

1 Computational modeling and 2 quantitative cell physiology reveal 3 central parameters for the 4 brassinosteroid-regulated cell 5 growth of the *Arabidopsis* root

6 Ruth Großholz^{1,2†}, Friederike Wanke^{3†}, Nina Glöckner^{3†}, Luiselotte Rausch³,
7 Leander Rohr³, Stefan Scholl¹, Emanuele Scacchi³, Amelie-Jette Spazierer³, Lana
8 Shabala⁴, Sergey Shabala^{4,5}, Karin Schumacher¹, Ursula Kummer^{1,2*‡}, Klaus
9 Harter^{3*‡}

***For correspondence:**

[ursula.kummer@bioquant.
uni-heidelberg.de](mailto:ursula.kummer@bioquant.uni-heidelberg.de) (UK); [klaus.
harter@zmbp.uni-tuebingen.de](mailto:klaus.harter@zmbp.uni-tuebingen.de) (KH)

†These authors contributed
equally to this work

‡These authors contributed
equally to this work

10 ¹Centre for Organismal Studies, Heidelberg University, Germany; ²BioQuant,
11 Heidelberg University, Germany; ³Center for Molecular Biology of Plants, University of
12 Tübingen, Germany; ⁴Tasmanian Institute for Agriculture, University of Tasmania,
13 Australia; ⁵International Research Centre for Environmental Membrane Biology, Foshan
14 University, Foshan, China

16 **Abstract** Brassinosteroids (BR) are key hormonal regulators of plant development. However,
17 whereas the individual components of BR perception and signaling are well characterized
18 experimentally, the question of how they can act and whether they are sufficient to carry out the
19 critical function of cellular elongation remains open. Here, we combined computational modeling
20 with quantitative cell physiology to understand the dynamics of the plasma membrane
21 (PM)-localized BR response pathway during the initiation of cell elongation in the epidermis of the
22 *Arabidopsis* root tip. The model, consisting of ordinary differential equations, comprises the BR
23 induced hyperpolarization of the PM, the acidification of the apoplast and subsequent cell wall
24 swelling. We demonstrated that the competence of the root epidermal cells for the BR response
25 predominantly depends on the amount and activity of H⁺-ATPases in the PM. The model further
26 predicted that an influx of cations is required to compensate for the shift of positive charges
27 caused by the apoplastic acidification. A potassium channel was subsequently identified and
28 experimentally characterized, fulfilling this function. Thus, we established the landscape of
29 components and parameters capable of triggering and guiding cellular elongation through the
30 fast response to BRs, a central process in plant development.

32 Introduction

33 Brassinosteroids (BRs) are plant steroid hormones that regulate a great variety of physiological and
34 developmental processes including elongation growth as well as environmental adaptations (*Müs-*
35 *sig et al., 2002; Clouse, 2002; Lv and Li, 2020; Wolf, 2020*). To achieve this, BR signal transduction
36 is closely linked with a multitude of other signaling pathways (*Lv and Li, 2020*).

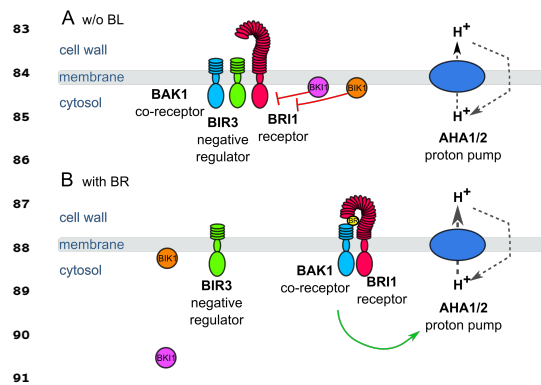
37 The canonical sequence of BR perception and signal transduction, which also leads to cell elon-
38 gation, is mediated by the plasma membrane (PM)-resident, nanocluster-organized receptor ki-
39 nase brassinosteroid-insensitive 1 (BRI1) and its co-receptor BRI1-activating kinase 1 (BAK1) as cen-
40 tral elements (*Lv and Li, 2020; Wolf, 2020*). The binding of BR to the receptor's extracellular domain
41 results in the re-arrangement of several proteins in the BRI1 nanocluster. This involves the release
42 of inhibitory mechanisms that include BRI1 kinase inhibitor 1 (BKI1) and BAK1-interacting receptor
43 like kinase 3 (BIR3) and leads to an increased interaction of BRI1 with BAK1 followed by a variety
44 of auto- and trans-phosphorylation events of their cytoplasmic domains. This cascade of events
45 eventually results in the establishment of the fully active BRI1 receptor complex.

46 Once the active complex is established, the BR response splits into two distinct downstream
47 pathways to trigger cell elongation (*Clouse, 2002, 2011*): A gene regulatory pathway leading to ex-
48 tensive transcriptional rearrangements that are realized *via* the kinase Brassinosteroid Insensitive
49 2 (BIN2), by the key transcription factors brassinazole resistant 1 (BZR1) and BR insensitive EMS
50 suppressor 1 (BES1) (*Lv and Li, 2020*). The second, faster pathway takes place in PM-resident BRI1
51 nanoclusters and leads to the upregulation of the proton pumping ATPase (AHA) activity (Fig. 1)
52 (*Caesar et al., 2011*). The enhanced activity of AHA results in the acidification of the apoplastic
53 space, hyperpolarization of the PM's membrane potential (E_m), activation of low pH-dependent
54 apoplastic enzymes, which finally weaken the wall's rigidity, causing wall swelling and eventually
55 the onset of cell elongation (*Elgass et al., 2009; Caesar et al., 2011; Witthöft et al., 2011; Witthöft*
56 *and Harter, 2011; Palmgren et al., 1991; Regenberget al., 1995; Baekgaard et al., 2005; Phyto et al.,*
57 *2019*). This sequence of signaling and reaction pathways allows for instance root cells in the elon-
58 gation zone (EZ) to grow four times their size in the meristematic zone (MZ) with a growth rate of
59 up to $0.7 \mu\text{m min}^{-1}$ (*Fasano et al., 2001; Verbelen et al., 2006*).

60 While the activation of the pathway is well understood qualitatively, the information on the
61 inactivation of the pathway is currently still sparse. The receptor BRI1 autophosphorylates at the
62 residue S891, which inhibits the receptor activity (*Oh et al., 2012*). However, the time-scale of this
63 phosphorylation is very slow, as it increases over the course of 12 h after stimulation with BR. The
64 dephosphorylation of this site is even slower, as residual phosphorylations can be detected 5 d
65 after inhibiting BR synthesis using brassinazole (*Oh et al., 2012*).

66 Despite the qualitative knowledge on the constituents, the BR perception and the canonical
67 signaling events, the dynamics of the system as a whole have yet to be examined quantitatively
68 (*Sankar et al., 2011; van Esse et al., 2012, 2013a,b; Allen and Ptashnyk, 2017*). Therefore, we em-
69 ployed computational modeling in combination with quantitative experimental data on the fast

70 BR response pathway in the PM, focusing on the epidermal cells of the *Arabidopsis* root tip as the
71 epidermis limits the rate of elongation (*Hacham et al., 2011*). The root tip is an excellent model sys-
72 tem for such a combined study because cells there first undergo a phase of cell division in the MZ
73 followed by a phase of growth in the EZ. The boundary from the MZ to the EZ is represented by the
74 transition zone (TZ). The formation of the TZ is characterized by the cytokinin-induced expression
75 of the *AHA1* and *AHA2* genes as a precondition for cell elongation in the EZ (*Pacifici et al., 2018*).
76 However BR is involved both in the control of both cell division and cell elongation in the differ-
77 ent zones, apparently also adding to the specific functional competence and behavior of the cells
78 along the axis of the root tip. However, the molecular determinants and processes establishing
79 this competence and their link to the cytokinin-caused gradient of growth competence are poorly
80 understood in terms of their quantitative dynamics. This lack of knowledge virtually provokes the
81 implementation of computational modeling.



