



HAL
open science

The cell surface is the place to be for brassinosteroid perception and responses

Charlotte Delesalle, Grégory Vert, Satoshi Fujita

► **To cite this version:**

Charlotte Delesalle, Grégory Vert, Satoshi Fujita. The cell surface is the place to be for brassinosteroid perception and responses. *Nature Plants*, 2024, 10 (2), pp.206-218. 10.1038/s41477-024-01621-2 . hal-04527873

HAL Id: hal-04527873

<https://hal.science/hal-04527873>

Submitted on 31 Mar 2024

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **THE CELL SURFACE IS THE PLACE TO BE FOR BRASSINOSTEROID**
2 **PERCEPTION AND RESPONSES**

3

4 Charlotte Delesalle¹, Grégory Vert¹ and Satoshi Fujita^{1*}

5

6 ¹ Plant Science Research Laboratory (LRSV), UMR5546 CNRS/Université Toulouse 3, 24
7 chemin de Borde Rouge, 31320 Auzeville Tolosane, France

8 * For correspondence: satoshi.fujita@univ-tlse3.fr

9

10

11

12

13

14 **Abstract**

15 Adjusting the micro-environment around the cell surface is critical to respond to external cues
16 or endogenous signals and to maintain cell activities. In plant cells, the plasma membrane is
17 covered by the cell wall and scaffolded with cytoskeletal networks, which altogether compose
18 the cell surface. It has long been known that these structures mutually interact, but the
19 mechanisms that integrate the whole system are still obscure. Here, we spotlight the
20 brassinosteroid (BR) plant hormone receptor BRI1 since it represents an outstanding model for
21 understanding cell surface signaling and regulation. We summarize how BRI1 activity and
22 dynamics are controlled by plasma membrane components and their associated factors to fine-
23 tune signaling. The downstream signals, in turn, manipulate cell surface structures by
24 transcriptional and post-translational mechanisms. Moreover, the changes in these
25 architectures impact BR signaling, resulting in a feedback loop formation. This review will
26 discuss how BRI1 and BR signaling function as central hubs to integrate cell surface regulation.

27

28 **Keywords**

29 Brassinosteroid receptor (BRI1) / Brassinosteroid signaling / Cell surface regulation / Post-
30 translational modification / Cytoskeletons / **Cell wall** / Feedback loop / Cell growth

31

32 Introduction

33 The plasma membrane (PM) provides a unique barrier and interface for exchanging chemicals
34 or information between extra- and intra-cellular environments to maintain cellular homeostasis.
35 These contradictory functions of the PM are supported by a water-impermeable lipid bilayer
36 and numerous membrane-integral or -surface proteins that facilitate the exchange of molecules
37 or signals. Cell surface receptors, such as G-protein coupled receptors (GPCRs), receptor
38 tyrosine kinases (RTKs), or receptor-like kinases (RLKs), are representative classes of
39 transmembrane proteins that play a crucial role in signal transduction at the cell surface.
40 Animals expanded GPCR and RTK families (over 800 GPCRs¹ and 58 RTKs² in humans),
41 while plants developed a wide variety of RLKs (over 600 RLK-family genes in *Arabidopsis*³
42 and over 1100 RLK-family genes in rice⁴). Among them, the brassinosteroid (BR) hormone
43 receptor BRASSINOSTEROID INSENSITIVE1 (BRI1)⁵ has been widely used as a model
44 receptor to better understand the molecular mechanisms of signal transduction in plants and its
45 interplay with the cell surface.

46

47 BRs are a group of polyhydroxylated sterols that show phytohormonal activities in many
48 aspects of the plant life cycle. For instance, *Arabidopsis bril* or other BR-defective mutants
49 show extreme dwarfism^{5,6}, cell proliferation defects⁷, abnormal vascular tissue differentiation⁸,
50 or impaired male fertility^{9,10}. Not limited to developmental or reproductive defects, BR-related
51 mutants also display impaired resistance to biotic and abiotic stresses^{11,12}. Similar to what is
52 observed in dicots, BRs also play significant roles in monocots. Several rice BR biosynthetic
53 mutants indeed show dwarf and fertility-related phenotypes^{13,14}. Null mutation of the rice
54 ortholog *OsBR11* leads to a severe defect in organ growth in shoots but a mild effect in root
55 growth, most likely due to the strong expression of its homologs¹⁵. A genetic screen in wheat
56 also identified a high-yield semi-dwarf strain, which is impaired in BR signaling, corroborating
57 the high importance of BR signaling in agriculture¹⁶.

58

59 BRI1 belongs to the LRR-RLK (leucine-rich repeat receptor kinase) subfamily X and plays a
60 central role in triggering BR signaling upon BR perception. BRI1 has both Ser/Thr and Tyr
61 kinase activities, and both enzymatic activities are critical for its functions^{17,18}. As already
62 reviewed in many articles¹⁹⁻²¹, the BR signaling pathway is one of the best-characterized
63 pathways in plant receptor signaling, particularly in the control of genomic responses. In the
64 absence of BRs, BIN2 (BRASSINOSTEROID INSENSITIVE 2) kinase²² suppresses BR-

65 triggered transcriptional responses by phosphorylating the transcription factors BES1 and
66 BZR1 (BRASSINOSTEROID-INSENSITIVE EMS SUPPRESSOR1 and BRASSINAZOLE
67 RESISTANT 1)^{23,24}. This phosphorylation has been shown to control BES1 and BZR1 at
68 different levels, all preventing downstream genomic responses. These include the inability to
69 bind target promoters²⁵, nuclear export/cytoplasmic retention²⁶⁻²⁹, or proteasome-dependent
70 degradation³⁰. The presence of BRs induces the formation of the BRI1-BAK1 (BRI1
71 ASSOCIATED KINASE, from LRR-RLK subfamily II) receptor complex³¹⁻³³. Full activation
72 of the BRI1-BAK1 complex allows signal transduction to BSKs (BR-SIGNALING KINASEs)
73 and CDGs (CONSTITUTIVE DIFFERENTIAL GROWTH proteins) RLCKs (receptor-like
74 cytoplasmic kinases)³⁴⁻³⁶. These RLCKs, in turn, phosphorylate and activate the BSU1 (BRI1
75 SUPPRESSOR 1) phosphatase that dephosphorylates BIN2, leading to BIN2 deactivation and
76 destabilization^{37,38}. Consequently, BES1 and BZR1 transcription factors are dephosphorylated
77 by PP2A phosphatases³⁹, resulting in their accumulation in the nucleus where they control the
78 transcription of thousands of BR-responsive genes^{25,27,28,38,40-42}. The intricate mechanism of
79 BR signal transduction from the cell surface to the nucleus is very well understood, but how
80 BR signaling regulates cell surface components or structures remains quite elusive compared
81 to other RLK signaling⁴³⁻⁴⁷. Recent advances shed light on how BR signaling impacts the
82 membrane surface environment and how cell surface cues regulate BRI1 and BR signaling.
83 This review will focus on the dynamics of BRI1 at the PM and the mutual relationships between
84 BR signaling and cell surface structures, such as cortical microtubules (MTs) and the cell wall.

85

86 **BRI1 regulation at the cell surface**

87 *Quantitative control of BRI1 at the PM*

88 BRI1 spans over diverse subcellular compartments, including PM, early endosomes (EE),
89 multivesicular bodies (MVB), and the vacuolar lumen⁴⁸. It has already been shown in animals
90 that the spatial distribution of membrane-bound receptors within cells is a critical determinant
91 of signaling activity⁴⁹. Several studies demonstrated that stabilization of BRI1 at the cell
92 surface by genetic perturbation yields hypersensitivity to BRs both in terms of physiological
93 signatures and BES1 phosphorylation status⁵⁰⁻⁵³, supporting the idea that BRI1 initiates
94 signaling from the PM. Only one pharmacological approach where BRI1 is trapped into
95 endosome-containing cellular structures suggested that BRI1 could fire from endosomes, but
96 this hypothesis lacks physiological evidence⁵⁴.

97 Intracellular trafficking appears to be one of the major ways to adjust the PM pool of BRI1.
98 BRI1 is believed to be constitutively internalized from the PM to EE in a ligand-independent
99 manner together with BAK1 since a BRI1 variant unable to bind BRs still shows comparable
100 internalization to the wild-type form⁵⁴⁻⁵⁶ (**Fig. 1a**), while some other receptors, such as FLS2
101 (FLAGELLIN INSENSITIVE 2) and SGN3 (SCHENGEN 3, also called GSO1 (GASSHO1)),
102 show ligand-induced endocytosis^{57,58}. This concept, however, remains obscure for BRI1
103 considering that several mechanisms driving BRI1 endocytosis, such as ubiquitination or the
104 recruitment of the E3 ligases PUB12/13 (Plant U-box type E3 ligase 12 and 13), appear to be
105 themselves BR-regulated^{51,59}.

106 Recent studies highlighted the numerous components involved in BRI1 endocytic machinery.
107 A pair of SNARE (Soluble N-ethylmaleimide sensitive factor attachment protein receptors)
108 family proteins, SYP22 (Q-SNARE) and VAMP727 (R-SNARE), regulates the PM pool of
109 BRI1⁶⁰. BRI1 endocytosis is executed by the AP-2- or T-PLATE-dependent clathrin-mediated
110 endocytosis (CME)^{50,61}, and the clathrin-independent pathway through FLOT1 (flotillin1)⁶²
111 (**Fig. 1a**). A recent report showed that a Tyr motif (YXXΦ, where Y stands for Tyr, X is any
112 amino acid, and Φ is a bulky hydrophobic residue) of BRI1 was critical for the CME-dependent
113 internalization through the interaction with an adaptor AP-2 subunit AP2M, similar to what has
114 also been characterized for plant nutrient transporters^{52,63}.

115 One important mechanism leading to BRI1 receptor internalization is polyubiquitination (**Fig.**
116 **1b**). More precisely, endocytosis of BRI1 largely relies on Lys63 (K63) polyubiquitination⁵¹,
117 as seen for other PM proteins⁶⁴⁻⁶⁶. The PUB12/13 E3 ubiquitin ligases and UBC35/36
118 (UBIQUITIN CONJUGATING ENZYMES 35 and 36) E2s have been demonstrated as main
119 actors of BRI1 Lys63 polyubiquitination *in vivo* and *in vitro*^{53,59,65}. *In vitro* experiments
120 showed that PUB13 phosphorylation by BRI1 is necessary for activating its ubiquitin ligase
121 activity⁵⁹. This result is consistent with the fact that the BRI1 kinase activity and its co-receptor
122 BAK1 are required for BRI1 constitutive ubiquitination and that BR depletion decreases BRI1
123 ubiquitination^{51,59}. So far, the precise ubiquitination sites on BRI1 are yet to be identified, but
124 introducing 25 Lys mutations in a BRI1 cytosolic domain severely abolished its internalization
125 and degradation, and yielded hyper-stimulated BR signaling⁵¹. Furthermore, BRI1 Lys63
126 polyubiquitination is reversibly removed by deubiquitination enzymes UBP12/13 (Ubiquitin
127 specific protease 12 and 13), enabling fine-tuning the degree of receptor polyubiquitination and
128 endocytosis⁶⁷ (**Fig. 1b**). These factors coordinately dictate the levels of active BRI1 at the cell
129 surface. Interestingly, PUB12/13 components also significantly impact the polyubiquitination

130 of the immune signaling FLS2 receptor⁶⁸, but the precise mechanisms determining the level of
131 ubiquitination for distinct receptors through PUB12/13 remain to be clarified.

132 A remarkable example of quantitative control of BRI1 at the PM takes place under higher
133 ambient growth temperatures. Elevated ambient temperature downregulates BR signal by
134 ubiquitin-mediated selective BRI1 degradation, which tunes root cell elongation under high
135 temperature condition^{69,70}. Recent work, however, revealed the interplay between
136 ubiquitination and another post-translational modification targeting BRI1, SUMOylation
137 (SMALL UBIQUITIN MODIFIER), in the control of BRI1 at warmer temperatures⁷⁰ (**Fig. 1b**).
138 Interestingly, SUMO was also reported in the case of FLS2⁷¹. *In vitro* experiments showed that
139 the SCE1 E2 SUMO conjugation enzyme SUMOylated FLS2, and the DESUMOYLATING
140 ISOPEPTIDASE 3a (Desi3a) SUMO protease removes SUMO from FLS2. *In planta*, the
141 SUMOylation level of FLS2 is increased in an flg22-dependent manner by degrading Desi3a,
142 which unleashes BIK1 (BOTRYTIS-INDUCED KINASE1) from the FLS2-BAK1 complex
143 and activates downstream pathways⁷¹. BRI1 is also SUMOylated *in planta*, but the identity of
144 the SUMO E2 or E3 catalyzing the SUMO conjugation to BRI1 is still uncharacterized.
145 Elevated temperature induces Desi3a protein accumulation, recruitment to BRI1, and BRI1
146 deSUMOylation⁷⁰. DeSUMOylated BRI1 shows higher affinity to BIK1, a negative regulator
147 of BR signaling⁷², and promotes BRI1 internalization. Both mechanisms limit the activation of
148 BRI1 upon elevated temperature and prevent an over-elongation of hypocotyls under such
149 conditions. An attractive possibility is that SUMOylation counteracts ubiquitination, as
150 SUMOylation sites on BRI1 likely overlap with ubiquitination targets, allowing plants to
151 modulate BR sensitivity in response to warmth. This regulatory mechanism represents an
152 excellent model for understanding how multi-posttranslational modifications compete,
153 converge, and determine BRI1 abundance at the PM.

