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Charlotte Delesalle, Grégory Vert, Satoshi FUJITA

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

3

4 Charlotte Delesalle<sup>1</sup>, Grégory Vert<sup>1</sup> and Satoshi Fujita<sup>1\*</sup>

5

6 <sup>1</sup> 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](mailto:satoshi.fujita@univ-tlse3.fr)

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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 GPCRs<sup>1</sup> and 58 RTKs<sup>2</sup> in humans),  
41 while plants developed a wide variety of RLKs (over 600 RLK-family genes in *Arabidopsis*<sup>3</sup>  
42 and over 1100 RLK-family genes in rice<sup>4</sup>). Among them, the brassinosteroid (BR) hormone  
43 receptor BRASSINOSTEROID INSENSITIVE1 (BRI1)<sup>5</sup> 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 bri1* or other BR-defective mutants  
49 show extreme dwarfism<sup>5,6</sup>, cell proliferation defects<sup>7</sup>, abnormal vascular tissue differentiation<sup>8</sup>,  
50 or impaired male fertility<sup>9,10</sup>. Not limited to developmental or reproductive defects, BR-related  
51 mutants also display impaired resistance to biotic and abiotic stresses<sup>11,12</sup>. 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<sup>13,14</sup>. 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 homologs<sup>15</sup>. 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 agriculture<sup>16</sup>.

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<sup>17,18</sup>. As already  
62 reviewed in many articles<sup>19-21</sup>, 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) kinase<sup>22</sup> suppresses BR-

65 triggered transcriptional responses by phosphorylating the transcription factors BES1 and  
66 BZR1 (BRASSINOSTEROID-INSENSITIVE EMS SUPPRESSOR1 and BRASSINAZOLE  
67 RESISTANT 1)<sup>23,24</sup>. 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 promoters<sup>25</sup>, nuclear export/cytoplasmic retention<sup>26-29</sup>, or proteasome-dependent  
70 degradation<sup>30</sup>. The presence of BRs induces the formation of the BRI1-BAK1 (BRI1  
71 ASSOCIATED KINASE, from LRR-RLK subfamily II) receptor complex<sup>31-33</sup>. 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)<sup>34-36</sup>. These RLCKs, in turn, phosphorylate and activate the BSU1 (BRI1  
75 SUPPRESSOR 1) phosphatase that dephosphorylates BIN2, leading to BIN2 deactivation and  
76 destabilization<sup>37,38</sup>. Consequently, BES1 and BZR1 transcription factors are dephosphorylated  
77 by PP2A phosphatases<sup>39</sup>, resulting in their accumulation in the nucleus where they control the  
78 transcription of thousands of BR-responsive genes<sup>25,27,28,38,40-42</sup>. 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 signaling<sup>43-47</sup>. 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 lumen<sup>48</sup>. 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 activity<sup>49</sup>. 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 status<sup>50-53</sup>, 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 evidence<sup>54</sup>.

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 form<sup>54-56</sup> (**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<sup>57,58</sup>. 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<sup>51,59</sup>.

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 BRI1<sup>60</sup>. BRI1 endocytosis is executed by the AP-2- or T-PLATE-dependent clathrin-mediated  
110 endocytosis (CME)<sup>50,61</sup>, and the clathrin-independent pathway through FLOT1 (flotillin1)<sup>62</sup>  
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<sup>52,63</sup>.

115 One important mechanism leading to BRI1 receptor internalization is polyubiquitination (**Fig.**  
116 **1b**). More precisely, endocytosis of BRI1 largely relies on Lys63 (K63) polyubiquitination<sup>51</sup>,  
117 as seen for other PM proteins<sup>64-66</sup>. 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*<sup>53,59,65</sup>. *In vitro* experiments  
120 showed that PUB13 phosphorylation by BRI1 is necessary for activating its ubiquitin ligase  
121 activity<sup>59</sup>. 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<sup>51,59</sup>. 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 signaling<sup>51</sup>. 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 endocytosis<sup>67</sup> (**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 receptor<sup>68</sup>, 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<sup>69,70</sup>. 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 temperatures<sup>70</sup> (**Fig. 1b**).  
138 Interestingly, SUMO was also reported in the case of FLS2<sup>71</sup>. *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 pathways<sup>71</sup>. 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 deSUMOylation<sup>70</sup>. DeSUMOylated BRI1 shows higher affinity to BIK1, a negative regulator  
147 of BR signaling<sup>72</sup>, 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 meet<sup>73</sup>. 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<sup>74,75</sup>.  
160 BRI1 also forms nanodomains at the PM, serving as hot spots for flotillin-dependent  
161 endocytosis<sup>62</sup>. 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 formation<sup>62</sup>. The BRI1 co-receptor, BAK1,  
164 also forms nanodomains at the PM, but its relevance for BR signaling remains to be  
165 characterized<sup>76</sup>.