82
83
84
85
86
87
88
89
90
91
92 **Figure 1.** Schematic overview of the key
93 constituents and processes of the plasma
94 membrane-associated fast BR response pathway
95 initiating early steps in cell elongation. A. Inactive
96 state: Co-localizing in a preformed nanocluster,
97 the inhibitors BIK1, BIK1 and BIR3 suppresses the
98 activity of BRI1 in the absence of BR keeping the
99 activity of H⁺ ATPases AHA1 and 2 at basic levels.
100 By interaction with BAK1, BIR3 blocks the access
101 of the co-receptor to BRI1. B. Active state: Upon
102 BR-binding to the receptor, the inhibitory
103 mechanisms of BIK1, BIK1 and BIR3 on BRI1 and
104 BAK1 are released causing the formation of the
105 active BRI1/BAK1 complex. The complex enhances
106 the AHA activity resulting in cell wall acidification,
107 plasma membrane hyperpolarization and
108 eventually onset of cell elongation. These key
109 constituents and qualitatively described processes
110 were used for the initial establishment of the
computational model at cellular.

While computational modeling is commonly used in biomedical research, it has been used much less frequently in the plant field (*Hübner et al., 2011; Holzheu and Kummer, 2020*). A few examples in plants include the modeling of auxin signaling (*Vernoux et al., 2011*) and transport pattern (*Band et al., 2014*), and parts of the BR signaling (*Sankar et al., 2011; van Esse et al., 2012, 2013a,b; Allen and Ptashnyk, 2017*). For instance, the modeling approach by van Esse *et al.* analyzed the link between the BR dose, gene expression and growth behavior in both the *Arabidopsis* root and shoot (*van Esse et al., 2012, 2013a,b*). However, none of the previous modeling approaches has been able to truly quantitatively depict cellular responses, make clear predictions about the cellular behavior or limiting constituents or processes.

In our study, we were able to determine how the constituents of the PM-resident fast BR response pathway work together and identified its rate-limiting elements applying an ordinary differential equations (ODE) approach. Substantiated by wet lab experiments, our computational approach led to a detailed kinetic model that describes the cellular response and explains the BR controlled differential growth behavior of the root cells on the basis of the differential AHA accumulation and activity. Furthermore, the model

111 predicted the existence of a cation influx across the PM that is crucial for the apoplastic acidification
112 and E_m hyperpolarization - subsequently narrowed down experimentally. Lastly, the model shows
113 of how the extent of the BR response can be fine-tuned by the level of the BIR3 inhibitor. Our
114 model proposes that the specific composition of the PM-resident BRI1 nanoclusters determines
115 the competence of the root cells to elongate in response to BR.

116 Results

117 A mathematical model of the fast BR response

118 To analyze the important steps and factors of the cell-specific, fast BR response in the root tip, we
119 developed a detailed mathematical model consisting of ODEs (Fig. 2). The model comprises four
120 cell compartments: the cytosol, the cell wall and the vacuole as three-dimensional compartments
121 as well as the PM as a two-dimensional compartment. The explicit inclusion of the PM as two-
122 dimensional compartment was prompted by the fact that most components of the BR perception
123 and initial processes are located in the membrane and the relevance of the membrane as a scaling
124 factor in this kind of system (*Holzheu et al., 2021*). The compartment sizes were set such that the
125 model initially describes the behavior of a single epidermis cell in the early EZ of the *A. thaliana*
126 root (*van Esse et al., 2011*) (see Appendix 1 Tab. 1).

127 The model captures the important components and steps of the fast BR response pathway. It
128 is set up in a way that an equilibrium state was reached before the system is stimulated with the
129 hormone by maintaining the system first without the hormone for 24 h. In this state, only a few
130 crucial reactions occur and carry a flux (v): the interaction between BIR3 and BAK1 (v_7) and BIR3 and
131 BRI1 (v_6), the proton leak from the cell wall into the cytoplasm (v_2), the basal activity of the ATPases
132 AHA1 and AHA2 (v_1) and the exchange of monovalent cations (here represented by potassium)
133 between cytoplasm and cell wall (v_4) and cytoplasm and vacuole (v_3). Modeling the basal state as
134 a physiologically plausible steady state ensures that the model describes the inactive state of the
135 BR response pathway accurately and that the interactions of BIR3 with BAK1 and BRI1 are in an
136 equilibrium.

137 The hormone is added to the model by an event triggered at 24 h. According to the current
138 state of knowledge, this initiates a number of molecular processes in the PM that occur almost
139 simultaneously (Fig. 2): binding of BR to BRI1 (v_9), the loss of BRI1 inhibition by its C-terminus (v_{12}),
140 the release of BK1 and BIK1 after phosphorylation (v_{10} and v_{11} , respectively) as well as the release
141 of BIR3 from BAK1, the establishment of the BAK1-BRI1 interaction via BR (v_{13}), and the auto- and
142 transphosphorylation of BAK1 and BRI1 (v_{14}). These spatial rearrangements and post-translational
143 modifications result in the active form of the BRI1 receptor complex, which immediately stimulates
144 the activity of H^+ -ATPases very likely by phosphorylation (*Minami et al., 2019*) (v_{15}). Further signaling
145 events occur later in time and lead to differential gene expression (*Lv and Li, 2020*). However, these
146 late events were not considered here for our modeling approach.

147 The main cell physiological output of this early sequence of events is the acidification of the
148 apoplastic space, the swelling of the cell wall and the hyperpolarization of the E_m . The latter is cal-
149 culated based on the net change in charge distribution of protons and potassium across the PM,

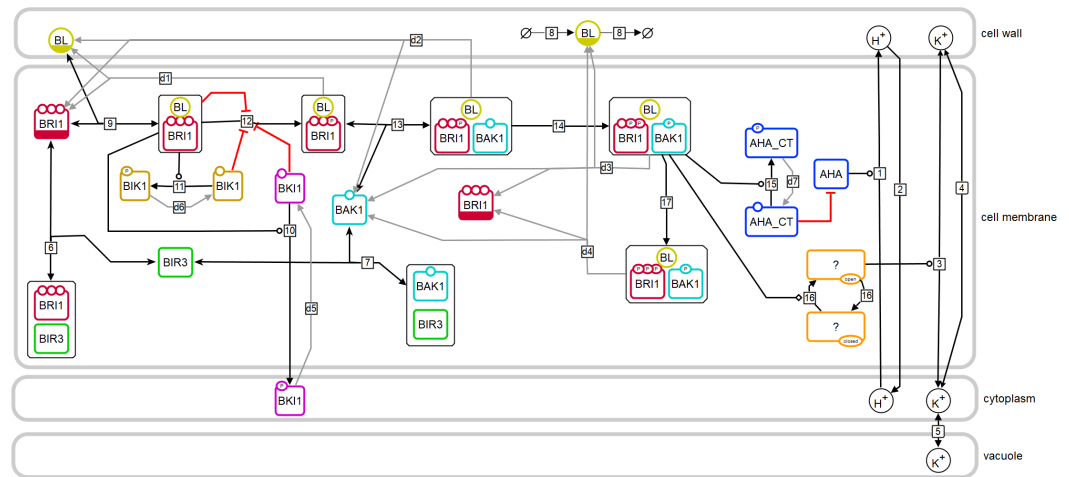


Figure 2. Model structure of the fast BR response pathway of *Arabidopsis thaliana*. Compartments are indicated by grey boxes. Smaller molecules are indicated by circles, proteins by rectangles. Potential sites for protein modifications are indicated by the small circles on the boundaries of the rectangles. Reactions, including substrates and products, are indicated by the arrows, with the reaction numbers noted in the small box. Reactions, which are required for the model to return to the initial state, are drawn in grey. A bar at the bottom of the circle or rectangle indicates that this entity appears more than once in the scheme.