154

155 *Cell surface nanodomains and BR signaling*

156 Receptor-like kinases are embedded in the PM where uncountable components meet⁷³. Recent
157 advances in microscopic techniques unveiled that specific proteins or lipids form nanodomains
158 at the PM within a sub-micrometer order. A prominent example is ROP6 nanodomain
159 formation upon auxin or hyperosmotic stress stimulation, leading to downstream activation^{74,75}.
160 BRI1 also forms nanodomains at the PM, serving as hot spots for flotillin-dependent
161 endocytosis⁶². Pharmacological disruption of sterol or sphingolipid distribution attenuates

162 BRI1 endocytosis and BES1 dephosphorylation, implying these membrane components are
163 crucial for BRI1 function by stabilizing nanodomain formation⁶². The BRI1 co-receptor, BAK1,
164 also forms nanodomains at the PM, but its relevance for BR signaling remains to be
165 characterized⁷⁶.

166 Some components in the BR pathways, like BIK1, are indeed shared by immune signaling
167 receptors, such as FLS2 or EFR^{72,77-79}, enabling plants to cross-regulate each pathway and to
168 provide trade-off mechanisms between defense and growth^{72,80,81}. A major question arises in
169 such a context: how do plants disentangle the different signaling pathways by sharing the same
170 components? Part of the answer relies on nanodomains that spatially separate signaling
171 complexes to avoid unfavorable signal interference (**Fig. 1c**). Single particle analysis by
172 variable angle epifluorescence microscopy (VAEM) revealed that BRI1 and FLS2 reside, in
173 fact, in distinct nanoclusters within the PM⁸². These particles show different dynamics at the
174 PM, and of note, the BRI1-BIK1 complex in nanodomains tends to localize along cortical MTs,
175 while the FLS2-BIK1 complex appears to be in distinct nanodomains with no correlation with
176 MTs (**Fig. 1c**). This observation might give us a clue to grasp how plants regulate distinct
177 signaling pathways using the same components. Similarly, BSK1 forms nanodomains with
178 BRI1 or FLS2, and the complexes behave differently after stimulation by corresponding
179 ligands⁸³. Overall, nanodomain formation now emerges as a critical step to emit different
180 outputs while sharing the same signaling components.

181

182 *Scaffolding function in BR signaling*

183 Quantitative proteomics estimated that 25.8 billion protein molecules are found in one plant
184 mesophyll cell, and 3.2 billion protein molecules exist in its cytosol⁷³. Under such crowded
185 conditions, gathering signaling components by scaffolding proteins or in nanodomains allows
186 them to initiate quick, efficient, and precise signal transduction from the PM to the nucleus, as
187 seen in yeast or humans⁸⁴. In plants, only a few examples are found for signal scaffolding
188 proteins, but the TTL (TETRATRICOPEPTIDE THIOREDOXIN-LIKE) family proteins⁸⁵
189 and BSK3 (BR-SIGNALING KINASE 3)⁸⁶ serve such a function in BR signaling. *ttl3* and
190 *bsk3* show hyposensitivity to BRs, supporting the positive roles of TTLs and BSK3 in BR
191 signaling^{85,86}. Mechanistically, both interact with central molecules in BR signaling, such as
192 BRI1, BSK1, BSU1, BIN2, and BZR1 for TTL3^{85,86}, enabling smooth signal relay. TTL
193 proteins interact with these components through TPR (TETRATRICOPEPTIDE REPEAT)
194 domains⁸⁵, while TPR domains in BSK3 are not required for its scaffolding activity, except

195 hetero/homo-oligomerization with BSK1/3⁸⁶. This is consistent with the fact that TPRs are not
196 absolutely required for its functions since a TRP-deleted BSK3 construct could complement
197 *bks3*⁸⁶. BR signaling enhances this scaffolding mechanism by upregulating *TTL* transcription
198 and promoting relocation from the cytosol to the PM⁸⁵.

199 TTL1, 3, and 4 proteins were also recently reported as CESA1 (Cellulose synthase A1)
200 interactors and confer abiotic stress tolerance to plants by maintaining CESAs at the PM during
201 stress^{87,88}. Therefore, TTL family scaffolding proteins also offer a chance for a deeper
202 understanding of a crossing point of stress response and BR signaling.

203

204 *Activity regulation by homo and heterodimerization of BRI1 and interaction with other* 205 *regulators*

206 Besides the quantitative regulation of BRI1 at the PM, BRI1 is also tightly controlled at the
207 activity level. BRI1 was early demonstrated to undergo BR-dependent homodimerization using
208 *in planta* biochemical approaches⁸⁹. BRI1 homodimerization is further supported by *in vitro*
209 data where purified BRI1 cytoplasmic kinase domain can homodimerize¹⁸, but not the
210 extracellular domain *in vitro*, regardless of the presence of ligands⁹⁰. Additionally, a BR-
211 independent homodimerization of BRI1 was brought to light using imaging analysis^{62,91}. BRI1
212 also possesses an autoinhibitory domain in its C-terminal domain. This autoinhibitory lock is
213 released by BR-induced inter-molecular phosphorylation of the BRI1 C-ter domain, suggesting
214 BRI1 homodimerization might be important for this regulation^{89,92}. Another
215 autophosphorylation site, Ser891, in the ATP-binding domain strongly suppresses the auto-
216 phosphorylation on Tyr residues, resulting in BR signal inhibition⁹³.

217 The BRI1-BAK1 complex formation represents another important regulatory mechanism.
218 BAK1 is sequestered away from BRI1 by BIR3 (BAK1-INTERACTING RECEPTOR-LIKE
219 KINASE 3) in the absence of ligand, as also observed for other BAK1 partners⁹⁴⁻⁹⁶ (**Fig. 1a**).
220 Ligand binding to BRI1 shifts the affinity of BAK1 toward BRI1 and allows BAK1 to form
221 the BR receptor complex with BRI1⁹⁷. Interestingly, BIR3 also binds to BRI1, arguing for the
222 existence of additional BIR3-dependent regulatory mechanisms targeting BRI1⁹⁸.

223 BRI1 interactors such as BKI1 (BRI1 KINASE INHIBITOR 1), a MEMBRANE-
224 ASSOCIATED KINASE REGULATOR (MAKR) family protein, also participate in the
225 confinement of BRI1 activity⁹⁹ (**Fig. 1a**). BKI1 prevents the complex formation of BRI1 and
226 BAK1, and its inhibitory activity largely depends on its association with PM¹⁰⁰. Four doublets
227 of KR (Lys-Arg) motifs of BKI1 ensure electrostatic interaction with specific lipids of the PM
228 in a “membrane hook” domain^{100,101}. Upon BR perception by BRI1, BKI1 is phosphorylated

229 by BRI1 on a tyrosine next to one KR motif and quickly disassociates from the PM, unlocking
230 the BR signaling pathway activation^{99,100}. Furthermore, a recent report demonstrated that an
231 evolutionary-conserved RING-type E3 Ub ligase in plants, namely ZnF, induces degradation
232 of the wheat TaBKI1, resulting in activating the downstream pathway¹⁶. Other RLK proteins
233 are known to be regulated either positively or negatively by MAKR family proteins^{102,103},
234 including the BKI1-interacting partner ER (ERECTA). BKI1 inhibits ER kinase activity and
235 disassociates from ER upon BR signaling activation, but not after EPF1/2 (EPIDERMAL
236 PATTERNING FACTOR 1/2) perception by ER. This mechanism adds one more complexity
237 to plant architecture development¹⁰⁴.

238 After the activation of the BR pathway, a PP2A phosphatase complex attenuates BR signaling
239 by dephosphorylating BRI1, as attested by PP2A mutant and overexpression phenotypes^{105,106}.
240 Of note, PP2A localization is regulated by reversible methylation on an A subunit of PP2A
241 through SBI1 (SUPPRESSOR OF BRI1) leucine carboxylmethyltransferase activity. Subunits
242 A, such as RCN1 (ROOTS CURL IN NAPHTHYLPHTHALAMIC ACID1), are generally
243 distributed in the cytosol, but methylation on RCN1 leads to PP2A complex formation to
244 membrane fractions at PM and/or endosomes, where BRI1 resides¹⁰⁵. BR signaling also
245 transcriptionally upregulates *SBI1* expression, which in turn dampens signaling¹⁰⁵. SBI1 and
246 PP2A are also required for BRI1 degradation¹⁰⁵, implying that dephosphorylation of BRI1 is
247 critical for diverting BRI1 to MVBs or lytic vacuolar compartments for degradation, although
248 SBI1 and PP2A might lead to BRI1 degradation by other mechanisms. These multi-lock
249 systems at distinct layers tightly regulate BRI1 activity or complex formation to suppress the
250 basal or excess BRI1 activity.

251 The activities of BR signaling components are not solely under the control of BR perception.
252 For example, other phytohormones crosstalk with BR signaling factors through post-
253 translational mechanisms. Salicylic acid, one of the stress-induced phytohormones, promotes
254 the detachment of BSKs from the PM by upregulating de-S-acylation enzymes, resulting in BR
255 pathway downregulation¹⁰⁷. The core regulators of abscisic acid (ABA) signaling ABI1 and 2
256 (ABA INSENSITIVE 1 and 2) phosphatases dephosphorylate BIN2, and BIN2 phosphorylates
257 SnRK2s (SNF1-Related Kinase 2)¹⁰⁸, thereby oscillating both signaling mechanisms. Further
258 study in such growth/stress response signaling cross points is critical to understand plant
259 resilience under complex environments.

260

261 **Interplay between BR signaling and cytoskeletal networks**

262 Two interplaying cytoskeletal networks in the cytosol and close contact with the PM, MTs and
263 the actin microfilaments (MF), participate in essential cell activities such as cell elongation,
264 cell division, and vesicle/organelle trafficking¹⁰⁹.

265 The cytoskeleton is a highly dynamic biopolymer, and its organization is tightly controlled by
266 external and internal cues¹¹⁰⁻¹¹³. This plastic nature of the cytoskeleton provides the essential
267 basis for morphogenesis, directional growth, and stress adaptation, as seen in stomatal closure
268 under high salinity stress^{114,115}, for example. Phytohormones, including BRs, are representative
269 endogenous regulators of the cytoskeleton¹¹⁶. BRs modulate the organization and dynamics of
270 MFs in response to gravity in *Arabidopsis*¹¹⁷, and other plants such as *Zea mays* and *Medicago*
271 *truncatula*¹¹⁸. However, the detailed molecular mechanisms underlying such regulation are still
272 obscure¹¹⁹. Here, we highlight recent advances in how BRs impact cytoskeletal network
273 regulation, particularly focusing on MTs.

274

275 *Transcriptional regulation*

276 Transcriptome analyses have already connected BRs and MTs/MFs, since the expression of
277 some tubulin (*TUB1* and *TUB8*) and actin (*ACT11*) genes are upregulated in the presence of
278 BRs¹⁹. A couple of studies have also highlighted the BR-induced transcriptional upregulation
279 of MAPs (Microtubule-Associated Proteins) that impacts MT dynamics and organ
280 developmental processes. The BZR1 transcription factor protein directly binds to promoter
281 regions of *MDP40* (*MICROTUBULE DESTABILIZING PROTEIN40*), *KATANIN1* (*KT1*,
282 encoding p60 subunit), and *KATANIN80(1/2/3/4)* (*KT80s*) and upregulates their transcription.
283 MDP40 promotes hypocotyl elongation, and KATANINs (KTN) support stamen filament cell
284 elongation for efficient fertilization through BR-triggered MT reorganization^{120,121}.

285 Not limited to uni-directional transcriptional regulation, BR signaling and MTs form a
286 feedback loop by controlling *CLASP* (*CLIP170-associated protein*) transcription (**Fig. 2d**).
287 *CLASP* encodes a MAP acting in transfacial MT bundle formation, particularly important to
288 maintain proper cell population in root division zone¹²². Ruan *et al.* demonstrated that the
289 transcription of *CLASP* was negatively regulated by BRs, which prolongs the transition from
290 the division zone to the elongation zone in the root meristems through the reorganization of
291 MTs. Conversely, CLASP modulates BR signaling through the delivery of BRI1 to the PM by
292 bridging BRI1-containing SORTING NEXIN1 (SNX1)-labeled endosomes and MTs¹²²⁻¹²⁵.
293 Thus, CLASP plays a pivotal role in the negative feedback loop in BR sensing by adjusting the
294 levels of BRI1 (**Fig. 2d, Fig. 3a**). Further analyses using super-resolution microscopy will

295 reveal how transfacial MTs, where CLASP preferentially accumulates, contribute to BRI1
296 secretion and distribution on the PM.