166 Some components in the BR pathways, like BIK1, are indeed shared by immune signaling  
167 receptors, such as FLS2 or EFR<sup>72,77-79</sup>, enabling plants to cross-regulate each pathway and to  
168 provide trade-off mechanisms between defense and growth<sup>72,80,81</sup>. 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 PM<sup>82</sup>. 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 ligands<sup>83</sup>. 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 cytosol<sup>73</sup>. 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 humans<sup>84</sup>. In plants, only a few examples are found for signal scaffolding  
188 proteins, but the TTL (TETRATRICOPEPTIDE THIOREDOXIN-LIKE) family proteins<sup>85</sup>  
189 and BSK3 (BR-SIGNALING KINASE 3)<sup>86</sup> 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<sup>85,86</sup>. Mechanistically, both interact with central molecules in BR signaling, such as  
192 BRI1, BSK1, BSU1, BIN2, and BZR1 for TTL3<sup>85,86</sup>, enabling smooth signal relay. TTL  
193 proteins interact with these components through TPR (TETRATRICOPEPTIDE REPEAT)  
194 domains<sup>85</sup>, while TPR domains in BSK3 are not required for its scaffolding activity, except



195 hetero/homo-oligomerization with BSK1/3<sup>86</sup>. 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*<sup>86</sup>. BR signaling enhances this scaffolding mechanism by upregulating *TTL* transcription  
198 and promoting relocation from the cytosol to the PM<sup>85</sup>.

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<sup>87,88</sup>. 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 approaches<sup>89</sup>. BRI1 homodimerization is further supported by *in vitro*  
209 data where purified BRI1 cytoplasmic kinase domain can homodimerize<sup>18</sup>, but not the  
210 extracellular domain *in vitro*, regardless of the presence of ligands<sup>90</sup>. Additionally, a BR-  
211 independent homodimerization of BRI1 was brought to light using imaging analysis<sup>62,91</sup>. 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<sup>89,92</sup>. Another  
215 autophosphorylation site, Ser891, in the ATP-binding domain strongly suppresses the auto-  
216 phosphorylation on Tyr residues, resulting in BR signal inhibition<sup>93</sup>.

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 partners<sup>94-96</sup> (**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 BRI1<sup>97</sup>. Interestingly, BIR3 also binds to BRI1, arguing for the  
222 existence of additional BIR3-dependent regulatory mechanisms targeting BRI1<sup>98</sup>.

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 activity<sup>99</sup> (**Fig. 1a**). BKI1 prevents the complex formation of BRI1 and  
226 BAK1, and its inhibitory activity largely depends on its association with PM<sup>100</sup>. 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<sup>100,101</sup>. 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<sup>99,100</sup>. 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 pathway<sup>16</sup>. Other RLK proteins  
233 are known to be regulated either positively or negatively by MAKR family proteins<sup>102,103</sup>,  
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 development<sup>104</sup>.

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<sup>105,106</sup>.  
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 resides<sup>105</sup>. BR signaling also  
245 transcriptionally upregulates *SBI1* expression, which in turn dampens signaling<sup>105</sup>. SBI1 and  
246 PP2A are also required for BRI1 degradation<sup>105</sup>, 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 downregulation<sup>107</sup>. 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)<sup>108</sup>, 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 trafficking<sup>109</sup>.

265 The cytoskeleton is a highly dynamic biopolymer, and its organization is tightly controlled by  
266 external and internal cues<sup>110-113</sup>. 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<sup>114,115</sup>, for example. Phytohormones, including BRs, are representative  
269 endogenous regulators of the cytoskeleton<sup>116</sup>. BRs modulate the organization and dynamics of  
270 MFs in response to gravity in *Arabidopsis*<sup>117</sup>, and other plants such as *Zea mays* and *Medicago*  
271 *truncatula*<sup>118</sup>. However, the detailed molecular mechanisms underlying such regulation are still  
272 obscure<sup>119</sup>. 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 BRs<sup>19</sup>. 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<sup>120,121</sup>.