150 the specific capacitance of the plasma membrane (*White et al., 1999*) and the membrane surface
 151 (*van Esse et al., 2011*) (see Appendix 1 Tab. 1). However, combining the available information de-
 152 rived from the literature resulted in preliminary model draft that was not able to reproduce the
 153 measured experimental data, for instance regarding the E_m hyperpolarization. Without a mech-
 154 anism to balance out the shift in charge distribution caused by the transported protons, even a
 155 modest acidification of the apoplast from a pH of 5.4 to 5.0 will result in a membrane hyperpolar-
 156 ization greater than the one observed experimentally (*Caesar et al., 2011*) as we describe below
 157 in detail (see also: Appendix 1 - example calculation of E_m and pH change). Consequently, we pos-
 158 tulated a cation channel in the model that opens upon activation of the BRI1 complex (v_{16}) and
 159 facilitates a monovalent cation influx (here represented by potassium) driven by the E_m (v_3).

160 In order to accurately model and simulate the fast BR response pathway, we therefore needed
 161 more experimental data about the PM-based BRI1 response module. Any remaining unknown
 162 model parameters were estimated based on the cell wall acidification (this study), E_m hyperpolar-
 163 ization (*Caesar et al., 2011*) and the qualitative overexpression behavior of BIR3 (*Imkampe et al.,*
 164 *2017*). To account for non-identifiable parameters, we investigated the parameter space by com-
 165 puting several independent model parameterizations that describe the experimental data equally
 166 well. All computational analyses were run with each model of the resulting ensemble of structurally
 167 identical models ($n=10$) to ascertain consistent results across parameter space.

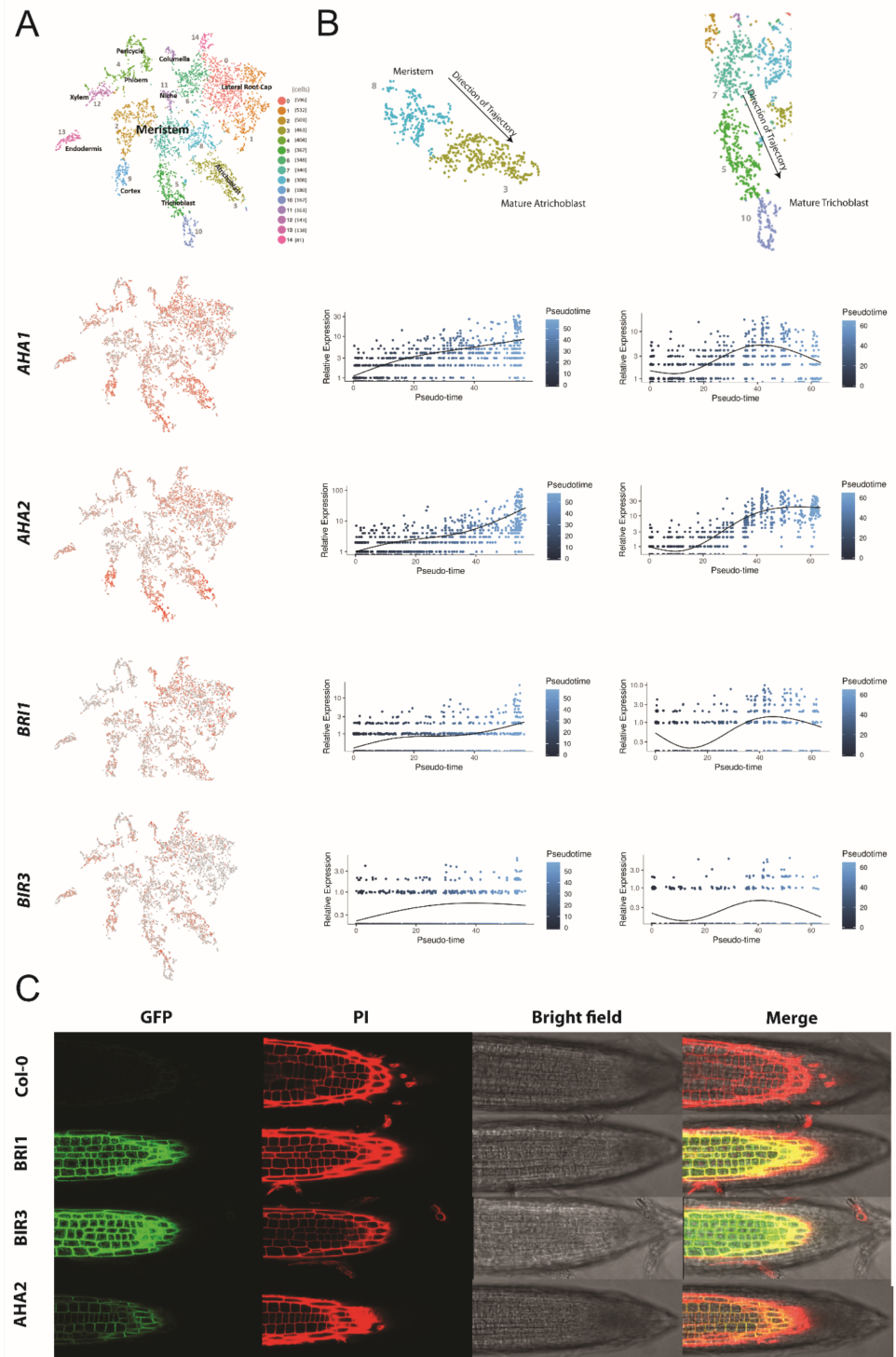
168 Quantification of signaling components

169 One experimental challenge for the refinement of the model was to quantify the central compo-
 170 nents of the pathway comprising predominantly BRI1, BAK1, BIR3 and AHA in the PM of epidermal
 171 cells of the root tip. Initially, we drew our attention on their steady-state transcript levels as they

172 were determined by high-throughput single cell RNA-sequencing (scRNA-Seq) of the different *Ara-*
173 *bidopsis* root cell types (Ma et al., 2020). Whereas *BRI1* and *BIR3* transcripts accumulated in all
174 cell types of the root more or less equally and did not alter much in their amount during cell de-
175 velopment along the root axis, *AHA2* and to much lower extent also *AHA1* transcripts were found
176 predominantly in the epidermal cells and the root cortex (Fig. 3 A). During root development, the
177 *AHA2* transcript amount but not those of *BRI1* and *BIR3* started to increase strongly in the cortex
178 and epidermis cells of the TZ and EZ (Fig. 3 B). This temporal transcript pattern was less prominent
179 for *AHA1* (Fig. 3 B) being in agreement with earlier observation that the *AHA1* promoter is not very
180 active in root epidermis cells. This indicates that *AHA1* does not play a prominent role in the control
181 of cell expansion (Merlot et al., 2007). Because its transcript accumulation was already induced by
182 protoplasting, no scRNA-Seq data could be used for *BAK1* (Ma et al., 2020).

