem:xylem differentiation ratio in shoots using the three BR receptors (BRI1, BRL1 and BRL3) [42]. In roots, inhibition of BR signaling in the *octopus (ops)* mutant leads to phloem discontinuity [43]. OPS is a PM protein specifically found in the phloem that binds and sequesters BIN2 at the PM (Figure 4) [43,44]. Because BIN2 inactivates BES1/BZR1 by phosphorylation in the nucleus [45], the sequestration of BIN2 by OPS specifically activates BR signaling in the phloem. As such, to our knowledge, OPS is the first cell-type specific regulator of BR signaling to be identified [43]. The BR-dependent mechanisms driving xylem differentiation in roots are still elusive but might resemble what has recently been uncovered in aerial parts. In cotyledons indeed, BRs control xylem differentiation through BES1 activation (Figure 4) [46]. However, a ligand/receptor pair called TRACHEARY-ELEMENT-DIFFERENTIATION-INHIBITORY-FACTOR (TDIF) and TDIF-RECEPTOR (TDR) antagonizes BR signaling in the procambium (Figure 4) [46]. TDIF is expressed in the phloem but perceived by TDR in the procambium, triggering the recruitment and activation of BIN2 by TDR [46,47]. BIN2 in turn phosphorylates and inactivates BES1, thereby preventing xylem differentiation (Figure 4) [46]. As such, the regulation of BES1/BZR1 by BIN2 is critical for phloem differentiation in the root and xylem differentiation in the shoot, although it is controlled by different pathways in these tissues. Whether the BIN2-mediated regulation of xylem differentiation demonstrated in cotyledons can be extended to roots remains an open question. Importantly, a direct interaction between TDIF and BIN2 has been reported in the context of lateral root initiation [47]. However in this case, BIN2 was proposed to act independently of BR signaling via the phosphorylation of AUXIN RESPONSE FACTORS (ARFs). Additional work is required to clarify the importance of BR-mediated BIN2 inactivation in the plethora of pathways BIN2 regulates [1].

BR signaling and the control of meristem size

Tissue specificity is also important for the BR control of meristem size, as reflected by BZR1-YFP expression pattern and subcellular localization, with high expression and nuclear localization in the epidermis of the transition/elongation zone (indicative of active BR signaling) and weaker cytoplasmic localization in the QC and surrounding initials (Figure 3)

[36]. In the QC, BZR1 and BES1 directly repress the expression of key root development regulators including BRASSINOSTEROIDS-AT-VASCULAR-AND-ORGANIZING-CENTER (BRAVO), a R2R3-MYB transcription factor, which represses QC division (Figure 3) [36,37]. BR signaling also induces the expression of ETHYLENE-RESPONSE-FACTOR-115 (ERF115), a positive regulator of QC division [48].

Transcriptomic profiling of BR-response genes in the root, together with analyses of direct BZR1 targets showed that BZR1 activates the transcription of its targets in the root transition/elongation zone, while it mainly represses genes in the QC and stem cells (Figure 3) [36]. In addition, this analysis suggests that BRs have antagonistic effects on gene expression compared to auxin, two hormonal pathways showing an inverted gradient of activity at the root tip (Figure 3) [36]. This observation came as a surprise, since BR and auxin are well documented to act synergistically in the shoot [1,49–52]. However, by analyzing BR genomic responses in a cell-type specific manner, Vragovic et al., (2015) nuanced this conclusion. Analysis of BR genomic responses in inner tissues of the meristem zone confirmed that BRs mostly repress genes [38]. On the other hand, they found that BR-induced genes in the epidermis are enriched in auxin-related genes and that auxin is required for BR-stimulated root growth in this tissue (Figure 3) [38]. This elegant study therefore illustrates how BR signaling can regulate different genomic and developmental programs based on tissue specific features, including different interaction with other hormonal pathways depending of the cellular context.

Conclusions and perspectives

Recent advances made on BRI1 activation and deactivation mechanisms in roots greatly complexify our view on the dynamics of BR perception. The knowledge gained on EGFR must certainly serve as a blueprint for dissecting the interplay between BRI1 dynamics and signaling, but differences are very likely to arise. The development of high-resolution imaging is slowly emerging in plants and will certainly provide invaluable insights into the spatial and temporal control of BRI1 internalization by CME and CIE, and into the relative contribution of endocytic-related proteins driving BRI1 endocytosis.