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 zone<sup>122</sup>. 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 MTs<sup>122-125</sup>.  
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<sup>126,127</sup>  
302 leads to MT disorganization and altered jigsaw puzzle shapes of pavement cells<sup>128</sup>. 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**)<sup>129,130</sup>. 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, respectively<sup>131</sup>. 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<sup>129,130</sup>. PHGAPs target ROP2, which  
314 prevents MT alignment in indent areas by inhibiting RIC1 (ROP Interactive CRIB motif-  
315 containing protein1)<sup>132</sup>. PHGAPs are preferentially accumulated at the anticlinal face of the  
316 pavement cell indent regions in MT- and BIN2-dependent manners<sup>129,130</sup>. 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 module<sup>133</sup>. On the  
319 other hand, when the BR signal is on, BIN2 is inactivated and degraded, allowing PHGAPs to  
320 stay dormant<sup>129</sup>. 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  $\alpha$  and  $\beta$ -tubulins *in vitro*<sup>128</sup>, 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 characterized<sup>134</sup>. Furthermore, a recent proximal labeling screen  
329 identified potential substrates of BIN2<sup>135</sup>. 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<sup>41,42</sup>. In addition to the  
343 transcriptional down-regulation of *CESA1*<sup>42</sup>, BIN2 also phosphorylates CESA1 and negatively  
344 regulates its activity<sup>136</sup>. BR treatment also suppresses gravitropism response in etiolated  
345 hypocotyls by reorientating cellulose microfibrils and changes in cell wall components<sup>137</sup>.  
346 Besides, the BR pathway plays multiple roles in determining the onset timing of secondary cell  
347 wall deposition<sup>138–140</sup>. 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.<sup>141,142</sup>. BR signaling  
355 manipulates the PD permeability by inducing callose accumulation, thereby restricting the  
356 diffusion of molecules through PD<sup>143</sup>. 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 PM<sup>143</sup>.

360 Of note, PD permeability impacts BR biosynthesis<sup>143</sup>. 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 step<sup>144</sup>. 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 pathway<sup>145</sup>. Furthermore, full complementation of the *cpd* BR biosynthetic mutant by  
366 epidermis-specific expression of *CPD* also supports such relocation idea<sup>8</sup>. 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 movements<sup>143</sup>. Together with the negative transcriptional feedback of biosynthesis  
370 genes<sup>144</sup>, 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 conditions<sup>146</sup>. Auxins are well-known inducers of apoplast  
378 acidification acting through the ABP1 (AUXIN BINDING PROTEIN1)-TMKs  
379 (TRANSMEMBRANE KINASES)-H<sup>+</sup>ATPase module. TMKs interact with the AHA1/2 H<sup>+</sup>-  
380 ATPase and phosphorylate their penultimate residues (Thr948/947, respectively)<sup>46,47,147</sup>,  
381 whereas PP2C-D (PROTEIN PHOSPHATASE2C-D) deactivates AHA1/2 by  
382 dephosphorylation<sup>148</sup>.

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

392 shows reduced sensitivity to BRs in root length<sup>151</sup>. Similarly, *SAUR19* overexpression leads to  
393 extracellular acidification<sup>148</sup>. 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 complex<sup>152</sup>.  
401 Recent reports described that the altered pH changed receptor-peptide ligand binding<sup>153</sup> or  
402 ligand activities<sup>154</sup>. 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<sup>155,156</sup>. Plants do  
413 not have obvious homologs of these molecules, but solid evidence indicates that plants do have  
414 cell wall surveillance systems<sup>157,158</sup>. Recent studies indeed revealed that different classes of  
415 proteins, such as mechano-sensitive Ca<sup>2+</sup> channels<sup>159</sup>, cell wall proteins<sup>160</sup>, receptor-like  
416 proteins<sup>161</sup>, GPI-anchored proteins<sup>162</sup>, or receptor-like kinases<sup>163-166</sup>, function in cell wall  
417 sensing. Mutations in these proteins often lead to abnormal cell shapes, defects in cell growth,  
418 or pollen tube rupture<sup>158</sup>.

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 Ca<sup>2+</sup> 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 pectins<sup>167</sup>. 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 growth<sup>168</sup>. 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 phenotype<sup>168</sup>. 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<sup>168,169</sup>,  
432 suggesting the phenotype of *PMEI5* overexpression is a result of the enhanced BR signaling  
433 pathway<sup>168</sup>.