297

298 *Non-transcriptional regulation*

299 Several studies have shown that BRs also control MT organization through post-translational
300 modifications. Such effects prominently appear in lobe-indent formation during leaf pavement
301 cell development. Genetic or pharmacological perturbation of BR biosynthesis/signaling^{126,127}
302 leads to MT disorganization and altered jigsaw puzzle shapes of pavement cells¹²⁸. However,
303 the detailed molecular mechanisms underlying the impact of BRs on MT regulation are still
304 missing.

305 Two recent complementary papers have nicely connected BR signaling to cortical MTs via
306 regulating ROP (Rho of plant) small GTPase signaling during pavement cell formation (**Fig.**
307 **2a**)^{129,130}. ROPs, which regulate pavement cell shapes, are small GTPases acting as molecular
308 switches from active to inactive states depending on GTP or GDP binding, respectively¹³¹. The
309 ROP activation cycle is carried out by GEFs (Guanine nucleotide Exchange Factors), and the
310 inactivation of ROPs is finely tuned by GAPs (GTPase-Activating Proteins) and GDIs (GDP
311 Dissociation Inhibitors). BRs were shown to impact MT organization through BIN2-mediated
312 phosphorylation of PHGAP1 and 2 (Pleckstrin Homology GAP1 and 2), whose double mutant
313 demonstrates vague lobe-indent patterns in pavement cells^{129,130}. PHGAPs target ROP2, which
314 prevents MT alignment in indent areas by inhibiting RIC1 (ROP Interactive CRIB motif-
315 containing protein1)¹³². PHGAPs are preferentially accumulated at the anticlinal face of the
316 pavement cell indent regions in MT- and BIN2-dependent manners^{129,130}. In the absence of
317 BRs, BIN2 activates and stabilizes PHGAPs, leading to ROP2 suppression, allowing MTs to
318 form well-ordered networks in indent regions through the ROP6-RIC1-KTN module¹³³. On the
319 other hand, when the BR signal is on, BIN2 is inactivated and degraded, allowing PHGAPs to
320 stay dormant¹²⁹. Then, active ROP2 prevents MT bundle formation by inhibiting ROP6,
321 leading to lobe formation. Thus, BRs modulate pavement cell lobe-indent formation through
322 MT regulation. This model also implies that local BR signal modulation occurs since BIN2
323 stays active only on the indent sides, possibly through feedback signal from ROP-related
324 factors, bundled MTs, or tensile stress to the BR pathways (**Fig. 2a, Fig. 3b**).

325 Previous reports also show that BIN2 phosphorylates both α and β -tubulins *in vitro*¹²⁸, and
326 some cytoskeletal proteins, such as TUA6, TUB4, and ACT2, showed mobility shifts on 2D
327 electrophoresis gels after BR treatments, implying that BRs induce some modifications on

328 these proteins that are yet to be characterized¹³⁴. Furthermore, a recent proximal labeling screen
329 identified potential substrates of BIN2¹³⁵. The candidate list includes various cytoskeleton-
330 associated proteins and PM proteins, suggesting the BR pathway impacts cell surface
331 regulation in several ways.

332

333 **Interplay between BR signaling and cell wall homeostasis**

334

335 Cell growth is an intricate process that requires precise regulation from both the inner- and
336 extracellular sides. As mentioned, BR signaling leads to complex cell shape formation in the
337 leaf epidermis by spatially controlling tip growth through cortical MT network regulation. BRs
338 also dictate cell growth by global transcriptional changes in cell wall biogenesis and
339 modification enzymes. For instance, several cell wall-remodeling genes, such as *CESA6*
340 (*CELLULOSE SYNTHASE A 6*), *XTH9*, *18*, *33* (*XYLOGLUCAN*
341 *ENDOTRANSGLUCOSYLASE/HYDROLASEs*), *EXPA8* (*EXPANSINA8*), and *PME2* *PME3*
342 (*PECTIN METHYLESTERASEs*), are direct targets of BES1/BZR1^{41,42}. In addition to the
343 transcriptional down-regulation of *CESA1*⁴², BIN2 also phosphorylates CESA1 and negatively
344 regulates its activity¹³⁶. BR treatment also suppresses gravitropism response in etiolated
345 hypocotyls by reorientating cellulose microfibrils and changes in cell wall components¹³⁷.
346 Besides, the BR pathway plays multiple roles in determining the onset timing of secondary cell
347 wall deposition^{138–140}. In this part, we will focus on how BRI1 engages the modulation and
348 sensing of the extracellular environment.

349 *Negative feedback loop between plasmodesmata aperture and BR signaling/biosynthesis*

350 Changes in cell wall composition determine the activity of cell surface structures such as
351 plasmodesmata (PD). PDs function as narrow channels for micro- and macro-molecules, such
352 as nutrients, signaling molecules, bacterial effectors, or viruses, between the cytoplasm of
353 neighboring cells. Their permeability responds to internal and external stimuli and is
354 predominantly regulated by callose deposition on the PD necks.^{141,142}. BR signaling
355 manipulates the PD permeability by inducing callose accumulation, thereby restricting the
356 diffusion of molecules through PD¹⁴³. BR-induced callose deposition appears to be mediated
357 by a transcriptional pathway, still, this does not exclude a possibility that the BRI1 complex
358 directly regulates callose synthase activity or other non-transcriptional mechanisms at the
359 PM¹⁴³.

360 Of note, PD permeability impacts BR biosynthesis¹⁴³. The metabolic pathway of BRs from
361 campesterol to brassinolide, the most bioactive BR, is well-documented, and a reductase and
362 several cytochrome P450 family mono-oxygenases are assigned to each step¹⁴⁴. Each enzyme
363 is expressed in different tissue layers around root meristem, despite some overlaps, indicating
364 that BR precursors should relocate across the layers to complete the full biosynthesis
365 pathway¹⁴⁵. Furthermore, full complementation of the *cpd* BR biosynthetic mutant by
366 epidermis-specific expression of *CPD* also supports such relocation idea⁸. This model is further
367 reinforced by elegant experiments combining a dominant callose synthase transgenic line that
368 over-accumulates callose and a fluorescently tagged BR precursor to directly visualize the
369 precursor movements¹⁴³. Together with the negative transcriptional feedback of biosynthesis
370 genes¹⁴⁴, callose deposition at plasmodesmata regulates BR production, constituting another
371 feedback in BR signaling regulation via the relocation of BRs and their precursors between
372 cells.

373

374 *Control of cell wall extensibility by acidification*

375 Acidifying apoplastic spaces is a critical step in expanding plant cells by increasing the
376 extensibility of the cell walls. Some cell wall loosening enzymes, such as expansins, possess
377 higher activity under acidic conditions¹⁴⁶. Auxins are well-known inducers of apoplast
378 acidification acting through the ABP1 (AUXIN BINDING PROTEIN1)-TMKs
379 (TRANSMEMBRANE KINASES)-H⁺ATPase module. TMKs interact with the AHA1/2 H⁺-
380 ATPase and phosphorylate their penultimate residues (Thr948/947, respectively)^{46,47,147},
381 whereas PP2C-D (PROTEIN PHOSPHATASE2C-D) deactivates AHA1/2 by
382 dephosphorylation¹⁴⁸.

383 Similar to auxin, BR treatments trigger apoplastic acidification by inducing phosphorylation of
384 the AHA1/2 H⁺-ATPases^{149–151}. Mechanistically, two non-mutually exclusive models are
385 proposed to explain the proton ATPase activation (**Fig. 2b**)^{149–151}. In the first model, the
386 activation of H⁺-ATPases relies on the canonical BR pathway through transcriptional
387 upregulation of PP2C inhibitors, *SAUR9/19* (*SMALL AUXIN UP RNA 9/19*)¹⁵⁰. In the second
388 model, BRI1 directly interacts with AHA1/2, and SAUR15 enhances the complex formation
389 to activate the proton ATPases by enhancing BRI1 activity¹⁵¹. Genetic evidence supports both
390 scenarios since *SAUR15* overexpression increases hypersensitivity to BRs by enhancing
391 extracellular acidification through upregulating H⁺-ATPase activity, and a *saur15* mutant

392 shows reduced sensitivity to BRs in root length¹⁵¹. Similarly, *SAUR19* overexpression leads to
393 extracellular acidification¹⁴⁸. It is still unclear how much both pathways contribute to apoplast
394 acidification and whether they crosstalk to coordinate proper cell elongation by adjusting cell
395 wall extensibility.

396 A modeling approach predicted that cation channels were involved in this pathway to
397 compensate for positive charges when protons are transported toward extracellular spaces.
398 CNGC10 (CYCLIC NUCLEOTIDE GATED CHANNEL 10) was proposed as a candidate
399 since *cngc10* mutants show defects in apoplastic acidification upon BR treatment. CNGC10
400 forms a complex with BRI1-BAK1, suggesting direct regulation by the receptor complex¹⁵².
401 Recent reports described that the altered pH changed receptor-peptide ligand binding¹⁵³ or
402 ligand activities¹⁵⁴. Remains to be determined whether the acidification matters to the BRI1-
403 BR binding capacity or BRI1-BR-BAK1 complex formation, and these points might be
404 essential to understand the homeostasis of apoplastic spaces.

405 *Sensing cell wall integrity*

406 Plant cell walls consist of carbohydrates, proteins, and other secondary metabolites. Cellulose,
407 cross-bridged by hemicellulose, provides tensile strength, while pectin confers elasticity to cell
408 walls. Transcriptional regulations and enzymatic activity changes allow plants to dynamically
409 remodel plant cell walls depending on developmental cues or stress responses, but how plant
410 cells monitor the cell wall status is still obscure. Yeast, which is also encapsulated in a cell
411 wall, has cell wall sensors, Wsc1-3, Mid1, and Mtl1 that monitor its mechanical properties and
412 trigger transcriptional changes to adapt to acute osmotic changes or heat shock^{155,156}. Plants do
413 not have obvious homologs of these molecules, but solid evidence indicates that plants do have
414 cell wall surveillance systems^{157,158}. Recent studies indeed revealed that different classes of
415 proteins, such as mechano-sensitive Ca²⁺ channels¹⁵⁹, cell wall proteins¹⁶⁰, receptor-like
416 proteins¹⁶¹, GPI-anchored proteins¹⁶², or receptor-like kinases¹⁶³⁻¹⁶⁶, function in cell wall
417 sensing. Mutations in these proteins often lead to abnormal cell shapes, defects in cell growth,
418 or pollen tube rupture¹⁵⁸.

419 An unexpected role of BRI1 in cell wall sensing emerged from a forward genetic approach
420 (**Fig. 2c**). Recent reports demonstrated that a BRI1-mediated cell wall surveillance system
421 monitors the homeostasis of pectin methyl esterification, affecting the biophysical properties
422 of pectins. Demethylesterified pectin binds to Ca²⁺ through its free carboxy groups, promoting
423 bridge formation between pectin chains. Of interest, super-resolution microscopic observation

424 clearly highlighted the biased distribution of methyl- and demethyl-esterified pectins¹⁶⁷. PMEs
425 (pectin methylesterases) and PMEIs (PME inhibitors) are critical proteins that determine the
426 pectin methyl esterification ratio. Disruption of the pectin methylesterification balance by
427 overexpression of *PMEI5* leads to wavy root growth¹⁶⁸. Wolf *et al.* used this wavy growth as a
428 phenotypic proxy to the methylesterification status of the cell walls and conducted a forward
429 suppressor screen of the PMElox phenotype¹⁶⁸. One of the suppressor mutants, called
430 *comfortable numb 1 (cnu1)*, has a mutation in the kinase domain of BRI1. Other *bri1* mutant
431 alleles, or the *det2* BR biosynthesis mutant, also suppress the PMElox phenotype^{168,169},
432 suggesting the phenotype of *PMEI5* overexpression is a result of the enhanced BR signaling
433 pathway¹⁶⁸.

434 Another component in this pathway was identified as CNU2, encoding RLP44 (Receptor Like
435 Protein 44), in the same suppressor screen. RLP44 stabilizes a ternary complex with BRI1-
436 BAK1 and enhances BR transcriptional responses as an auxiliary component of the pathway¹⁶¹
437 (**Fig. 2c**). RLP44 is phosphorylated in BR/cell wall homeostasis-dependent manners, which is
438 required for its functionality in cell wall maintenance^{161,170}. Since phosphorylated RLP44 tends
439 to stay at the PM, while a non-phosphorylated form mainly resides in endosomes, phospho-
440 status of RLP44 may affect BR signal initiation in this context¹⁷¹. In parallel, RLP44 also
441 strengthens the PSKR (phytosulfokine receptor)-BAK1 complex formation, which mediates
442 procambium/xylem patterning together with BRI1¹⁷². This fact raises the question of how
443 RLP44 balances BRI1 and PSKR activities. Indeed, plants harboring the *bri1^{cnu4}* mutation
444 display abnormal xylem pattern formation due to the imbalance of competition between
445 BRI1^{cnu4}, which has tighter interaction with RLP44, and PSKR¹⁶⁹. Distinct from BR signaling,
446 the phospho-status of RLP44 does not affect PSK signaling, suggesting RLP44
447 phosphorylation status may not explain the competition¹⁷⁰. Moreover, a recent study also
448 suggested that BRI1 plays a role in the xylem patterning process partially independent of BRs⁵⁵.
449 Further study may reveal the nature of microenvironmental cues or components that govern
450 the competition over RLP44 during xylem patterning.