A wealth of new information is also rapidly accumulating on the role of BRs in root development. The emerging picture suggests that coherent root growth is balanced by opposite tissue-specific BR effects. Future challenges will aim at understanding how BR signaling in these different tissues contributes to both the robustness and the plasticity of root system architecture. For example, remodeling of the root system in response to phosphate starvation was recently shown to involve changes in BR metabolism and requires the activity of both BES1 and BZR1 [53]. Such studies on the role of BRs in environmental interactions, both on petri dishes but also on soil, are eagerly awaited. Finally, addressing the importance of BR-tissue specific signaling at the level of root system architecture and in the context of environmental interaction will be a major challenge in the coming years.

Acknowledgements

We thank Youssef Belkhadir, Matthieu Platre, Laïa Armengot, and Sara Martins for critical comments on the manuscript. We apologize to researchers whose work could not be cited here due to space limitations. Y.J. is funded

by grants from European Research Council (3363360-APPL) and Marie Curie Action (PCIG-GA-2011-303601); G.V. by grants from Marie Curie Action (PCIG-GA-2012-334021) and Agence Nationale de la Recherche (ANR-13-JSV2-0004-01).

References

1. Belkhadir Y, Jaillais Y. The molecular circuitry of brassinosteroid signaling. *New Phytol.* 2015; 206:522–540. [PubMed: 25615890]
2. Singh AP, Savaldi-Goldstein S. Growth control: brassinosteroid activity gets context. *J Exp Bot.* 2015; 66:1123–1132. [PubMed: 25673814]
3. Liu Y, Li J. Endoplasmic reticulum-mediated protein quality control in Arabidopsis. *Front Plant Sci.* 2014; 5:162. [PubMed: 24817869]
4. Liu Y, Zhang C, Wang D, Su W, Liu L, Wang M, Li J. EBS7 is a plant-specific component of a highly conserved endoplasmic reticulum-associated degradation system in Arabidopsis. *Proc Natl Acad Sci U S A.* 2015; 112:12205–12210. [PubMed: 26371323]
5. Geldner N, Hyman DL, Wang X, Schumacher K, Chory J. Endosomal signaling of plant steroid receptor kinase BRI1. *Genes Dev.* 2007; 21:1598–1602. [PubMed: 17578906]
6. Russinova E, Borst JW, Kwaaitaal M, Cano-Delgado A, Yin Y, Chory J, de Vries SC. Heterodimerization and endocytosis of Arabidopsis brassinosteroid receptors BRI1 and AtSERK3 (BAK1). *Plant Cell.* 2004; 16:3216–3229. [PubMed: 15548744]
7. Luo Y, Scholl S, Doering A, Zhang Y, Irani NG, Di Rubbo S, Neumetzler L, Krishnamoorthy P, Van Houtte I, Mylle E, et al. V-ATPase activity in the TGN/EE is required for exocytosis and recycling in Arabidopsis. *Nature Plants.* 2015; 1 [• This paper highlights the reduced ability of *det3* to acidify the TGN/EE compartments and links it to impaired secretion and recycling of BRI1 in the cell.]