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 pathway<sup>161</sup>  
437 (**Fig. 2c**). RLP44 is phosphorylated in BR/cell wall homeostasis-dependent manners, which is  
438 required for its functionality in cell wall maintenance<sup>161,170</sup>. 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 context<sup>171</sup>. In parallel, RLP44 also  
441 strengthens the PSKR (phytosulfokine receptor)-BAK1 complex formation, which mediates  
442 procambium/xylem patterning together with BRI1<sup>172</sup>. This fact raises the question of how  
443 RLP44 balances BRI1 and PSKR activities. Indeed, plants harboring the *bri1<sup>cnu4</sup>* mutation  
444 display abnormal xylem pattern formation due to the imbalance of competition between  
445 BRI1<sup>cnu4</sup>, which has tighter interaction with RLP44, and PSKR<sup>169</sup>. Distinct from BR signaling,  
446 the phospho-status of RLP44 does not affect PSK signaling, suggesting RLP44  
447 phosphorylation status may not explain the competition<sup>170</sup>. Moreover, a recent study also  
448 suggested that BRI1 plays a role in the xylem patterning process partially independent of BRs<sup>55</sup>.  
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 signals<sup>173</sup>. 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 signaling<sup>174</sup>. 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 growth<sup>175</sup>. 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 growth<sup>176</sup>.

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 cellulose<sup>177</sup>, 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 size<sup>178</sup>. Of note, epidermal specific BRI1 expression in *bri1*  
483 mutant rescues the root growth and limits the stele area<sup>8</sup>. 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 shoots<sup>179</sup>. 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<sup>179–181</sup>. 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 *qua1-2* (*quasimodo 1-2*) in hypocotyl epidermal cells, suggesting BRs  
496 reduce epidermal constraint<sup>179</sup>. 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*<sup>182</sup>, 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 mechanisms<sup>21</sup>. 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

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541 Conceptualization, C.D., G.V., and S.F.; writing – original draft, C.D.; writing – review &  
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### 543 **Declaration of interests**

544 No interests are declared.

545

546

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### 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, BIK1, 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.