451 A recent report provides mechanistic insights into how BRI1 functions in cell-surface
452 continuum signaling with another RLK in rice. OsWAK11 (WALL ASSOCIATED KINASE
453 11) was identified as a negative growth regulator and as an interactor of OsBRI1. OsWAK11
454 monitors pectin methylation status by preferentially binding to methylesterified pectins.
455 Demethylesterified pectin suppresses BR signaling through BRI1 phosphorylation by
456 OsWAK11. On the other hand, the methylesterified rich situation leads to OsWAK11 reduction,

457 allowing BRI1 to initiate signals¹⁷³. OsWAK11 phosphorylates a monocot-specific motif in
458 OsBRI1, suggesting this pectin sensing module is unique to monocots. How the pectin status
459 enhances BR signaling is still an open question in Arabidopsis, but the recent identification of
460 RFO1 (RESISTANCE TO FUSARIUM OXYSPORUM1), a WAK-like receptor-like kinase,
461 may fill the gap (**Fig. 2c**). *rfo1* mutant suppresses the PMElox phenotype like BR-defective
462 mutants by modulating the transcription of BR-related genes. In addition, RFO1 directly binds
463 to demethylesterified pectins, supporting the idea that RFO1 may connect pectins to BR-
464 signaling¹⁷⁴. Further studies on direct interaction between RFO1 and BRI1-BAK1-RLP44, and
465 divergent mechanisms in cell wall sensing among plant species are awaited.

466

467 **Coordination of organ growth**

468 Coordinated cell growth in each tissue is one of the critical processes for multicellular
469 organisms to determine organ shape, to keep their integrity, and to achieve biased organ growth.
470 For example, the root endodermis-specific expression of a dominant negative form of DELLA
471 protein, a gibberellin and other growth signal integrating factor, severely attenuates whole root
472 growth¹⁷⁵. On the other hand, ERECTA-EPFL4/6 (EPIDERMAL PATTERNING FACTOR-
473 LIKE 4/6) receptor-peptide pairs determine inflorescence stem height by modulating cortex
474 cell proliferation and growth¹⁷⁶.

475 Several lines of evidence prove that BR signaling coordinates organ growth by adjusting
476 physical constraint forces. For example, the proper balance of responses to BRs in root hair
477 and non-hair cells is crucial since an imbalanced BR-induced cell growth triggers ethylene
478 responses and accumulation of crystalline cellulose¹⁷⁷, resulting in inadequate whole root
479 growth. Quantitative 3D cell growth analysis also revealed that cells in each tissue layer display
480 distinct responses to BRs, which are essential for proper radial root meristem growth.
481 Additionally, BR treatment decreases the stele area in WT roots, while BR signaling
482 perturbation increases the stele size¹⁷⁸. Of note, epidermal specific BRI1 expression in *bri1*
483 mutant rescues the root growth and limits the stele area⁸. These observations suggest that the
484 epidermal layer provides BR-dependent mechanical constraint. A recent study uncovered that
485 BRs are also involved in organ integrity through coordinated tissue growth in the carnivorous
486 aquatic plant *Utricularia gibba* (commonly called floating bladderwort) and terrestrial plant
487 shoots¹⁷⁹. In this study, a genetic screen identified dwarf and extra-dwarf mutants, both
488 presenting short internodes, small leaves, small traps, and distorted vascular tissue.

489 Computational modeling predicted that the wavy vascular tissue phenotype in the dwarf mutant
490 resulted from the physical constraint of retarded epidermal layer growth. Consistent with the
491 dwarf phenotypes, the responsible gene was a cytochrome P450 90B1 enzyme, a homolog of
492 *DWF4* (*DWARF4*) in Arabidopsis^{179–181}. In line with this observation, treatment with a BR
493 biosynthesis inhibitor recapitulates *dwarf* mutant phenotypes of *U. gibba*, while BR treatment
494 rescued the mutant phenotypes. Furthermore, a mutation in Arabidopsis *DWF4* enhances the
495 crack phenotype in *qual-2* (*quasimodo 1-2*) in hypocotyl epidermal cells, suggesting BRs
496 reduce epidermal constraint¹⁷⁹. Modeling analysis revealed that cell wall extensibility through
497 BR-induced apoplast acidification could be one of the determinants of such coordination, but
498 this does not exclude the possibility that other BR-related mechanisms also drive the process.
499 Decoding BR response under tension in each tissue may support the unwiring of these
500 interactions. Thus, BRs impact not only the adjustment of the microenvironment around the
501 cell surface but also regulate the whole apoplast continuum to coordinate organ growth.

502

503 **Conclusion and perspectives**

504 Since the identification of BRs as “growth-promoting chemicals” from *B.napa*¹⁸², forward
505 genetic approaches in Arabidopsis or rice, biochemical characterization, and many other
506 physiological analyses unraveled the critical roles of BRs in plant life cycles and the detailed
507 underlying molecular mechanisms²¹. Besides these developmental and physiological aspects,
508 BRI1 serves as a model of choice to many cell biologists because of its unique dynamic
509 regulation through post-translational modifications, particularly in the vicinity of the cell
510 surface (**Fig. 1**). Together with the dynamic quantitative regulation of BRI1, a multi-layered
511 interaction network fine tunes the activity of BRI1 or BRI1-BAK1 complex formation at the
512 PM (**Fig. 1**), oscillating the level of BR signaling to regulate cell surface structures through
513 transcriptional and non-transcriptional mechanisms (**Fig. 2**).

514 Many questions are yet to be answered, from the factors binding to and decoding the post-
515 translational modification codes of BRI1 controlling its activity and dynamics, to the
516 mechanisms driving the diffusion, nanodomain formation/maintenance, or intracellular sorting
517 of BRI1. Recent technical advances in high- and super-resolution microscopy uncovered
518 nanodomain formation of BRI1 and will be instrumental in understanding better this new facet
519 of BRI1 biology.

520 Signals from BRI1 are emitted from nanodomains, but how these signals can modulate the
521 spatially restricted cell wall status changes is still obscure. For example, methyl- and demethyl-
522 esterified pectins distribute ununiformly, suggesting pectin modification status sensing is
523 locally activated. As mentioned above, transcriptional feedback is thought to maintain cell wall
524 status, but it is still unclear how transcriptional feedback can achieve local cell wall
525 modifications. Combining recent advanced methods, such as proximal labeling-based spatial
526 proteomes, super-resolution microscopy techniques, and all conventional approaches will help
527 us approach the molecular machinery for local cell wall modification with unprecedented
528 resolutions. Thus, BRI1 and its signaling are still inspiring us to discover novel concepts in cell
529 biology.

530

531 **Acknowledgments**

532 We apologize to authors whose work is related to brassinosteroid-driven cell surface regulation
533 that could not be cited either because of my oversight or space limitation. The figures were
534 created with BioRender.com. We thank Steven Moussu for the critical reading of the
535 manuscript. This work was supported by a Ph.D. fellowship from the University Toulouse 3-
536 Paul Sabatier to C.D., research grants from Agence Nationale de la Recherche (ANR-17-CE20-
537 0026-01 to G.V. and ANR-22-CE13-0021-01 to S.F.), and the French Laboratory of Excellence
538 (project “TULIP” grant nos. ANR-10-LABX-41 and ANR-11-IDEX-0002-02 to G.V.).

539

540 **Author contributions**

541 Conceptualization, C.D., G.V., and S.F.; writing – original draft, C.D.; writing – review &
542 editing, C.D., G.V., and S.F.; visualization, C.D., S.F.; supervision, S.F. and G.V.

543 **Declaration of interests**

544 No interests are declared.

545

546

- 547 1. Congreve, M., de Graaf, C., Swain, N. A. & Tate, C. G. Impact of GPCR Structures on Drug
548 Discovery. *Cell* **181**, 81–91 (2020).

- 549 2. Robinson, D. R., Wu, Y.-M. & Lin, S.-F. The protein tyrosine kinase family of the human genome.
550 *Oncogene* **19**, 5548–5557 (2000).
- 551 3. Shiu, S.-H. & Bleecker, A. B. Receptor-like kinases from Arabidopsis form a monophyletic gene
552 family related to animal receptor kinases. *Proc. Natl. Acad. Sci.* **98**, 10763–10768 (2001).
- 553 4. Shiu, S.-H. *et al.* Comparative Analysis of the Receptor-Like Kinase Family in Arabidopsis and
554 Rice[W]. *Plant Cell* **16**, 1220–1234 (2004).
- 555 5. Li, J. & Chory, J. A Putative Leucine-Rich Repeat Receptor Kinase Involved in Brassinosteroid
556 Signal Transduction. *Cell* **90**, 929–938 (1997).
- 557 6. Choe, S. *et al.* The Arabidopsis *dwf7/ste1* Mutant Is Defective in the $\Delta 7$ Sterol C-5 Desaturation
558 Step Leading to Brassinosteroid Biosynthesis. *Plant Cell* **11**, 207–221 (1999).
- 559 7. Nakaya, M., Tsukaya, H., Murakami, N. & Kato, M. Brassinosteroids Control the Proliferation of
560 Leaf Cells of Arabidopsis thaliana. *Plant Cell Physiol.* **43**, 239–244 (2002).
- 561 8. Savaldi-Goldstein, S., Peto, C. & Chory, J. The epidermis both drives and restricts plant shoot
562 growth. *Nature* **446**, 199–202 (2007).
- 563 9. Ye, Q. *et al.* Brassinosteroids control male fertility by regulating the expression of key genes
564 involved in Arabidopsis anther and pollen development. *Proc. Natl. Acad. Sci.* **107**, 6100–6105
565 (2010).
- 566 10. Vogler, F., Schmalzl, C., Englhart, M., Bircheneder, M. & Sprunck, S. Brassinosteroids promote
567 Arabidopsis pollen germination and growth. *Plant Reprod.* **27**, 153–167 (2014).
- 568 11. Krishna, P., Prasad, B. D. & Rahman, T. Brassinosteroid Action in Plant Abiotic Stress Tolerance.
569 in *Brassinosteroids: Methods and Protocols* (eds. Russinova, E. & Caño-Delgado, A. I.) 193–202
570 (Springer, 2017). doi:10.1007/978-1-4939-6813-8_16.
- 571 12. Planas-Riverola, A. *et al.* Brassinosteroid signaling in plant development and adaptation to
572 stress. *Development* **146**, dev151894 (2019).

- 573 13. Hong, Z. *et al.* Loss-of-function of a rice brassinosteroid biosynthetic enzyme, C-6 oxidase,
574 prevents the organized arrangement and polar elongation of cells in the leaves and stem. *Plant*
575 *J.* **32**, 495–508 (2002).
- 576 14. Mori, M. *et al.* Isolation and Characterization of a Rice Dwarf Mutant with a Defect in
577 Brassinosteroid Biosynthesis. *Plant Physiol.* **130**, 1152–1161 (2002).
- 578 15. Nakamura, A. *et al.* The Role of OsBRI1 and Its Homologous Genes, OsBRL1 and OsBRL3, in
579 Rice. *Plant Physiol.* **140**, 580–590 (2006).
- 580 16. Song, L. *et al.* Reducing brassinosteroid signalling enhances grain yield in semi-dwarf wheat.
581 *Nature* **617**, 118–124 (2023).
- 582 17. Oh, M.-H. *et al.* Tyrosine phosphorylation of the BRI1 receptor kinase emerges as a component
583 of brassinosteroid signaling in Arabidopsis. *Proc. Natl. Acad. Sci.* **106**, 658–663 (2009).
- 584 18. Bojar, D. *et al.* Crystal structures of the phosphorylated BRI1 kinase domain and implications
585 for brassinosteroid signal initiation. *Plant J.* **78**, 31–43 (2014).
- 586 19. Vert, G., Nemhauser, J. L., Geldner, N., Hong, F. & Chory, J. MOLECULAR MECHANISMS OF
587 STEROID HORMONE SIGNALING IN PLANTS. *Annu. Rev. Cell Dev. Biol.* **21**, 177–201 (2005).
- 588 20. Jaillais, Y. & Vert, G. Brassinosteroid signaling and BRI1 dynamics went underground. *Curr.*
589 *Opin. Plant Biol.* **33**, 92–100 (2016).
- 590 21. Nolan, T. M., Vukašinović, N., Liu, D., Russinova, E. & Yin, Y. Brassinosteroids: Multidimensional
591 Regulators of Plant Growth, Development, and Stress Responses[OPEN]. *Plant Cell* **32**, 295–318
592 (2020).
- 593 22. Li, J., Nam, K. H., Vafeados, D. & Chory, J. BIN2, a New Brassinosteroid-Insensitive Locus in
594 Arabidopsis. *Plant Physiol.* **127**, 14–22 (2001).
- 595 23. He, J.-X., Gendron, J. M., Yang, Y., Li, J. & Wang, Z.-Y. The GSK3-like kinase BIN2 phosphorylates
596 and destabilizes BZR1, a positive regulator of the brassinosteroid signaling pathway in
597 Arabidopsis. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 10185–10190 (2002).