8. Di Rubbo S, Irani NG, Kim SY, Xu ZY, Gadeyne A, Dejonghe W, Vanhoutte I, Persiau G, Eeckhout D, Simon S, et al. The clathrin adaptor complex AP-2 mediates endocytosis of brassinosteroid insensitive1 in Arabidopsis. *Plant Cell.* 2013; 25:2986–2997. [PubMed: 23975899]
9. Gou X, Yin H, He K, Du J, Yi J, Xu S, Lin H, Clouse SD, Li J. Genetic evidence for an indispensable role of somatic embryogenesis receptor kinases in brassinosteroid signaling. *PLoS Genet.* 2012; 8:e1002452. [PubMed: 22253607]
10. Hothorn M, Belkhadir Y, Dreux M, Dabi T, Noel JP, Wilson IA, Chory J. Structural basis of steroid hormone perception by the receptor kinase BRI1. *Nature.* 2011; 474:467–471. [PubMed: 21666665]
11. Irani NG, Di Rubbo S, Mylle E, Van den Begin J, Schneider-Pizon J, Hnilikova J, Sisa M, Buyst D, Vilarraza-Blasi J, Szatmari AM, et al. Fluorescent castasterone reveals BRI1 signaling from the plasma membrane. *Nat Chem Biol.* 2012; 8:583–589. [PubMed: 22561410]
12. Jaillais Y, Belkhadir Y, Balsemao-Pires E, Dangl JL, Chory J. Extracellular leucine-rich repeats as a platform for receptor/coreceptor complex formation. *Proc Natl Acad Sci U S A.* 2011; 108:8503–8507. [PubMed: 21464298]
13. Santiago J, Henzler C, Hothorn M. Molecular mechanism for plant steroid receptor activation by somatic embryogenesis co-receptor kinases. *Science.* 2013; 341:889–892. [PubMed: 23929946]
14. Bucherl CA, van Esse GW, Kruis A, Luchtenberg J, Westphal AH, Aker J, van Hoek A, Albrecht C, Borst JW, de Vries SC. Visualization of BRI1 and BAK1(SERK3) membrane receptor heterooligomers during brassinosteroid signaling. *Plant Physiol.* 2013; 162:1911–1925. [PubMed: 23796795]
15. Jaillais Y, Hothorn M, Belkhadir Y, Dabi T, Nimchuk ZL, Meyerowitz EM, Chory J. Tyrosine phosphorylation controls brassinosteroid receptor activation by triggering membrane release of its kinase inhibitor. *Genes Dev.* 2011; 25:232–237. [PubMed: 21289069]
16. Wang X, Chory J. Brassinosteroids regulate dissociation of BKI1, a negative regulator of BRI1 signaling, from the plasma membrane. *Science.* 2006; 313:1118–1122. [PubMed: 16857903]
17. Wang L, Li H, Lv X, Chen T, Li R, Xue Y, Jiang J, Jin B, Baluska F, Samaj J, et al. Spatiotemporal Dynamics of the BRI1 Receptor and its Regulation by Membrane Microdomains in Living Arabidopsis Cells. *Mol Plant.* 2015; 8:1334–1349. [PubMed: 25896454] [• The internalization of BRI1 is shown to be driven by both clathrin-dependent and microdomain-based processes, and ligand binding pushes BRI1 towards the clathrin-independent endocytic route.]