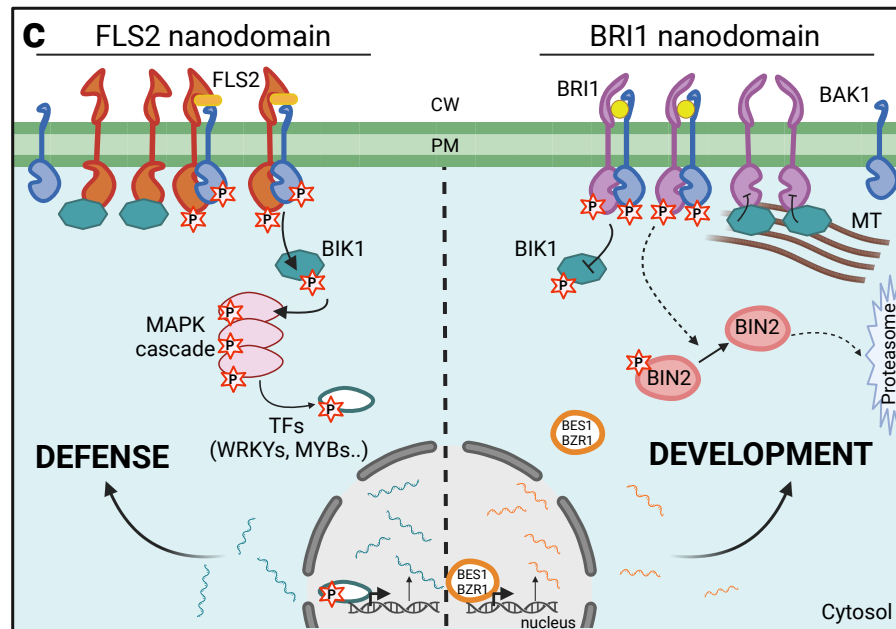
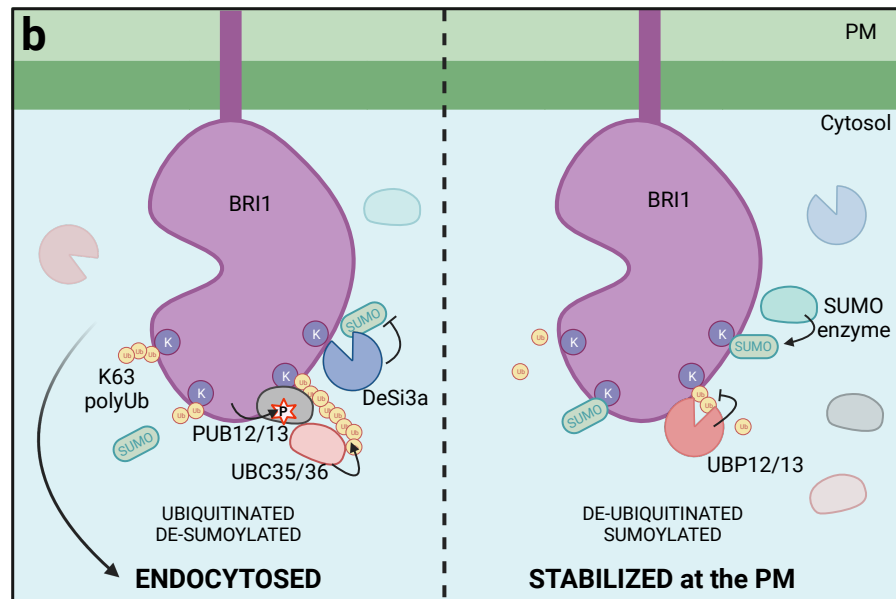
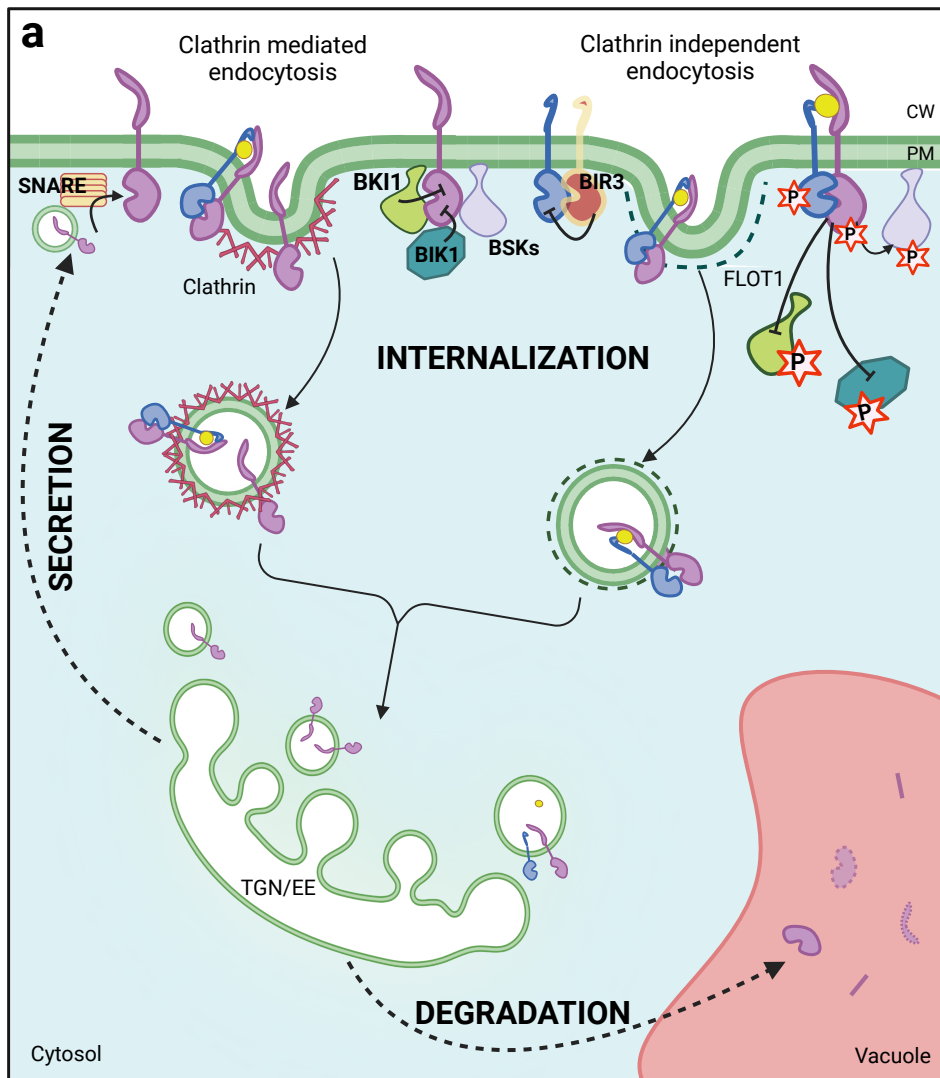
### Figure 2 Schematic representation of cell surface regulation by the BR pathway in *Arabidopsis thaliana* seedling development