183 On the basis of the scRNA-Seq data we focused our further studies on the *in vivo* protein quan-
184 tification of the GFP fusions of *BRI1*, *BAK1*, *BIR3* and *AHA2* in developing epidermal cells along the
185 root tip axis. For the PM of cells of the early EZ, the amount of *BRI1*-GFP was already quantified to
186 around 11 receptor molecules per μm^2 and for *BAK1*-GFP to 5 co-receptors per μm^2 for *BAK1*-GFP
187 by van Esse et al. (2011). To complete this data set, we applied quantitative CLSM for the quantifi-
188 cation of *BIR3*-GFP and *AHA2*-GFP in the epidermal root cells of published transgenic *Arabidopsis*
189 lines that express the fusion protein under the respective native promoter (Fuglsang et al., 2014;
190 Imkampe et al., 2017). As these GFP fusion proteins carry the identical fluorophore version, their
191 fluorescence intensity can be set in relation to the *BRI1*-GFP intensity and, thus, to the *BRI1*-GFP
192 receptor amount in the PM. The quantification of GFP fluorescence was performed in 50 x 50 μm
193 areas at the epidermis along the root tip, as shown exemplarily in Fig. 3C. The amount of *BRI1*-GFP
194 and *BAK1*-GFP did not alter much in the epidermal cells along the root axis, as it was reported be-
195 fore (Fig. 3 D) (van Esse et al., 2011). A relative homogeneous fluorescence intensity distribution
196 was also observed for *BIR3*-GFP that translated to about 17 inhibitor molecules per μm^2 PM area in
197 the MZ and 14 in the early EZ (Fig. 3 D). In contrast, there was a significant gradient of *AHA2*-GFP flu-
198 orescence intensity along the root axis, being comparatively low in the MZ (with 4 *AHA2* molecules
199 per μm^2 PM area) but high in the late EZ / maturation zone (with about 10 *AHA2* molecules per
200 μm^2 PM area) (Fig. 3 D). A relatively sharp alteration of the *AHA2*-GFP amount was detected for the
201 TZ (Fig. 3 D). If the amount of *AHA2*-GFP and *BIR3*-GFP molecules was set in ratio to the number
202 *BRI1*-GFP molecules in the PM along the root tip axis, there was no alteration with respect to *BIR3*
203 (ratio: about 1.35), but a strong increase regarding *AHA2* from 0.28 in the MZ to up to 5 in the late
204 EZ.

205 Our significantly improved spatio-temporal refinement of previous data (Pacifci et al., 2018)
206 by scRNA-Seq and quantitative CLSM demonstrate a coincidence of *AHA2* protein accumulation
207 with the onset of growth in the EZ. These results suggest that the *AHA2* protein accumulation and
208 probably activity pattern may be regulatory related to normal and BR-regulated root growth along
209 the root tip axis. This hypothesis is particularly plausible given that *AHA2* interact physically with
210 *BRI1* and *BAK1* *in vivo* (Caesar et al., 2011; Ladwig et al., 2015; Yuan et al., 2018).



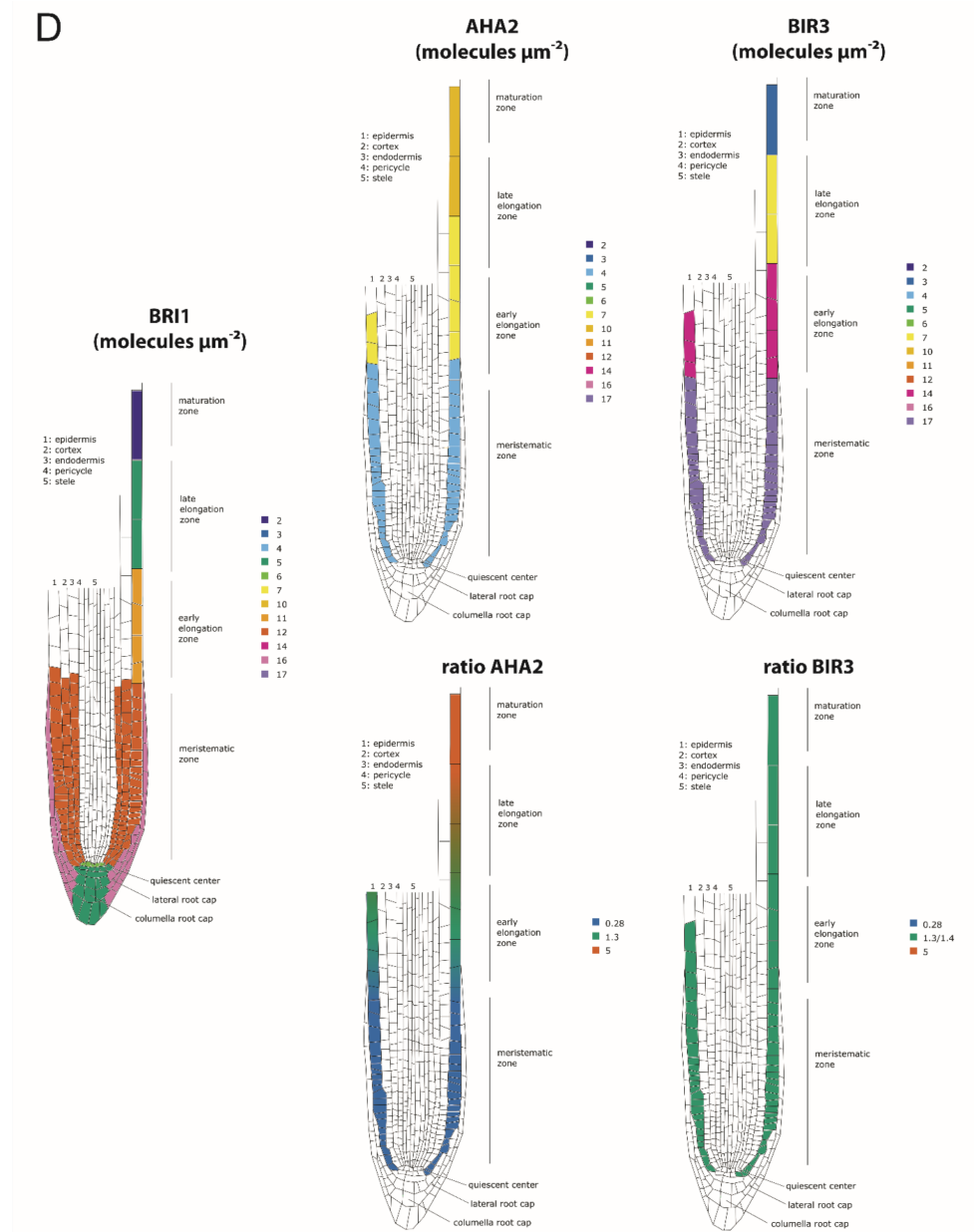


Figure 3. The constituents of the BRI1 nanocluster are spatio-temporally differentially expressed in the epidermal cells along the *Arabidopsis* root tip axis. A. *AHA1*, *AHA2*, *BIR3* and *BRI1* transcript levels in the different cell types of the *Arabidopsis* root tip derived from scRNA-Seq data (Ma et al., 2020). The atrichoblasts and trichoblasts together represent the epidermal cells. B. Developmental trajectories of *AHA1*, *AHA2*, *BIR3* and *BRI1* transcript accumulation along the root tip (Ma et al., 2020). The transition from the meristematic to the EZ is at a pseudotime value of around 30. C. Example of quantification of the GFP fluorescence of the *AHA2*, *BIR3* and *BRI1* fusion proteins, here in the plasma membrane of the meristematic region of the root epidermis in wild type *Arabidopsis* (Col-0 accession) and the respective transgenic lines. Left to right: GFP channel, PI channel, bright field, merged channels. The red box represents a $50 \mu\text{m} \times 50 \mu\text{m}$ area chosen for the measurement here. D. *Upper panel.* Number of the indicated GFP fusion proteins (molecules per μm^2) in the plasma membrane of epidermal cells along the root tip axis. The values for *BRI1*-GFP and *BAK1*-GFP were taken from the literature (van Esse et al., 2011). *Lower panel.* The same but here the ratios of *BRI1*-GFP/*AHA2*-GFP and *BRI1*-GFP/*BIR3*-GFP molecules in the plasma membrane are given.