18. Gadeyne A, Sanchez-Rodriguez C, Vanneste S, Di Rubbo S, Zauber H, Vanneste K, Van Leene J, De Winne N, Eeckhout D, Persiau G, et al. The TPLATE adaptor complex drives clathrin-mediated endocytosis in plants. *Cell*. 2014; 156:691–704. [PubMed: 24529374]
19. Martins S, Dohmann EM, Cayrel A, Johnson A, Fischer W, Pojer F, Satiat-Jeunemaitre B, Jaillais Y, Chory J, Geldner N, et al. Internalization and vacuolar targeting of the brassinosteroid hormone receptor BRI1 are regulated by ubiquitination. *Nat Commun*. 2015; 6:6151. [PubMed: 25608221] [•• This study reports on a new post-translational modification controlling BRI1 and BR signaling. Ubiquitination of BRI1, under the form of K63 polyubiquitin chains, is shown to contribute to BRI1 internalization and to be crucial for BRI1 degradation in the vacuole.]
20. Goh LK, Huang F, Kim W, Gygi S, Sorkin A. Multiple mechanisms collectively regulate clathrin-mediated endocytosis of the epidermal growth factor receptor. *J Cell Biol*. 2010; 189:871–883. [PubMed: 20513767]
21. Sigismund S, Woelk T, Puri C, Maspero E, Tacchetti C, Transidico P, Di Fiore PP, Polo S. Clathrin-independent endocytosis of ubiquitinated cargos. *Proc Natl Acad Sci U S A*. 2005; 102:2760–2765. [PubMed: 15701692]
22. Sigismund S, Algisi V, Nappo G, Conte A, Pascolutti R, Cuomo A, Bonaldi T, Argenzio E, Verhoeve LG, Maspero E, et al. Threshold-controlled ubiquitination of the EGFR directs receptor fate. *EMBO J*. 2013; 32:2140–2157. [PubMed: 23799367]
23. Sigismund S, Argenzio E, Tosoni D, Cavallaro E, Polo S, Di Fiore PP. Clathrin-mediated internalization is essential for sustained EGFR signaling but dispensable for degradation. *Dev Cell*. 2008; 15:209–219. [PubMed: 18694561]
24. Cardona-Lopez X, Cuyas L, Marin E, Rajulu C, Irigoyen ML, Gil E, Puga MI, Bligny R, Nussaume L, Geldner N, et al. ESCRT-III-Associated Protein ALIX Mediates High-Affinity Phosphate Transporter Trafficking to Maintain Phosphate Homeostasis in Arabidopsis. *Plant Cell*. 2015; 27:2560–2581. [PubMed: 26342016]
25. Balla T. Phosphoinositides: tiny lipids with giant impact on cell regulation. *Physiol Rev*. 2013; 93:1019–1137. [PubMed: 23899561]
26. Lemmon MA. Membrane recognition by phospholipid-binding domains. *Nat Rev Mol Cell Biol*. 2008; 9:99–111. [PubMed: 18216767]
27. Munnik T, Nielsen E. Green light for polyphosphoinositide signals in plants. *Curr Opin Plant Biol*. 2011; 14:489–497. [PubMed: 21775194]
28. Platre MP, Jaillais Y. Guidelines for the Use of Protein Domains in Acidic Phospholipid Imaging. *Methods Mol Biol*. 2016; 1376:175–194. [PubMed: 26552684]
29. Simon ML, Platre MP, Assil S, van Wijk R, Chen WY, Chory J, Dreux M, Munnik T, Jaillais Y. A multi-colour/multi-affinity marker set to visualize phosphoinositide dynamics in Arabidopsis. *Plant J*. 2014; 77:322–337. [PubMed: 24147788]
30. Simon ML, Platre MP, Marques-Bueno MM, Armengot L, Stanislas T, Bayle V, Caillaud MC, Jaillais Y. A PtdIns(4)P-driven electrostatic field controls cell membrane identity and signalling in plants. *Nat Plants*. 2016; 2:16089. [PubMed: 27322096] [•• This study reveals that BK11 is targeted to the PM by the anionic phospholipid PI4P and that BK11 phosphorylation by BRI1 may trigger membrane dissociation through an electrostatic switch mechanism.]
31. McLaughlin S, Murray D. Plasma membrane phosphoinositide organization by protein electrostatics. *Nature*. 2005; 438:605–611. [PubMed: 16319880]
32. Ahearn IM, Haigis K, Bar-Sagi D, Philips MR. Regulating the regulator: post-translational modification of RAS. *Nat Rev Mol Cell Biol*. 2012; 13:39–51.
33. Fridman Y, Elkouby L, Holland N, Vragovic K, Elbaum R, Savaldi-Goldstein S. Root growth is modulated by differential hormonal sensitivity in neighboring cells. *Genes Dev*. 2014; 28:912–920. [PubMed: 24736847]
34. Gonzalez-Garcia MP, Vilarrosa-Blasi J, Zhiponova M, Divol F, Mora-Garcia S, Russinova E, Cano-Delgado AI. Brassinosteroids control meristem size by promoting cell cycle progression in Arabidopsis roots. *Development*. 2011; 138:849–859. [PubMed: 21270057]
35. Hacham Y, Holland N, Butterfield C, Ubeda-Tomas S, Bennett MJ, Chory J, Savaldi-Goldstein S. Brassinosteroid perception in the epidermis controls root meristem size. *Development*. 2011; 138:839–848. [PubMed: 21270053]