- 598 24. Zhao, J. *et al.* Two Putative BIN2 Substrates Are Nuclear Components of Brassinosteroid
599 Signaling. *Plant Physiol.* **130**, 1221–9 (2002).
- 600 25. Vert, G. & Chory, J. Downstream nuclear events in brassinosteroid signalling. *Nature* **441**, 96–
601 100 (2006).
- 602 26. Gampala, S. S. *et al.* An Essential Role for 14-3-3 Proteins in Brassinosteroid Signal Transduction
603 in Arabidopsis. *Dev. Cell* **13**, 177–189 (2007).
- 604 27. Yin, Y. *et al.* BES1 Accumulates in the Nucleus in Response to Brassinosteroids to Regulate
605 Gene Expression and Promote Stem Elongation. *Cell* **109**, 181–191 (2002).
- 606 28. Wang, Z. Y. *et al.* Nuclear-localized BZR1 mediates brassinosteroid-induced growth and
607 feedback suppression of brassinosteroid biosynthesis. *Dev. Cell* **2**, 505–513 (2002).
- 608 29. Ryu, H. *et al.* Nucleocytoplasmic Shuttling of BZR1 Mediated by Phosphorylation Is Essential in
609 Arabidopsis Brassinosteroid Signaling. *Plant Cell* **19**, 2749–2762 (2007).
- 610 30. Wang, R. *et al.* Nucleocytoplasmic trafficking and turnover mechanisms of BRASSINAZOLE
611 RESISTANT1 in Arabidopsis thaliana. *Proc. Natl. Acad. Sci.* **118**, e2101838118 (2021).
- 612 31. Li, J. *et al.* BAK1, an Arabidopsis LRR receptor-like protein kinase, interacts with BRI1 and
613 modulates brassinosteroid signaling. *Cell* **110**, 213–222 (2002).
- 614 32. Nam, K. H. & Li, J. BRI1/BAK1, a Receptor Kinase Pair Mediating Brassinosteroid Signaling. *Cell*
615 **110**, 203–212 (2002).
- 616 33. Santiago, J., Henzler, C. & Hothorn, M. Molecular Mechanism for Plant Steroid Receptor
617 Activation by Somatic Embryogenesis Co-Receptor Kinases. *Science* **341**, 889–892 (2013).
- 618 34. Wang, X. *et al.* Sequential Transphosphorylation of the BRI1/BAK1 Receptor Kinase Complex
619 Impacts Early Events in Brassinosteroid Signaling. *Dev. Cell* **15**, 220–235 (2008).
- 620 35. Tang, W. *et al.* Brassinosteroid-Signaling Kinases (BSKs) mediate signal transduction from the
621 receptor kinase BRI1 in Arabidopsis. *Science* **321**, 557–560 (2008).

- 622 36. Kim, T.-W., Guan, S., Burlingame, A. L. & Wang, Z.-Y. The CDG1 Kinase Mediates
623 Brassinosteroid Signal Transduction from BRI1 Receptor Kinase to BSU1 Phosphatase and
624 GSK3-like Kinase BIN2. *Mol. Cell* **43**, 561–571 (2011).
- 625 37. Mora-García, S. *et al.* Nuclear protein phosphatases with Kelch-repeat domains modulate the
626 response to brassinosteroids in Arabidopsis. *Genes Dev.* **18**, 448–460 (2004).
- 627 38. Kim, T.-W. *et al.* Brassinosteroid signal transduction from cell-surface receptor kinases to
628 nuclear transcription factors. *Nat. Cell Biol.* **11**, 1254–1260 (2009).
- 629 39. Tang, W. *et al.* PP2A activates brassinosteroid-responsive gene expression and plant growth by
630 dephosphorylating BZR1. *Nat. Cell Biol.* **13**, 124–131 (2011).
- 631 40. Müssig, C., Fischer, S. & Altmann, T. Brassinosteroid-Regulated Gene Expression. *Plant Physiol.*
632 **129**, 1241–1251 (2002).
- 633 41. Sun, Y. *et al.* Integration of Brassinosteroid Signal Transduction with the Transcription Network
634 for Plant Growth Regulation in Arabidopsis. *Dev. Cell* **19**, 765–777 (2010).
- 635 42. Yu, X. *et al.* A brassinosteroid transcriptional network revealed by genome-wide identification
636 of BES1 target genes in Arabidopsis thaliana. *Plant J.* **65**, 634–646 (2011).
- 637 43. Kadota, Y. *et al.* Direct Regulation of the NADPH Oxidase RBOHD by the PRR-Associated Kinase
638 BIK1 during Plant Immunity. *Mol. Cell* **54**, 43–55 (2014).
- 639 44. Li, L. *et al.* The FLS2-Associated Kinase BIK1 Directly Phosphorylates the NADPH Oxidase RbohD
640 to Control Plant Immunity. *Cell Host Microbe* **15**, 329–338 (2014).
- 641 45. Fujita, S. *et al.* SCHENGEN receptor module drives localized ROS production and lignification in
642 plant roots. *EMBO J.* **39**, e103894 (2020).
- 643 46. Lin, W. *et al.* TMK-based cell-surface auxin signalling activates cell-wall acidification. *Nature*
644 **599**, 278–282 (2021).
- 645 47. Li, L. *et al.* Cell surface and intracellular auxin signalling for H⁺ fluxes in root growth. *Nature*
646 **599**, 273–277 (2021).

- 647 48. Viotti, C. *et al.* Endocytic and Secretory Traffic in Arabidopsis Merge in the Trans-Golgi
648 Network/Early Endosome, an Independent and Highly Dynamic Organelle. *Plant Cell* **22**, 1344–
649 1357 (2010).
- 650 49. Sutkeviciute, I. & Vilardaga, J.-P. Structural insights into emergent signaling modes of G
651 protein-coupled receptors. *J. Biol. Chem.* **295**, 11626–11642 (2020).
- 652 50. Di Rubbo, S. *et al.* The Clathrin Adaptor Complex AP-2 Mediates Endocytosis of
653 BRASSINOSTEROID INSENSITIVE1 in Arabidopsis. *Plant Cell* **25**, 2986–2997 (2013).
- 654 51. Martins, S. *et al.* Internalization and vacuolar targeting of the brassinosteroid hormone
655 receptor BRI1 are regulated by ubiquitination. *Nat. Commun.* **6**, 6151 (2015).
- 656 52. Liu, D. *et al.* Endocytosis of BRASSINOSTEROID INSENSITIVE1 Is Partly Driven by a Canonical
657 Tyr-Based Motif. *Plant Cell* **32**, 3598–3612 (2020).
- 658 53. Saeed, B. *et al.* K63-linked ubiquitin chains are a global signal for endocytosis and contribute to
659 selective autophagy in plants. *Curr. Biol.* **33**, 1337-1345.e5 (2023).
- 660 54. Geldner, N., Hyman, D. L., Wang, X., Schumacher, K. & Chory, J. Endosomal signaling of plant
661 steroid receptor kinase BRI1. *Genes Dev.* **21**, 1598–1602 (2007).
- 662 55. Neubus Claus, L. A. *et al.* BRASSINOSTEROID INSENSITIVE1 internalization can occur
663 independent of ligand binding. *Plant Physiol.* kiad005 (2023) doi:10.1093/plphys/kiad005.
- 664 56. Russinova, E. *et al.* Heterodimerization and Endocytosis of Arabidopsis Brassinosteroid
665 Receptors BRI1 and AtSERK3 (BAK1). *Plant Cell* **16**, 3216–3229 (2004).
- 666 57. Robatzek, S., Chinchilla, D. & Boller, T. Ligand-induced endocytosis of the pattern recognition
667 receptor FLS2 in Arabidopsis. *Genes Dev.* **20**, 537–542 (2006).
- 668 58. Doblas, V. G. *et al.* Root diffusion barrier control by a vasculature-derived peptide binding to
669 the SGN3 receptor. *Science* **355**, 280–284 (2017).
- 670 59. Zhou, J. *et al.* Regulation of Arabidopsis brassinosteroid receptor BRI1 endocytosis and
671 degradation by plant U-box PUB12/PUB13-mediated ubiquitination. *Proc. Natl. Acad. Sci.* **115**,
672 E1906–E1915 (2018).

- 673 60. Zhang, L. *et al.* SYP22 and VAMP727 regulate BRI1 plasma membrane targeting to control plant
674 growth in Arabidopsis. *New Phytol.* **223**, 1059–1065 (2019).
- 675 61. Gadeyne, A. *et al.* The TPLATE Adaptor Complex Drives Clathrin-Mediated Endocytosis in
676 Plants. *Cell* **156**, 691–704 (2014).
- 677 62. Wang, L. *et al.* Spatiotemporal Dynamics of the BRI1 Receptor and its Regulation by Membrane
678 Microdomains in Living Arabidopsis Cells. *Mol. Plant* **8**, 1334–1349 (2015).
- 679 63. Yoshinari, A. *et al.* Polar Localization of the Borate Exporter BOR1 Requires AP2-Dependent
680 Endocytosis1. *Plant Physiol.* **179**, 1569–1580 (2019).
- 681 64. Leitner, J. *et al.* Lysine63-linked ubiquitylation of PIN2 auxin carrier protein governs hormonally
682 controlled adaptation of Arabidopsis root growth. *Proc. Natl. Acad. Sci.* **109**, 8322–8327 (2012).
- 683 65. Johnson, A. & Vert, G. Unraveling K63 Polyubiquitination Networks by Sensor-Based
684 Proteomics1. *Plant Physiol.* **171**, 1808–1820 (2016).
- 685 66. Dubeaux, G., Neveu, J., Zelazny, E. & Vert, G. Metal Sensing by the IRT1 Transporter-Receptor
686 Orchestrates Its Own Degradation and Plant Metal Nutrition. *Mol. Cell* **69**, 953-964.e5 (2018).
- 687 67. Luo, Y. *et al.* Deubiquitinating enzymes UBP12 and UBP13 stabilize the brassinosteroid
688 receptor BRI1. *EMBO Rep.* **23**, e53354 (2022).
- 689 68. Lu, D. *et al.* Direct Ubiquitination of Pattern Recognition Receptor FLS2 Attenuates Plant Innate
690 Immunity. *Science* **332**, 1439–1442 (2011).
- 691 69. Martins, S. *et al.* Brassinosteroid signaling-dependent root responses to prolonged elevated
692 ambient temperature. *Nat. Commun.* **8**, 309 (2017).
- 693 70. Naranjo-Arcos, M. *et al.* SUMO/deSUMOylation of the BRI1 brassinosteroid receptor
694 modulates plant growth responses to temperature. *Proc. Natl. Acad. Sci.* **120**, e2217255120
695 (2023).
- 696 71. Orosa, B. *et al.* SUMO conjugation to the pattern recognition receptor FLS2 triggers
697 intracellular signalling in plant innate immunity. *Nat. Commun.* **9**, 5185 (2018).

- 698 72. Lin, W. *et al.* Inverse modulation of plant immune and brassinosteroid signaling pathways by
699 the receptor-like cytoplasmic kinase BIK1. *Proc. Natl. Acad. Sci. U. S. A.* **110**, 12114–12119
700 (2013).
- 701 73. Heinemann, B., Künzler, P., Eubel, H., Braun, H.-P. & Hildebrandt, T. M. Estimating the number
702 of protein molecules in a plant cell: protein and amino acid homeostasis during drought. *Plant*
703 *Physiol.* **185**, 385–404 (2021).
- 704 74. Platre, M. P. *et al.* Developmental control of plant Rho GTPase nano-organization by the lipid
705 phosphatidylserine. *Science* **364**, 57–62 (2019).
- 706 75. Smokvarska, M. *et al.* A Plasma Membrane Nanodomain Ensures Signal Specificity during
707 Osmotic Signaling in Plants. *Curr. Biol.* **30**, 4654-4664.e4 (2020).
- 708 76. Gronnier, J. *et al.* Regulation of immune receptor kinase plasma membrane nanoscale
709 organization by a plant peptide hormone and its receptors. *eLife* **11**, e74162 (2022).
- 710 77. Chinchilla, D. *et al.* A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant
711 defence. *Nature* **448**, 497–500 (2007).
- 712 78. Heese, A. *et al.* The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity
713 in plants. *Proc. Natl. Acad. Sci.* **104**, 12217–12222 (2007).
- 714 79. Schulze, B. *et al.* Rapid Heteromerization and Phosphorylation of Ligand-activated Plant
715 Transmembrane Receptors and Their Associated Kinase BAK1*. *J. Biol. Chem.* **285**, 9444–9451
716 (2010).
- 717 80. Belkhadir, Y., Yang, L., Hetzel, J., Dangl, J. L. & Chory, J. The growth–defense pivot: crisis
718 management in plants mediated by LRR-RK surface receptors. *Trends Biochem. Sci.* **39**, 447–
719 456 (2014).
- 720 81. Ortiz-Morea, F. A., He, P., Shan, L. & Russinova, E. It takes two to tango – molecular links
721 between plant immunity and brassinosteroid signalling. *J. Cell Sci.* **133**, jcs246728 (2020).
- 722 82. Bücherl, C. A. *et al.* Plant immune and growth receptors share common signalling components
723 but localise to distinct plasma membrane nanodomains. *eLife* **6**, e25114 (2017).