211 **Modeling predicts the H⁺-ATPases being crucial regulators of the extracellular pH**
212 **in the BR/BRI1 response**

213 To test the hypothesis formulated above, we decided to investigate the functional role of AHA in
214 the context of BR-regulated signaling activity both experimentally and computationally. Here, we
215 first sought to quantify and analyze the response in the early EZ. With the key components of the
216 H⁺ homeostasis and nanocluster quantified, we were able to tailor the model to represent a single
217 epidermis cell in the early EZ. By further using a combination of dose-response data and time-
218 course measurements to fit the remaining unknown model parameters, we then should be able
219 to analyze both the overall response and the temporal dynamics of the BR signaling module.

220 To measure the dose-response behavior and the time-course response to BR stimulation ex-
221 perimentally, we relied on the salt 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium (HPTS), a non-
222 invasive dye that incorporates into the plant cell wall and enables the ratiometric fluorescence read-
223 out of the pH conditions at cellular resolution (*Barbez et al., 2017*). To determine the apoplastic
224 pH conditions 60 min after brassinolide (BL) application in the EZ, we performed a dose-response
225 analysis. A significant decrease of the apoplastic pH was observed already at a BL concentration
226 of 0.1 nM that continued up to a concentration of 10 nM (Fig. 4 A). Higher concentrations of BL
227 did not further increase the cellular response in the EZ. This behavior is reproduced by the model
228 ensemble (Fig. 4 A).

229 To capture not only the overall response to BL stimulation in the EZ but also its temporal dynam-
230 ics, we further performed time-course measurements of the apoplastic pH in response to 10 nM
231 BL using HPTS. Here, we observed a rapid acidification within 10 min of hormone application that is
232 maintained for the remainder of the experiment (Fig.4 B). This observation was again reproduced
233 by the model ensemble (Fig. 4 B). At the same time, we could also capture the cell wall swelling
234 in the model that has been observed in response to BL application previously (*Elgass et al., 2009*;
235 *Caesar et al., 2011*) (Fig 4 C).

236 Using this model ensemble that specifically describes the behavior of a single epidermis cell
237 in the early EZ, we analyzed the importance of the individual model components and parameters
238 for the cell physiological response by calculating the scaled sensitivities. In particular, this means
239 that we calculated the relative change of the cell wall acidification in response to relative changes
240 in model parameters while simulating the BR response stimulated with 10 nM BL for 5 min and 60
241 min. The results of the sensitivity analysis for all model parameterizations ($n = 10$) are summarized
242 in figure 5, where a positive influence on the BR response is denoted in green, no influence is de-
243 noted in white and a negative influence is denoted by red, with the color saturation indicating the
244 strength of the control. Notably, at the beginning of the BR response the initial concentrations of
245 the receptor BRI1 and the proton pumps have a large impact. In addition, parameters influencing
246 proton extrusion such as the degree of inhibition and the pump activity of the ATPases strongly
247 control the early BR response across all model parameterizations (Fig. 5). The sensitivities of the
248 acidification 60 min after BL application in turn show a greater control of down-regulating elements
249 such as the inhibitory phosphorylation of the receptor (Appendix 1 Figure 2), though the amount
250 of proton pumps as well as their activity remain important. In combination with the quantifica-

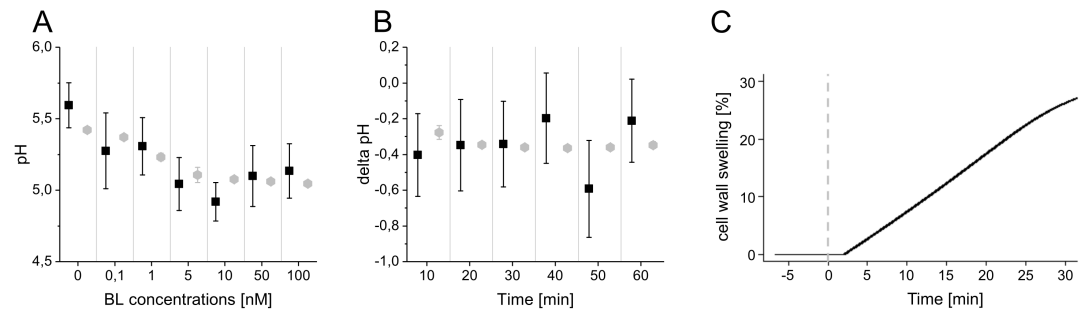


Figure 4. The computational model quantitatively and dynamically captures the sensitivity and kinetics of apoplastic acidification in *Arabidopsis* epidermal cells of the root early EZ in response to BL. A. HPTS-staining visualized (black quadrats) and computationally simulated (grey diamonds) dose-response behavior of apoplastic pH. Real or virtual BL incubation was done for 60 min. Error bars represent SE ($n \geq 14$) for the experimental data and SD for the simulations of different model parameterizations ($n = 10$). B. HPTS-staining visualized (black quadrats) and computationally simulated (grey diamonds) time-course of apoplastic pH change in response to 10 nM BL. Error bars represent SEM ($n \geq 16$) for the experimental data and SD for the simulations of different model parameterizations ($n = 10$). C. Computationally simulated time course of relative wall swelling in response to 10 nM BL. The virtual addition of BL at time 0 is indicated by the vertical dashed line.

251 tion data, this strongly supports our hypothesis that the proton pumps are the key elements that
 252 determine the competency of cells to respond to BR stimulation and react with elongation growth.

253 In consequence, the cells in the MZ should show a higher starting pH and react less strongly to
 254 BR stimulation due to the lower expression levels of AHA2. To predict the behavior of an epidermis
 255 cell in the MZ, we adjusted the model ensemble to instead represent a single epidermis cell in
 256 the MZ in terms of protein concentrations and compartment sizes. This model ensemble shows
 257 both a higher resting pH (Fig. 6 A) and a reduced response to BR stimulation as evident in the
 258 dose-response behavior that was confirmed experimentally by HPTS visualization (Fig. 6 B). At BL
 259 concentrations of 5 and 10 nM, the experimental pH values appear to be lower than those of the
 260 computational model (Fig. 6 A). This could indicate that further proton pumps besides AHA2 or
 261 other acidification mechanisms are activated by BL in the epidermal cells of the MZ.