36. Chaiwanon J, Wang ZY. Spatiotemporal brassinosteroid signaling and antagonism with auxin pattern stem cell dynamics in *Arabidopsis* roots. *Curr Biol*. 2015; 25:1031–1042. [PubMed: 25866388] [• Besides the well-known synergism displayed by BRs and auxin in shoots, this paper unravels the complex antagonism of the two hormones in roots to regulate cell elongation and root growth. This paper also reveals patterned-BR signaling in roots, which is mainly regulated by BR catabolism.]
37. Vilarrasa-Blasi J, Gonzalez-Garcia MP, Frigola D, Fabregas N, Alexiou KG, Lopez-Bigas N, Rivas S, Jauneau A, Lohmann JU, Benfey PN, et al. Regulation of plant stem cell quiescence by a brassinosteroid signaling module. *Dev Cell*. 2014; 30:36–47. [PubMed: 24981610]
38. Vragovic K, Sela A, Friedlander-Shani L, Fridman Y, Hacham Y, Holland N, Bartom E, Mockler TC, Savaldi-Goldstein S. Translatome analyses capture of opposing tissue-specific brassinosteroid signals orchestrating root meristem differentiation. *Proc Natl Acad Sci U S A*. 2015; 112:923–928. [PubMed: 25561530] [•• Spatiotemporal translatomes of *Arabidopsis* roots reveal tissue-specific responses in gene expression to brassinosteroids and highlights the contribution of different root cell layers to growth. This paper also reveals how BRs orchestrate root growth by regulating locally auxin production and transport, which then act non-autonomously across the different root tissues.]
39. Wolf S, van der Does D, Ladwig F, Sticht C, Kolbeck A, Schurholz AK, Augustin S, Keinath N, Rausch T, Greiner S, et al. A receptor-like protein mediates the response to pectin modification by activating brassinosteroid signaling. *Proc Natl Acad Sci U S A*. 2014; 111:15261–15266. [PubMed: 25288746]
40. Cheng Y, Zhu W, Chen Y, Ito S, Asami T, Wang X. Brassinosteroids control root epidermal cell fate via direct regulation of a MYB-bHLH-WD40 complex by GSK3-like kinases. *Elife*. 2014
41. Kuppusamy KT, Chen AY, Nemhauser JL. Steroids are required for epidermal cell fate establishment in *Arabidopsis* roots. *Proc Natl Acad Sci U S A*. 2009; 106:8073–8076. [PubMed: 19416891]
42. Cano-Delgado A, Yin Y, Yu C, Vafeados D, Mora-Garcia S, Cheng JC, Nam KH, Li J, Chory J. BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in *Arabidopsis*. *Development*. 2004; 131:5341–5351. [PubMed: 15486337]
43. Anne P, Azzopardi M, Gissot L, Beaubiat S, Hematy K, Palauqui JC. OCTOPUS Negatively Regulates BIN2 to Control Phloem Differentiation in *Arabidopsis thaliana*. *Curr Biol*. 2015; 25:2584–2590. [PubMed: 26387715] [•• This study reports that OPS acts as a cell-type specific modulator of BR signaling in the phloem, by sequestrating the negative regulator BIN2 at the plasma membrane, thereby activating BR signaling in this tissue.]
44. Truernit E, Bauby H, Belcram K, Barthelemy J, Palauqui JC. OCTOPUS, a polarly localised membrane-associated protein, regulates phloem differentiation entry in *Arabidopsis thaliana*. *Development*. 2012; 139:1306–1315. [PubMed: 22395740]
45. Vert G, Chory J. Downstream nuclear events in brassinosteroid signalling. *Nature*. 2006; 441:96–100. [PubMed: 16672972]
46. Kondo Y, Ito T, Nakagami H, Hirakawa Y, Saito M, Tamaki T, Shirasu K, Fukuda H. Plant GSK3 proteins regulate xylem cell differentiation downstream of TDIF-TDR signalling. *Nat Commun*. 2014; 5:3504. [PubMed: 24662460]
47. Cho H, Ryu H, Rho S, Hill K, Smith S, Audenaert D, Park J, Han S, Beeckman T, Bennett MJ, et al. A secreted peptide acts on BIN2-mediated phosphorylation of ARFs to potentiate auxin response during lateral root development. *Nat Cell Biol*. 2014; 16:66–76. [PubMed: 24362628]
48. Heyman J, Cools T, Vandenbussche F, Heyndrickx KS, Van Leene J, Vercauteren I, Vanderauwera S, Vandepoele K, De Jaeger G, Van Der Straeten D, et al. ERF115 controls root quiescent center cell division and stem cell replenishment. *Science*. 2013; 342:860–863. [PubMed: 24158907]
49. Jaillais Y, Chory J. Unraveling the paradoxes of plant hormone signaling integration. *Nat Struct Mol Biol*. 2010; 17:642–645. [PubMed: 20520656]
50. Nemhauser JL, Mockler TC, Chory J. Interdependency of brassinosteroid and auxin signaling in *Arabidopsis*. *PLoS Biol*. 2004; 2:E258. [PubMed: 15328536]
51. Vert G, Chory J. Crosstalk in cellular signaling: background noise or the real thing? *Dev Cell*. 2011; 21:985–991. [PubMed: 22172668]