- 724 83. Su, B. *et al.* Dynamic spatial reorganization of BSK1 complexes in the plasma membrane
725 underpins signal-specific activation for growth and immunity. *Mol. Plant* **14**, 588–603 (2021).
- 726 84. Good, M. C., Zalatan, J. G. & Lim, W. A. Scaffold Proteins: Hubs for Controlling the Flow of
727 Cellular Information. *Science* **332**, 680–686 (2011).
- 728 85. Amorim-Silva, V. *et al.* TTL Proteins Scaffold Brassinosteroid Signaling Components at the
729 Plasma Membrane to Optimize Signal Transduction in Arabidopsis. *Plant Cell* **31**, 1807–1828
730 (2019).
- 731 86. Ren, H. *et al.* BRASSINOSTEROID-SIGNALING KINASE 3, a plasma membrane-associated scaffold
732 protein involved in early brassinosteroid signaling. *PLoS Genet.* **15**, e1007904 (2019).
- 733 87. Lakhssassi, N. *et al.* The Arabidopsis TETRATRICOPEPTIDE THIOREDOXIN-LIKE Gene Family Is
734 Required for Osmotic Stress Tolerance and Male Sporogenesis1[C][W][OA]. *Plant Physiol.* **158**,
735 1252–1266 (2012).
- 736 88. Kesten, C. *et al.* Peripheral membrane proteins modulate stress tolerance by safeguarding
737 cellulose synthases. *Sci. Adv.* **8**, eabq6971 (2022).
- 738 89. Wang, X. *et al.* Autoregulation and Homodimerization Are Involved in the Activation of the
739 Plant Steroid Receptor BRI1. *Dev. Cell* **8**, 855–865 (2005).
- 740 90. Hothorn, M. *et al.* Structural basis of steroid hormone perception by the receptor kinase BRI1.
741 *Nature* **474**, 467–471 (2011).
- 742 91. Hink, M. A., Shah, K., Russinova, E., de Vries, S. C. & Visser, A. J. W. G. Fluorescence Fluctuation
743 Analysis of Arabidopsis thaliana Somatic Embryogenesis Receptor-Like Kinase and
744 Brassinosteroid Insensitive 1 Receptor Oligomerization. *Biophys. J.* **94**, 1052–1062 (2008).
- 745 92. Wang, X. *et al.* Identification and Functional Analysis of in Vivo Phosphorylation Sites of the
746 Arabidopsis BRASSINOSTEROID-INSENSITIVE1 Receptor Kinase. *Plant Cell* **17**, 1685–1703
747 (2005).

- 748 93. Oh, M.-H., Wang, X., Clouse, S. D. & Huber, S. C. Deactivation of the Arabidopsis
749 BRASSINOSTEROID INSENSITIVE 1 (BRI1) receptor kinase by autophosphorylation within the
750 glycine-rich loop. *Proc. Natl. Acad. Sci.* **109**, 327–332 (2012).
- 751 94. Liu, Y., Huang, X., Li, M., He, P. & Zhang, Y. Loss-of-function of Arabidopsis receptor-like kinase
752 BIR1 activates cell death and defense responses mediated by BAK1 and SOBIR1. *New Phytol.*
753 **212**, 637–645 (2016).
- 754 95. Ma, C. *et al.* Structural basis for BIR1-mediated negative regulation of plant immunity. *Cell Res.*
755 **27**, 1521–1524 (2017).
- 756 96. Halter, T. *et al.* The Leucine-Rich Repeat Receptor Kinase BIR2 Is a Negative Regulator of BAK1
757 in Plant Immunity. *Curr. Biol.* **24**, 134–143 (2014).
- 758 97. Hohmann, U., Nicolet, J., Moretti, A., Hothorn, L. A. & Hothorn, M. The SERK3 elongated allele
759 defines a role for BIR ectodomains in brassinosteroid signalling. *Nat. Plants* **4**, 345–351 (2018).
- 760 98. Imkamp, J. *et al.* The Arabidopsis Leucine-Rich Repeat Receptor Kinase BIR3 Negatively
761 Regulates BAK1 Receptor Complex Formation and Stabilizes BAK1. *Plant Cell* **29**, 2285–2303
762 (2017).
- 763 99. Wang, X. & Chory, J. Brassinosteroids Regulate Dissociation of BKI1, a Negative Regulator of
764 BRI1 Signaling, from the Plasma Membrane. *Science* **313**, 1118–1122 (2006).
- 765 100. Jaillais, Y. *et al.* Tyrosine phosphorylation controls brassinosteroid receptor activation by
766 triggering membrane release of its kinase inhibitor. *Genes Dev.* **25**, 232–237 (2011).
- 767 101. Simon, M. L. A. *et al.* A PtdIns(4)P-driven electrostatic field controls cell membrane identity and
768 signalling in plants. *Nat. Plants* **2**, 1–10 (2016).
- 769 102. Kang, Y. H. & Hardtke, C. S. Arabidopsis MAKR5 is a positive effector of BAM3-dependent
770 CLE45 signaling. *EMBO Rep.* **17**, 1145–1154 (2016).
- 771 103. Marquès-Bueno, M. M. *et al.* Auxin-Regulated Reversible Inhibition of TMK1 Signaling by
772 MAKR2 Modulates the Dynamics of Root Gravitropism. *Curr. Biol.* **31**, 228–237.e10 (2021).

- 773 104. Wang, D. *et al.* BKI1 Regulates Plant Architecture through Coordinated Inhibition of the
774 Brassinosteroid and ERECTA Signaling Pathways in Arabidopsis. *Mol. Plant* **10**, 297–308 (2017).
- 775 105. Wu, G. *et al.* Methylation of a Phosphatase Specifies Dephosphorylation and Degradation of
776 Activated Brassinosteroid Receptors. *Sci. Signal.* **4**, ra29–ra29 (2011).
- 777 106. Wang, R. *et al.* The Brassinosteroid-Activated BRI1 Receptor Kinase Is Switched off by
778 Dephosphorylation Mediated by Cytoplasm-Localized PP2A B' Subunits. *Mol. Plant* **9**, 148–157
779 (2016).
- 780 107. Liu, X. *et al.* Salicylic acid attenuates brassinosteroid signaling via protein de-S-acylation. *EMBO*
781 *J. n/a*, e112998 (2023).
- 782 108. Wang, H. *et al.* Abscisic Acid Signaling Inhibits Brassinosteroid Signaling through Dampening
783 the Dephosphorylation of BIN2 by ABI1 and ABI2. *Mol. Plant* **11**, 315–325 (2018).
- 784 109. Kost, B. & Chua, N.-H. The Plant Cytoskeleton: Vacuoles and Cell Walls Make the Difference.
785 *Cell* **108**, 9–12 (2002).
- 786 110. Hamant, O. *et al.* Developmental Patterning by Mechanical Signals in Arabidopsis. *Science* **322**,
787 1650–1655 (2008).
- 788 111. Fujita, S. *et al.* An Atypical Tubulin Kinase Mediates Stress-Induced Microtubule
789 Depolymerization in Arabidopsis. *Curr. Biol.* **23**, 1969–1978 (2013).
- 790 112. Oda, Y. & Fukuda, H. Initiation of Cell Wall Pattern by a Rho- and Microtubule-Driven Symmetry
791 Breaking. *Science* **337**, 1333–1336 (2012).
- 792 113. Lindeboom, J. J. *et al.* A mechanism for reorientation of cortical microtubule arrays driven by
793 microtubule severing. *Science* **342**, 1245533 (2013).
- 794 114. Qian, D. *et al.* Arabidopsis ADF5 promotes stomatal closure by regulating actin cytoskeleton
795 remodeling in response to ABA and drought stress. *J. Exp. Bot.* **70**, 435–446 (2019).
- 796 115. Takatani, S. *et al.* Microtubule Response to Tensile Stress Is Curbed by NEK6 to Buffer Growth
797 Variation in the Arabidopsis Hypocotyl. *Curr. Biol.* **30**, 1491-1503.e2 (2020).

- 798 116. Catterou, M. *et al.* Brassinosteroids, microtubules and cell elongation in *Arabidopsis thaliana*.
799 II. Effects of brassinosteroids on microtubules and cell elongation in the *bul1* mutant. *Planta*
800 **212**, 673–683 (2001).
- 801 117. Lanza, M. *et al.* Role of Actin Cytoskeleton in Brassinosteroid Signaling and in Its Integration
802 with the Auxin Response in Plants. *Dev. Cell* **22**, 1275–1285 (2012).
- 803 118. de Bang, L. *et al.* Brassinosteroids Inhibit Autotropic Root Straightening by Modifying
804 Filamentous-Actin Organization and Dynamics. *Front. Plant Sci.* **11**, (2020).
- 805 119. Blume, Ya. B., Krasylenko, Yu. A. & Yemets, A. I. Effects of phytohormones on the cytoskeleton
806 of the plant cell. *Russ. J. Plant Physiol.* **59**, 515–529 (2012).
- 807 120. Wang, X. *et al.* *Arabidopsis* MICROTUBULE DESTABILIZING PROTEIN40 Is Involved in
808 Brassinosteroid Regulation of Hypocotyl Elongation. *Plant Cell* **24**, 4012–4025 (2012).
- 809 121. Wang, J. *et al.* Brassinosteroid signals cooperate with katanin-mediated microtubule severing
810 to control stamen filament elongation. *EMBO J.* **n/a**, e111883 (2022).
- 811 122. Ambrose, C., Allard, J. F., Cytrynbaum, E. N. & Wasteneys, G. O. A CLASP-modulated cell edge
812 barrier mechanism drives cell-wide cortical microtubule organization in *Arabidopsis*. *Nat.*
813 *Commun.* **2**, 430 (2011).
- 814 123. Ruan, Y. *et al.* The Microtubule-Associated Protein CLASP Sustains Cell Proliferation through a
815 Brassinosteroid Signaling Negative Feedback Loop. *Curr. Biol.* **28**, 2718–2729.e5 (2018).
- 816 124. Ambrose, C. *et al.* CLASP Interacts with Sorting Nexin 1 to Link Microtubules and Auxin
817 Transport via PIN2 Recycling in *Arabidopsis thaliana*. *Dev. Cell* **24**, 649–659 (2013).
- 818 125. Jaillais, Y., Fobis-Loisy, I., Miège, C. & Gaude, T. Evidence for a sorting endosome in *Arabidopsis*
819 root cells. *Plant J.* **53**, 237–247 (2008).
- 820 126. De Rybel, B. *et al.* Chemical Inhibition of a Subset of *Arabidopsis thaliana* GSK3-like Kinases
821 Activates Brassinosteroid Signaling. *Chem. Biol.* **16**, 594–604 (2009).
- 822 127. Min, Y. K. *et al.* New lead compounds for brassinosteroid biosynthesis inhibitors. *Bioorg. Med.*
823 *Chem. Lett.* **9**, 425–430 (1999).

- 824 128. Liu, X. *et al.* Brassinosteroids regulate pavement cell growth by mediating BIN2-induced
825 microtubule stabilization. *J. Exp. Bot.* **69**, 1037–1049 (2018).
- 826 129. Zhang, C. *et al.* ROPGAP-dependent interaction between brassinosteroid and ROP2-GTPase
827 signaling controls pavement cell shape in Arabidopsis. *Curr. Biol.* **32**, 518-531.e6 (2022).
- 828 130. Lauster, T. *et al.* Arabidopsis pavement cell shape formation involves spatially confined
829 ROPGAP regulators. *Curr. Biol.* **32**, 532-544.e7 (2022).
- 830 131. Feiguelman, G., Fu, Y. & Yalovsky, S. ROP GTPases Structure-Function and Signaling Pathways.
831 *Plant Physiol.* **176**, 57–79 (2018).
- 832 132. Fu, Y., Gu, Y., Zheng, Z., Wasteneys, G. & Yang, Z. Arabidopsis Interdigitating Cell Growth
833 Requires Two Antagonistic Pathways with Opposing Action on Cell Morphogenesis. *Cell* **120**,
834 687–700 (2005).
- 835 133. Lin, D. *et al.* Rho GTPase Signaling Activates Microtubule Severing to Promote Microtubule
836 Ordering in Arabidopsis. *Curr. Biol.* **23**, 290–297 (2013).
- 837 134. Deng, Z. *et al.* A Proteomic Study of Brassinosteroid Response in Arabidopsis. *Mol. Cell.*
838 *Proteomics MCP* **6**, 2058–2071 (2007).
- 839 135. Kim, T.-W. *et al.* Mapping the signaling network of BIN2 kinase using TurboID-mediated biotin
840 labeling and phosphoproteomics. *Plant Cell* **35**, 975–993 (2023).
- 841 136. Sánchez-Rodríguez, C. *et al.* BRASSINOSTEROID INSENSITIVE2 negatively regulates cellulose
842 synthesis in Arabidopsis by phosphorylating cellulose synthase 1. *Proc. Natl. Acad. Sci.* **114**,
843 3533–3538 (2017).
- 844 137. Somssich, M. *et al.* Brassinosteroids Influence Arabidopsis Hypocotyl Gravidresponses through
845 Changes in Mannans and Cellulose. *Plant Cell Physiol.* **62**, 678–692 (2021).
- 846 138. Wang, W., Sun, Y., Li, G. & Zhang, S. Brassinosteroids promote parenchyma cell and secondary
847 xylem development in sugar beet (*Beta vulgaris* L.) root. *Plant Direct* **5**, e340 (2021).
- 848 139. Li, M. *et al.* Brassinosteroid signaling restricts root lignification by antagonizing SHORT-ROOT
849 function in Arabidopsis. *Plant Physiol.* **190**, 1182–1198 (2022).