262 **Experimental evaluation confirm the predicted relevance of the H⁺-ATPases for the** 263 **extracellular pH control in the BR/BRI1 response**

264 To confirm the predictions of the model experimentally, we used both HPTS and microelectrode
 265 ion flux estimation (MIFE) measurements, another non-invasive experimental method that allows
 266 for contact-free, real-time, electrophysiological measurements of H⁺ fluxes at the surface of roots
 267 by using an H⁺-specific electrode that mainly reflects the ATPase activity in the underlying tissues
 268 (Newman, 2001; Fuglsang et al., 2014). Confirming previous results (Staal et al., 2011), our MIFE
 269 measurements along the Arabidopsis root tip revealed a net H⁺ influx at the MZ, which then was
 270 drastically reduced in the EZ implying higher H⁺ ATPase activity in this region (Fig. 7 A). These
 271 differential H⁺ fluxes translate in a pH gradient along the surface of the root tip with the MZ less
 272 acidic and the elongation more acidic (Staal et al., 2011). Using HPTS, we could substantiate the

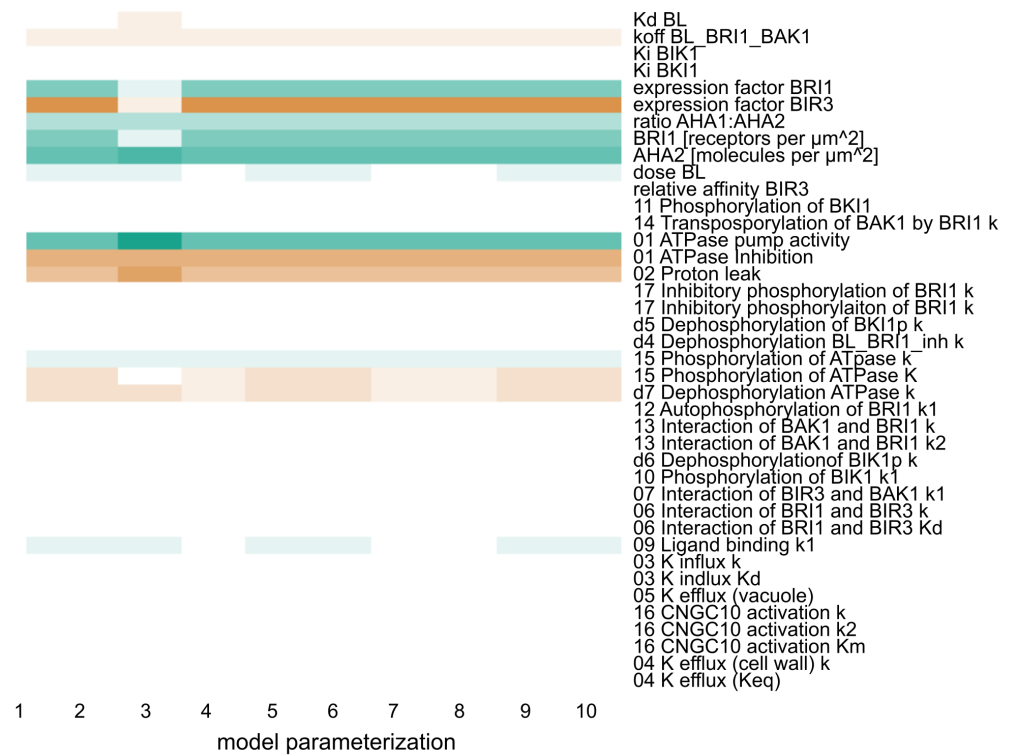


Figure 5. Computational calculation of scaled sensitivities of the cell wall acidification predicts AHA2 activity and molecules in the PM as well as BRI1 expression and molecules in the PM to be the deciding factors for the competence of *Arabidopsis* epidermal root cells to elongate in response to 5 min BL application for all parameterizations of the model. A positive influence is shown in green, a neutral in white and a negative in red, with the color saturation indicating the strength of the influence.

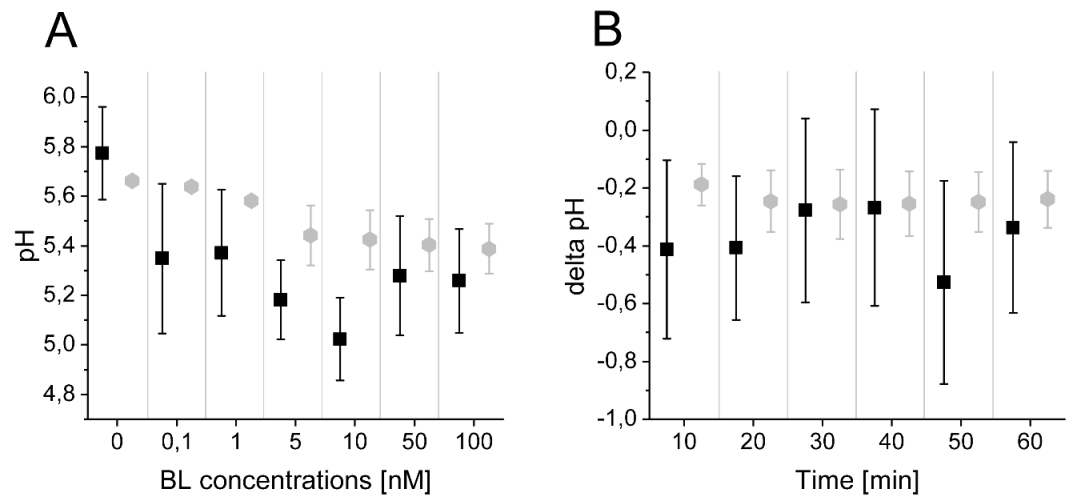


Figure 6. The computational model captures the sensitivity and kinetics of apoplastic acidification in *Arabidopsis* epidermal cells of the root MZ in response to BL. A. HPTS-staining visualized (black quadrats) and computationally simulated (grey diamonds) dose-response behavior of apoplastic pH. Experimental and virtual BL incubation was done for 60 min before HPTS measurements. Error bars represent SE ($n \geq 16$) in the experimental approach and SD for the simulations of different model parameterizations ($n = 10$). B. HPTS-staining visualized (black quadrats) and computationally simulated (grey diamonds) time-course of apoplastic pH change in response to 10 nM BL. Error bars represent SEM ($n \geq 16$) in the experimental approach and SD for the simulations of different model parameterizations ($n = 10$).

273 MIFE results and confirm the observation of *Barbez et al. (2017)* that there is an apoplastic pH
274 gradient of the epidermal root cells from the MZ (less acidic) to the EZ (more acidic) (Fig. 7 B).

275 To address the question whether the establishment of the resting pH gradient and the differen-
276 tial changes of the pH conditions upon external BL application depend on fully functional BRI1, we
277 used the *bri1-301* mutant for further HPTS and MIFE measurements. In the *bri1-301* mutant a BRI1
278 version with a reduced kinase activity is expressed, which causes a weak defective root growth
279 phenotype at ambient temperature (*Lv et al., 2018; Zhang et al., 2018*). This less-pronounced *bri1-*
280 *301* phenotype allows HPTS and MIFE measurements technically comparable to those of wild type
281 plants. As shown in figure 7 C, the BL-induced changes in the apoplastic pH observed for wild type
282 were significantly lower in the *bri1-301* mutant. The HPTS data were again supported by our MIFE
283 measurements: The wild type cells of the EZ showed an increase in the net H^+ efflux upon applica-
284 tion of 10 nM BL, which continued over the measurement period of 20 min, whilst the cells of the
285 *bri1-301* mutant responded much less (Fig. 7 D).

286 In summary, the concordant results of our experimental approaches substantiate the predic-
287 tion of the mathematical model that the enhanced level of H^+ ATPase amount and activity in rela-
288 tion to the number of BRI1 receptors define the BR-regulated apoplastic acidification and linked
289 hyperpolarization of the E_m . Moreover, the maintenance of the pH gradient and H^+ fluxes along
290 the root tip axis and the BL regulation of alterations depend on kinase-active BRI1.