52. Vert G, Walcher CL, Chory J, Nemhauser JL. Integration of auxin and brassinosteroid pathways by Auxin Response Factor 2. *Proc Natl Acad Sci U S A*. 2008; 105:9829–9834. [PubMed: 18599455]
53. Singh AP, Fridman Y, Friedlander-Shani L, Tarkowska D, Strnad M, Savaldi-Goldstein S. Activity of the brassinosteroid transcription factors BRASSINAZOLE RESISTANT1 and BRASSINOSTEROID INSENSITIVE1-ETHYL METHANESULFONATE-SUPPRESSOR1/BRASSINAZOLE RESISTANT2 blocks developmental reprogramming in response to low phosphate availability. *Plant Physiol*. 2014; 166:678–688. [PubMed: 25136063]

Figure 1. BRI1 trafficking to and from the cell surface.

ER, endoplasmic reticulum; TGN/EE, trans-Golgi network/early endosomes; MVB, multivesicular body; ERQC, ER quality control protein response; ERAD, ER-associated degradation; BRI1, BRASSINOSTEROID-INSENSITIVE-1; BAK1, BRI1-ASSOCIATED-KINASE-1; LRR, Leucine-Rich Repeat; ESCRT, ENDOSOMAL-SORTING-COMPLEX-REQUIRED-FOR-TRANSPORT; TPC, TPLATE-COMPLEX; AP-2, ADAPTOR-COMPLEX-2; Ub, K63-linked polyubiquitination. Question marks, ubiquitination may drive clathrin-dependent and/or -independent BRI1 endocytosis. Black arrows represent

trafficking pathways, green and purple arrows indicate the recruitment of BRI1 into CME and CIE internalization pathways, respectively. Note that although CIE opens an interesting new window to dissect BRI1 trafficking, to date CME remains the main demonstrated internalization route of BRI1.

Figure 2. Regulation of BR receptor complex activation by the antagonistic action of BRs and BKI1.

Left, inhibited BRI1 receptor in the absence of ligand. Note that BAK1 may interact with BRI1 in this context, but has not been drawn for sake of clarity. Right, ligand-activated receptor complex. PM, Plasma Membrane; BRI1, BRASINOSTEROID-INSENSITIVE-1; BAK1, BRI1-ASSOCIATED-KINASE-1; BKI1, BRI1-KINASE-INHIBITOR-1; PI4P, phosphatidylinositol 4-phosphate; [KR], Lysine and/or Arginine doublet. Kinase domains are represented by kidney-shape figures, the juxtamembrane segments are in orange, activation loops in red and C-terminal tails in brown. The area highlighted in red at the

bottom of BRI1 kinase domain corresponds to the BKI1-binding surface and putative BAK1 interaction area. Phosphorylated residues are represented by orange circle labeled with the letter P, and lipid phosphorylation by yellow circles.

Figure 3. Tissue-specific features of BR-regulated root meristem size.

Left, schematic representation of an Arabidopsis root tip longitudinal section. Arrows indicate activation, blunt-ended lines indicate inhibition and bullet-ended lines indicate the different zone of the root. BZR1 expression pattern and subcellular localization is represented in yellow as indicated in the box at the bottom left corner. BES1, BR-INSENSITIVE-EMS-SUPPRESSOR-1; BZR1, BRASSINAZOLE-RESISTANT-1; BRAVO, BRASSINOSTEROIDS-AT-VASCULAR-AND-ORGANIZING-CENTER; ERF115, ETHYLENE-RESPONSE-FACTOR-115; QC, Quiescent Center.

Figure 4. Role of BR signaling in root tissue patterning.

Top left, schematic representation of an Arabidopsis root tip transversal section. Top right, regulation of BIN2 activity by OPS in phloem, TDR in cambium and BR signaling in xylem. Note that the role of BRs in the regulation of xylem differentiation has only been demonstrated in aerial parts. Bottom, regulation of TTG1 and EGL3 by BIN2 to control root epidermal patterning. Note that BR signaling is not specific of trichoblast cells and occurs in both cell types. As such, modulation of BR signaling and/or biosynthesis might control the atrichoblast/trichoblast ratio. This regulation might therefore be relevant during environmental interactions since root hair production is highly constrained by the root environment. Arrows and blunt-ended lines indicate inhibition and activation, respectively.

Red crosses represent the release of BES1/BZR1 inhibition by BIN2 inactivation. BRs, Brassinosteroids; BRI1, BR-INSENSITIVE-1; BRLs, BRI1-LIKEs; BES1, BR-INSENSITIVE-EMS-SUPPRESSOR-1; BZR1, BRASSINAZOLE-RESISTANT-1; BIN2, BR-INSENSITIVE-2; OPS, OCTOPUS; TDIF, TRACHEARY-ELEMENT-DIFFERENTIATION-INHIBITORY-FACTOR; TDR, TDIF-RECEPTOR; pBES1/pBZR1, phosphorylated form of BES1/BZR1; TTG1, TRANSPARENT-TESTA-GLABRA1; EGL3, ENHANCER-OF-GLABRA3.