- 850 140. Zhuang, Y. *et al.* MYB42 inhibits hypocotyl cell elongation by coordinating brassinosteroid
851 homeostasis and signalling in *Arabidopsis thaliana*. *Ann. Bot.* **129**, 403–413 (2021).
- 852 141. Vatén, A. *et al.* Callose Biosynthesis Regulates Symplastic Trafficking during Root Development.
853 *Dev. Cell* **21**, 1144–1155 (2011).
- 854 142. Sager, R. E. & Lee, J.-Y. Plasmodesmata at a glance. *J. Cell Sci.* **131**, jcs209346 (2018).
- 855 143. Wang, Y. *et al.* Plasmodesmata mediate cell-to-cell transport of brassinosteroid hormones.
856 *Nat. Chem. Biol.* 1–11 (2023) doi:10.1038/s41589-023-01346-x.
- 857 144. Clouse, S. D. Brassinosteroids. *Arab. Book* **2002**, (2002).
- 858 145. Vukašinić, N. *et al.* Local brassinosteroid biosynthesis enables optimal root growth. *Nat.*
859 *Plants* **7**, 619–632 (2021).
- 860 146. Takahashi, K., Hirata, S., Kido, N. & Katou, K. Wall-Yielding Properties of Cell Walls from
861 Elongating Cucumber Hypocotyls in Relation to the Action of Expansin. *Plant Cell Physiol.* **47**,
862 1520–1529 (2006).
- 863 147. Friml, J. *et al.* ABP1–TMK auxin perception for global phosphorylation and auxin canalization.
864 *Nature* **609**, 575–581 (2022).
- 865 148. Spartz, A. K. *et al.* SAUR Inhibition of PP2C-D Phosphatases Activates Plasma Membrane H⁺-
866 ATPases to Promote Cell Expansion in *Arabidopsis*[C][W]. *Plant Cell* **26**, 2129–2142 (2014).
- 867 149. Caesar, K. *et al.* A fast brassinolide-regulated response pathway in the plasma membrane of
868 *Arabidopsis thaliana*. *Plant J.* **66**, 528–540 (2011).
- 869 150. Minami, A., Takahashi, K., Inoue, S., Tada, Y. & Kinoshita, T. Brassinosteroid Induces
870 Phosphorylation of the Plasma Membrane H⁺-ATPase during Hypocotyl Elongation in
871 *Arabidopsis thaliana*. *Plant Cell Physiol.* **60**, 935–944 (2019).
- 872 151. Li, M. *et al.* SAUR15 interaction with BRI1 activates plasma membrane H⁺-ATPase to promote
873 organ development of *Arabidopsis*. *Plant Physiol.* **189**, 2454–2466 (2022).

- 874 152. Großholz, R. *et al.* Computational modeling and quantitative physiology reveal central
875 parameters for brassinosteroid-regulated early cell physiological processes linked to elongation
876 growth of the Arabidopsis root. *eLife* **11**, e73031 (2022).
- 877 153. Liu, L. *et al.* Extracellular pH sensing by plant cell-surface peptide-receptor complexes. *Cell* **185**,
878 3341-3355.e13 (2022).
- 879 154. Diaz-Ardila, H. N., Gujas, B., Wang, Q., Moret, B. & Hardtke, C. S. pH-dependent CLE peptide
880 perception permits phloem differentiation in Arabidopsis roots. *Curr. Biol.* **33**, 597-605.e3
881 (2023).
- 882 155. Doblas, V. G., Gonneau, M. & Höfte, H. Cell wall integrity signaling in plants: Malectin-domain
883 kinases and lessons from other kingdoms. *Cell Surf.* **3**, 1–11 (2018).
- 884 156. Mishra, R., Minc, N. & Peter, M. Cells under pressure: how yeast cells respond to mechanical
885 forces. *Trends Microbiol.* **30**, 495–510 (2022).
- 886 157. Engelsdorf, T. *et al.* The plant cell wall integrity maintenance and immune signaling systems
887 cooperate to control stress responses in Arabidopsis thaliana. *Sci Signal* **11**, eaao3070 (2018).
- 888 158. Wolf, S. Cell Wall Signaling in Plant Development and Defense. *Annu. Rev. Plant Biol.* **73**, 323–
889 353 (2022).
- 890 159. Haswell, E. S., Peyronnet, R., Barbier-Brygoo, H., Meyerowitz, E. M. & Frachisse, J.-M. Two
891 MscS Homologs Provide Mechanosensitive Channel Activities in the Arabidopsis Root. *Curr.*
892 *Biol.* **18**, 730–734 (2008).
- 893 160. Mecchia, M. A. *et al.* RALF4/19 peptides interact with LRX proteins to control pollen tube
894 growth in Arabidopsis. *Science* **358**, 1600–1603 (2017).
- 895 161. Wolf, S. *et al.* A receptor-like protein mediates the response to pectin modification by
896 activating brassinosteroid signaling. *Proc. Natl. Acad. Sci.* **111**, 15261–15266 (2014).
- 897 162. Ge, Z. *et al.* LLG2/3 Are Co-receptors in BUPS/ANX-RALF Signaling to Regulate Arabidopsis
898 Pollen Tube Integrity. *Curr. Biol.* **29**, 3256-3265.e5 (2019).

- 899 163. Hématy, K. *et al.* A Receptor-like Kinase Mediates the Response of Arabidopsis Cells to the
900 Inhibition of Cellulose Synthesis. *Curr. Biol.* **17**, 922–931 (2007).
- 901 164. Miyazaki, S. *et al.* ANXUR1 and 2, Sister Genes to FERONIA/SIRENE, Are Male Factors for
902 Coordinated Fertilization. *Curr. Biol.* **19**, 1327–1331 (2009).
- 903 165. Tang, W. *et al.* Mechano-transduction via the pectin-FERONIA complex activates ROP6 GTPase
904 signaling in Arabidopsis pavement cell morphogenesis. *Curr. Biol.* **32**, 508-517.e3 (2022).
- 905 166. Ge, Z. *et al.* Arabidopsis pollen tube integrity and sperm release are regulated by RALF-
906 mediated signaling. *Science* **358**, 1596–1600 (2017).
- 907 167. Haas, K. T., Wightman, R., Meyerowitz, E. M. & Peaucelle, A. Pectin homogalacturonan
908 nanofilament expansion drives morphogenesis in plant epidermal cells. *Science* **367**, 1003–
909 1007 (2020).
- 910 168. Wolf, S., Mravec, J., Greiner, S., Mouille, G. & Höfte, H. Plant Cell Wall Homeostasis Is Mediated
911 by Brassinosteroid Feedback Signaling. *Curr. Biol.* **22**, 1732–1737 (2012).
- 912 169. Holzwart, E. *et al.* A Mutant Allele Uncouples the Brassinosteroid-Dependent and Independent
913 Functions of BRASSINOSTEROID INSENSITIVE 11[OPEN]. *Plant Physiol.* **182**, 669–678 (2020).
- 914 170. Garnelo Gómez, B., Holzwart, E., Shi, C., Lozano-Durán, R. & Wolf, S. Phosphorylation-
915 dependent routing of RLP44 towards brassinosteroid or phytosulfokine signalling. *J. Cell Sci.*
916 **134**, jcs259134 (2021).
- 917 171. Garnelo Gómez, B., Holzwart, E., Shi, C., Lozano-Durán, R. & Wolf, S. Phosphorylation-
918 dependent routing of RLP44 towards brassinosteroid or phytosulfokine signalling. *J. Cell Sci.*
919 **134**, jcs259134 (2021).
- 920 172. Holzwart, E. *et al.* BRI1 controls vascular cell fate in the Arabidopsis root through RLP44 and
921 phytosulfokine signaling. *Proc. Natl. Acad. Sci.* **115**, 11838–11843 (2018).
- 922 173. Yue, Z.-L. *et al.* The receptor kinase OsWAK11 monitors cell wall pectin changes to fine-tune
923 brassinosteroid signaling and regulate cell elongation in rice. *Curr. Biol.* **32**, 2454-2466.e7
924 (2022).

- 925 174. Huerta, A. I. *et al.* The WAK-like protein RFO1 acts as a sensor of the pectin methylation status
926 in Arabidopsis cell walls to modulate root growth and defense. *Mol. Plant* **16**, 865–881 (2023).
- 927 175. Ubeda-Tomás, S. *et al.* Root growth in Arabidopsis requires gibberellin/DELLA signalling in the
928 endodermis. *Nat. Cell Biol.* **10**, 625–628 (2008).
- 929 176. Uchida, N. *et al.* Regulation of inflorescence architecture by intertissue layer ligand–receptor
930 communication between endodermis and phloem. *Proc. Natl. Acad. Sci.* **109**, 6337–6342
931 (2012).
- 932 177. Fridman, Y. *et al.* Root growth is modulated by differential hormonal sensitivity in neighboring
933 cells. *Genes Dev.* **28**, 912–920 (2014).
- 934 178. Fridman, Y. *et al.* The root meristem is shaped by brassinosteroid control of cell geometry. *Nat.*
935 *Plants* **7**, 1475–1484 (2021).
- 936 179. Kelly-Bellow, R. *et al.* Brassinosteroid coordinates cell layer interactions in plants via cell wall
937 and tissue mechanics. *Science* **380**, 1275–1281 (2023).
- 938 180. Azpiroz, R., Wu, Y., LoCascio, J. C. & Feldmann, K. A. An Arabidopsis Brassinosteroid-Dependent
939 Mutant Is Blocked in Cell Elongation. *Plant Cell* **10**, 219–230 (1998).
- 940 181. Choe, S. *et al.* The DWF4 gene of Arabidopsis encodes a cytochrome P450 that mediates
941 multiple 22 α -hydroxylation steps in brassinosteroid biosynthesis. *Plant Cell* **10**, 231–243
942 (1998).
- 943 182. Grove, M. D. *et al.* Brassinolide, a plant growth-promoting steroid isolated from *Brassica napus*
944 pollen. *Nature* **281**, 216–217 (1979).
- 945

Figure 1 Schematic representation of BRI1 levels and activity controls at the plasma membrane

- a. BRI1 activity is negatively regulated by numerous protein partners such as BSKs, BKI1, and BIK1, all interacting with BRI1 at the plasma membrane (PM) when the BR pathway is off. BAK1 is also sequestered away from BRI1 by BIR3. BRs induce the formation of the BRI1-BAK1 complex and initiate downstream signaling, resulting in the release of inhibitors. The BRI1 pool at the PM is adjusted by **the balance of exocytosis, endocytosis and degradation**. Two endocytotic pathways, the clathrin-mediated and -independent endocytosis, remove BRI1 from the PM. Endocytosed BRI1 reaches the trans-Golgi network/early endosomes (TGN/EE) and is transported to the lytic vacuole for degradation.
- b. Post-translational modifications on lysine residues (K) of BRI1 cytoplasmic domain are signals regulating its dynamics. The UBC35/36 (E2) and PUB12/13 (E3) machinery leads to Lys63 (K63) polyubiquitination of BRI1, which is enhanced by BRs, driving BRI1 endocytosis. The UBP12/13 deubiquitinating enzymes stabilize BRI1 at the PM by removing ubiquitin moieties from BRI1. SUMOylation also stabilizes BRI1 at the PM, and deSUMOylation by DeSi3a enhances BRI1 endocytosis. Since both PTMs target the same residues, competition between PTMs likely happens to fine tune BRI1 cell surface levels.
- c. BRI1 and FLS2 form distinct nanodomains within the PM, acting as platforms to amplify signal and drive responses towards development or defense. BIK1 has different impacts on the two pathways. BRI1-BIK1 interaction colocalizes with CMTs and negatively affects the BR pathway. After sensing BRs, the BRI1-BAK1 complex and BIK1 trans-phosphorylate each other, leading to the release of the BIK1 in the cytosol. Consequently, the negative regulator BIN2 is degraded by the proteasome, allowing BES1/BZR1 transcription factors to initiate transcriptional changes. Conversely, the BIK1 phosphorylation induced by FLS2 activates the MAPK cascade and defense responses.