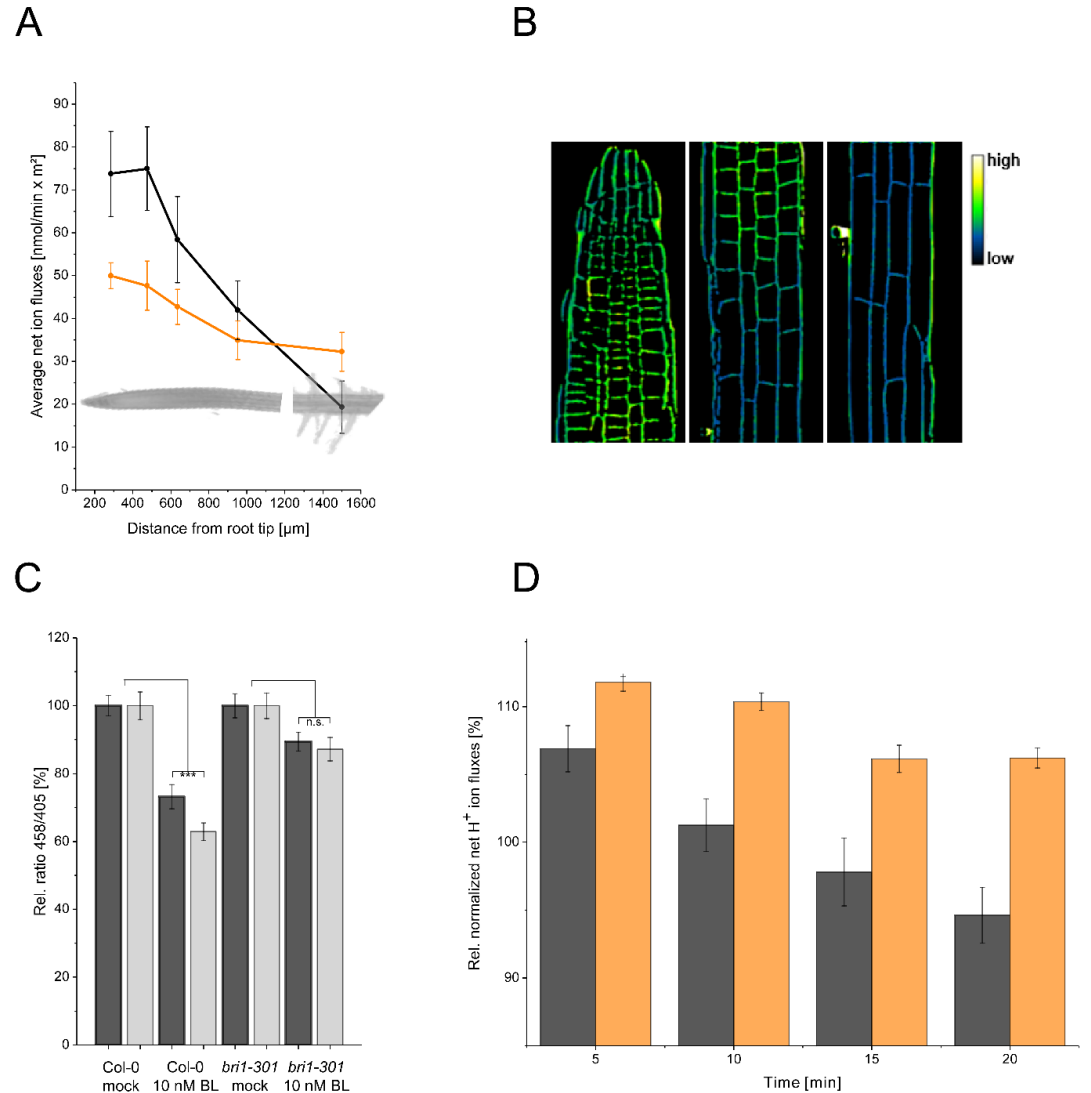


Figure 7. The resting apoplastic pH gradient of epidermal root cells along the axis and its regulation by BR depends on kinase-active BRI1. **A.** MIFE recording of the H⁺ fluxes along the root axis of *Arabidopsis* wild type (black line) and *bri1-301* mutant (yellow line) plants. Measurements were performed every 100 μm from 250 μm of the root tip off to the root hair zone. Error bars represent SD (n = 3). **B.** Representative image of the apoplastic pH of epidermal cells along the root axis of wild type *Arabidopsis* using HPTS-staining starting with the MZ (left) over the TZ/early EZ (middle) to the late EZ (right). **C.** Comparison of the relative apoplastic pH (ratio 458/405) of epidermal root cells in the MZ (black bars) and EZ (grey bars) of wild type and *bri1-301* mutant plants after 60 min of BL (10 nM) or mock treatment, visualized by HPTS staining. The data derived from the mock treatments of the respective line were set to 100. Error bars represent SE. Statistical evaluations were performed by ANOVA followed by Tukey-Kramer HSD post hoc test. The black asterisks indicate statistically significant differences (***: P < 0.001); ns: not significant. **D.** Relative H⁺ fluxes at the EZ of wild type (black bars) and *bri1-301* mutant (yellow bars) plants between 5 and 20 min after application of 10 nM BL recorded by MIFE. The flux directly before the addition of BL was set to 100. The increase in net influx after probe application is due to a disturbance of the H⁺ conditions at the root surface, which is observed with any treatment. Error bars represent SD (n = 3).

291 **Modeling predicts a cation channel for charge compensation during H⁺ export and** 292 **PM hyperpolarization**

293 The great value of mathematical modeling and prediction is especially demonstrated after we cal-
294 culated the membrane potential derived from the pH value changes in the apoplastic cell space
295 of the root tip upon BL treatment and compared it with the previously experimentally determined
296 E_m changes (*Caesar et al., 2011*). The calculated E_m change induced by the change in charge dis-
297 tribution due the acidification of the apoplastic space was much stronger as the measured one
298 (Fig. 8 A and Appendix 1 - example calculation of E_m and pH change based on membrane area,
299 specific membrane capacitance and transported charges): An acidification from pH 5.4 to pH 5.0
300 in response to 10 nM BL corresponds to a E_m change of approximately 28 mV, as opposed to the
301 experimentally measured 7.2 mV (*Caesar et al., 2011*). According to the prediction of our model,
302 this discrepancy values was eliminated, if an import of monovalent cations such as potassium (K⁺),
303 which predominantly contributes to the E_m of the PM in plant cells (*Higinbotham, 1973*), took place
304 in parallel to the ATPase generated H⁺ extrusion. Against the background that BAK1 and AHA2
305 interact with a cation channel of the cyclic nucleotide-gated ion channel (CNGC) family in the phy-
306 tosulfo kinase receptor 1-mediated growth response (CNGC17; (*Ladwig et al., 2015*)), we searched in
307 the literature and the Arabidopsis eFP browser (*Sullivan et al., 2019*) for a CNGC member, which
308 is expressed in the root, localizes to the PM, imports K⁺ ions, and is functionally linked to cell ex-
309 pansion, and identified CNGC10 (*Borsics et al., 2007; Christopher et al., 2007; Duszyn et al., 2019*).
310 When CNGC10 and its K⁺ transport properties were integrated into our model, the discrepancy
311 between the calculated and measured value was gone (Fig. 8 B). This suggests that the CNGC10-
312 mediated influx of potassium counteracts the ATPase-caused efflux of H⁺ into the apoplast in the
313 root tip.

314 To test whether CNGC10 is able to interact with components of the BRI1 nanocluster such as
315 BRI1, BAK1 and AHA2, Förster resonance energy transfer by fluorescence lifetime imaging (FRET-
316 FLIM) analyses in transiently transformed *Nicotiana benthamiana* leaf cells and yeast mating-based
317 split-ubiquitin (mbSUS) assays were performed. The growth of yeast cells on interaction selective
318 media and the reduction of the GFP fluorescence lifetime (FLT) revealed a spatially very close as-
319 sociation (below 13 nm; (*Glöckner et al., 2020*)) and interaction, respectively, of CNGC10 with BRI1,
320 BAK1 and AHA2 (Fig. 8 C-E). To test whether CNGC10 functions in the fast BR response pathway,
321 we analyzed the BL-induced apoplastic pH change in two independent *ngc10* loss-of-function lines
322 (*Jin et al., 2015; Borsics et al., 2007*) compared to the corresponding wild type (Col-0). In contrast
323 to the wild type both mutants did not acidify the apoplast of the cells in the EZ upon application
324 of 10 nM BL (Fig. 8 F), whilst the mutant cells of the MZ behaved like wild type (Appendix 1 Fig. 3).
325 These data indicate that CNGC10 is the major K⁺ channel to maintain the E_m homeostasis of the
326 PM during BL-induced apoplastic acidification primarily in the EZ and appears to be an additional
327 constituent of the elongation growth-related BRI1 nanocluster.