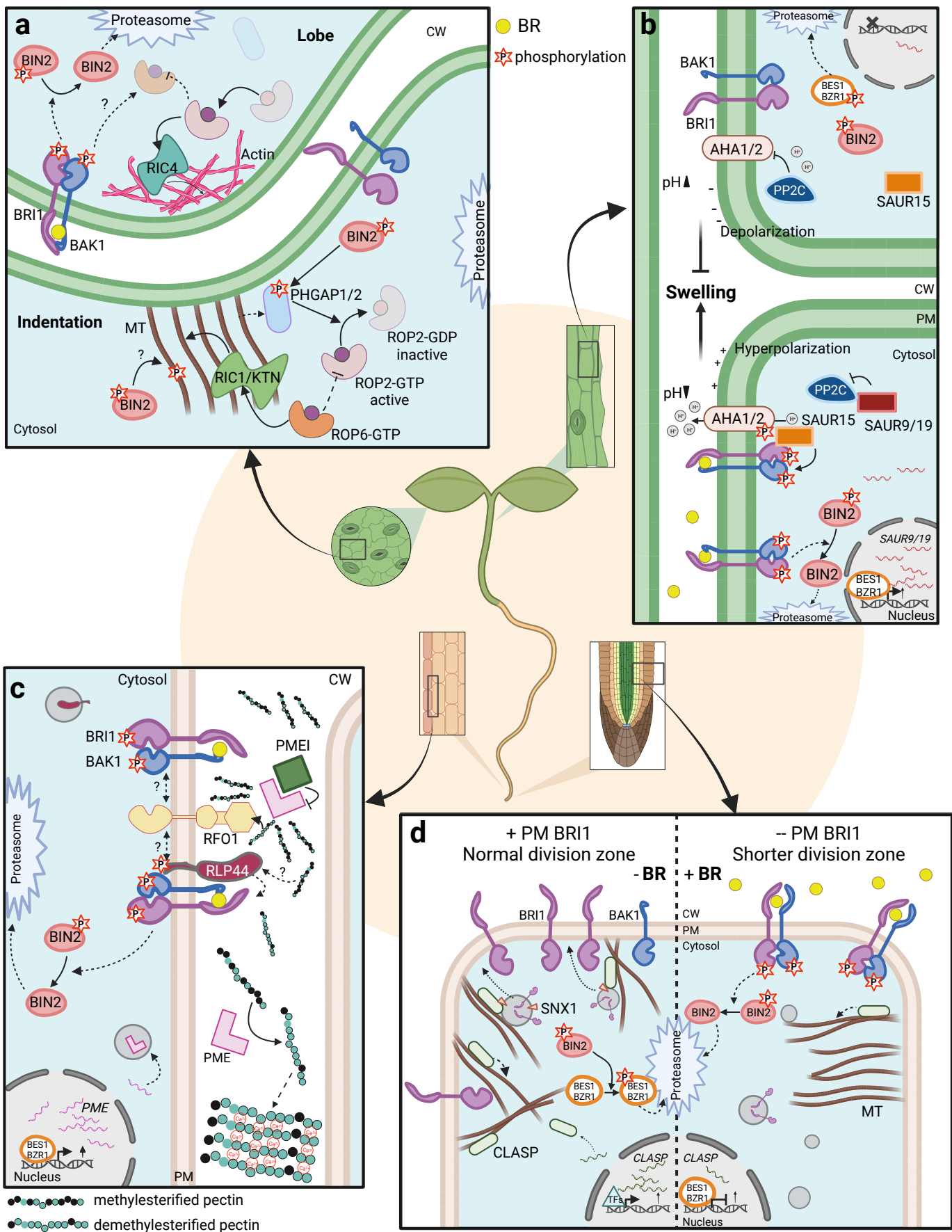
- a. BRs modulate the formation of lobes and indentations in the leave pavement cells. In indent areas, the BR pathway is locally off, allowing BIN2 to be active and to phosphorylate PHGAPs, inhibitors of ROP2, specifically localized at the anticlinal face of indent regions in a microtubule- and BIN2-dependent 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 2