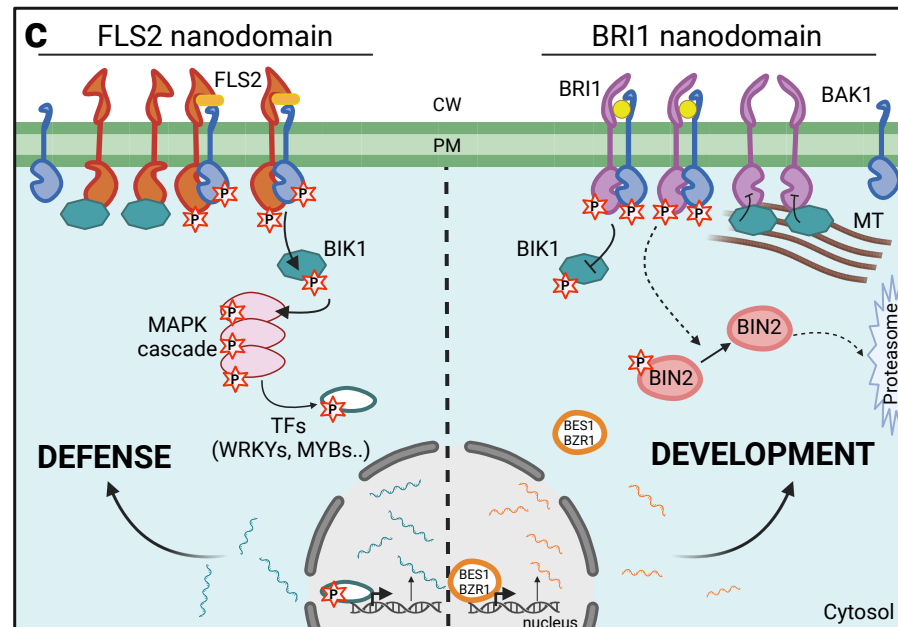
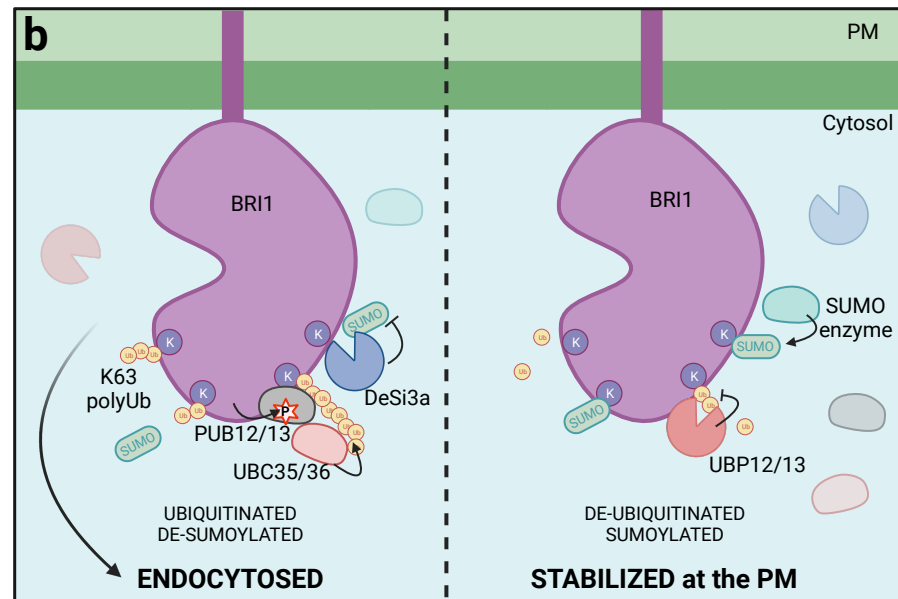
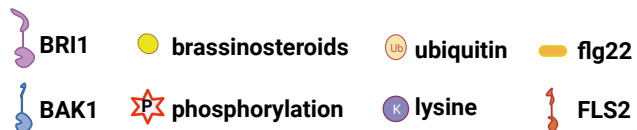
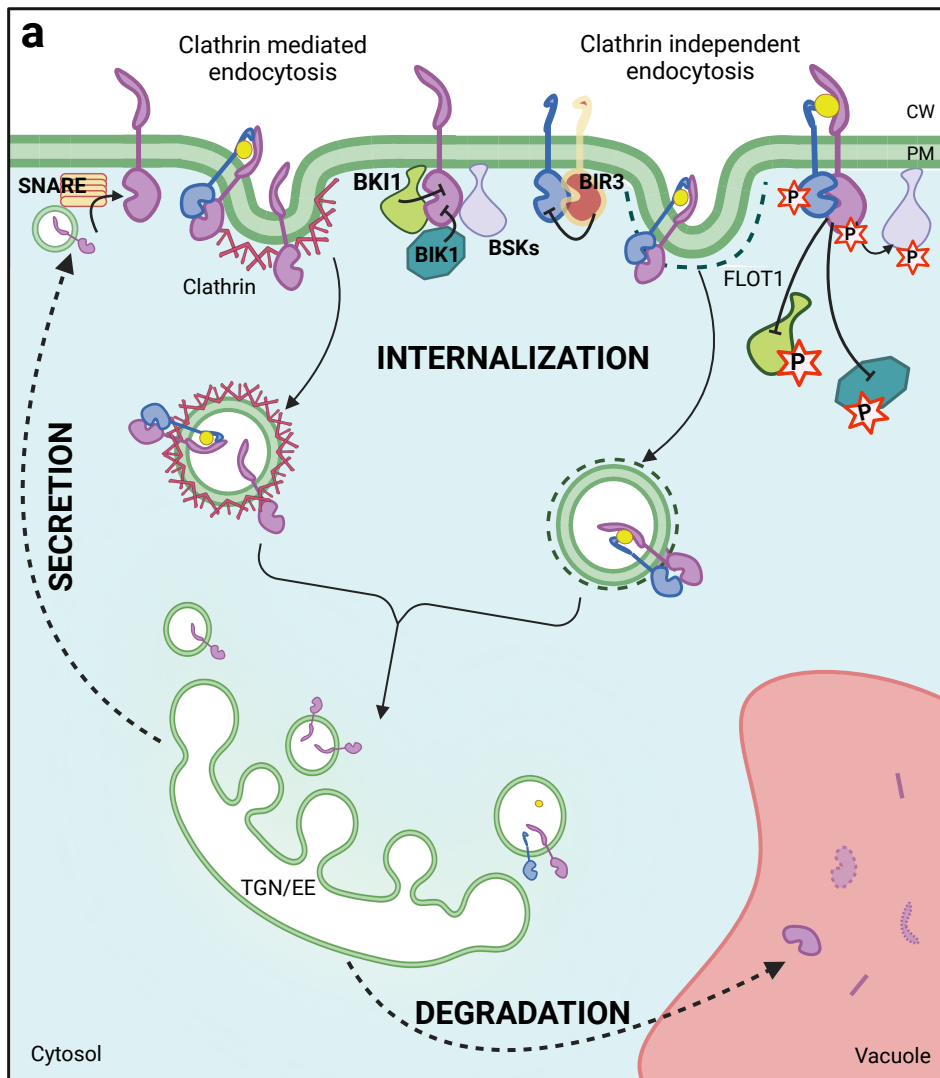
Figure 2 Schematic representation of cell surface regulation by the BR pathway in *Arabidopsis thaliana* seedling development

- a. BRs modulate the formation of lobes and indentations in the leave pavement cells. In indent areas, the BR pathway is locally off, allowing BIN2 to be active and to phosphorylate PHGAPs, inhibitors of ROP2, specifically localized at the anticlinal face of indent regions in a microtubule- and BIN2-dependent manner. ROP6 activation acts on the CMTs through its effector RIC1/KTN, making the convex form. BIN2 also phosphorylates tubulins *in vitro*, but its effects are still unknown. In the lobe regions, BIN2 is inhibited by the canonical BR pathway, leading to the accumulation of an active form of ROP2, which acts on the actin cytoskeleton through RIC4 and promotes expansion. Reciprocal inhibitions between ROP2 and ROP6 also maintain spatially restricted ROP activation.
- b. BR-induced apoplastic acidification through AHA1 promotes cell wall swelling in hypocotyl cells. In the absence of BRs, PP2C inhibits AHA1, leading to less acidic pH conditions. When BR signaling turns on, *SAUR9/19*, targets of BES1/BZR1, are transcriptionally upregulated and inhibit PP2C, supporting apoplastic acidification. Furthermore, BRI1 forms a complex with SAUR15 and AHA1, increasing AHA1 phosphorylation and enhancing proton ATPase activity, resulting in altered CW enzyme activities and membrane polarization.
- c. Cell wall integrity sensing in root epidermal cells partially depends on BRI1. PMEs and their inhibitor PMEIs control the degree of homogalacturonan methylation. PME inhibition results in a higher ratio of methylated pectin. The altered pectin methylation ratio activates the RLP44-BAK1-BRI1 complex, probably through RFO1. *PMEs* are transcriptionally upregulated by BES1/BZR1 directly, balancing the ratio between the two forms of homogalacturonan.
- d. BRI1 and CLASP form a negative feedback loop. When the BR pathway is off, CLASP allows the transport of BRI1 to the PM via the SNX1 retromer that depends on the MT network. In the presence of BRs, *CLASP* transcription is directly downregulated by BES1/BZR1, leading to the

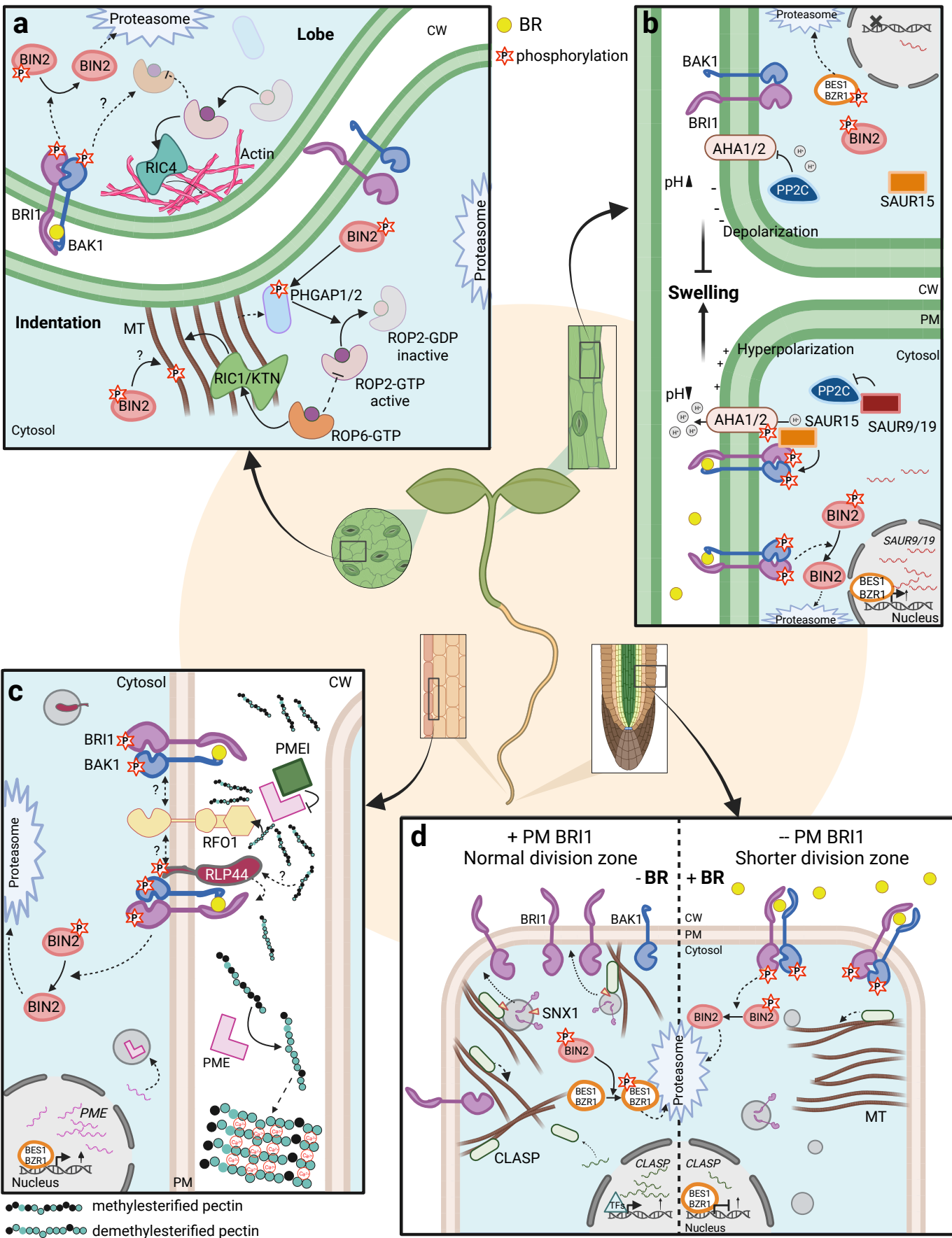
reorientation of the MTs and less BRI1 transport to the PM. This feedback loop finetunes the division zone size in the root tips.

Figure 3 BR controls the feedback loop coupled by several mechanisms

- a.** BRI1 and CLASP constitute a negative feedback loop by a transcription-vesicle transport coupled mechanism. Please also refer to Fig. 2d for the detailed mechanisms.
- b.** BIN2 kinase enhances the ROP-MT feedback loop through PHGAP focalization at indent sites and activation (left). In the lobe areas, active ROP2 promotes actin cytoskeleton network formation (right). Active ROP2 and ROP6 are highlighted by color. Please also refer to Fig. 2a for the detailed mechanisms.



Delesalle et al. Figure 1



Delesalle et al. Figure 2