328 **Computational modeling enables the in silico analysis of BIR3 function**

329 To further demonstrate the performance of our model, we investigated the function of the in-
330 hibitor BIR3 in the activity modulation of the BRI1 nanocluster in more detail *in silico*. The basis

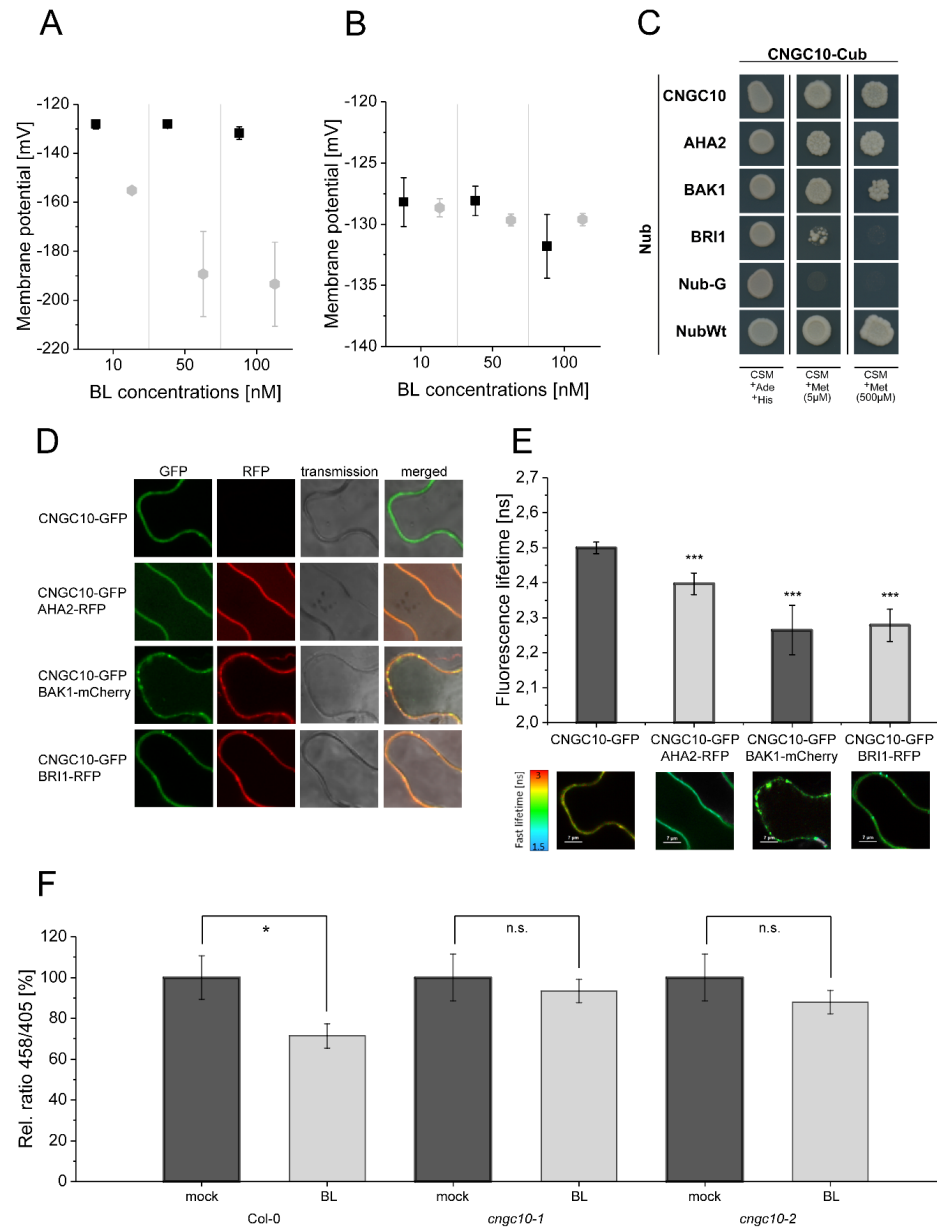


Figure 8. The computational model predicts the existence of a potassium channel, likely to be CNGC10, to maintain the homeostasis of the plasma membrane potential and apoplasmic pH in *Arabidopsis* epidermal root cells of the early EZ. A. Modeled E_m in the presence of different different BL concentrations without the integration of potassium import (grey diamonds) in comparison to the published experimental data (black quadrats; *Caesar et al. (2011)*) after 20 min of BL treatment. B. Modeled E_m in the presence of different BL concentrations with the integration of the CNGC10 potassium channel (grey diamonds) in comparison to the published experimental data (black quadrats; *Caesar et al. (2011)*). Error bars in A and B represent SEM ($n \geq 4$) in the experimental approach and SD of simulation results of the different model parameterizations. C. CNGC10 forms homomers and interacts with BAK1 and AHA2 in the yeast mating-based split-ubiquitin system. The indicated combinations of Cub and Nub fusion constructs were transformed into yeast cells. Yeast cells were then grown either on media selective for the presence of the plasmids (CSM +Ade,+His) or on interaction selective media with two different concentrations (5 μ M, 500 μ M) of methionine (CSM +Met). The combination of CNGC10-Cub with Nub-G served as negative and that with NubWT as positive control. D. CNGC10 colocalizes with AHA2, BAK1 and BRI1 in the plasma membrane of plant cells. Representative confocal images of transiently transformed tobacco epidermal leaf cells expressing the indicated fusion proteins.

Figure 8. (continued) E. CNGC10 is spatially closely associated with AHA2, BAK1 and BRI1 in the plasma membrane of plant cells. Fluorescence lifetime imaging microscopy (FLIM) analysis comparing the different Förster resonance energy transfer (FRET) pairs. *Top*: FLIM measurements of transiently transformed tobacco epidermal leaf cells expressing the CNGC10-GFP donor fusion with the indicated RFP or mCherry acceptor fusions. Error bars indicate SD ($n \geq 21$). Statistical evaluations were performed by a Kruskal-Wallis test followed by Steel-Dwass post hoc test. The black asterisks indicate statistically significant differences (***: $P \leq 0.0001$). *Bottom*: Heat maps of representative plasma membrane areas used for FLIM measurements. The donor lifetimes of CNGC10 are color-coded according the scale at the left. F. Comparison of the relative apoplastic pH (ratio 458/405) of epidermal root cells in the EZ of wild type and two independent *cngc10* mutant plants after 60 min of BL (10 nM) or mock treatment, visualized by HPTS staining. The data derived from the mock treatments of the respective line were set to 100. Error bars represent SE ($n \geq 8$). Statistical evaluations were performed as described in Fig. 7C. The black asterisk indicates statistically significant differences (*: $P = 0.021$); n.s.: not significant.

331 for the focus on BIR3 were the observations by *Imkampe et al. (2017)* regarding the activity of the
332 BR signaling in BIR3 as well as BIR3 and BRI1 overexpressing plant in the parameter estimation:
333 The pathway should be inactive (= no acidification), when BIR3 is overexpressed, whilst the addi-
334 tional overexpression of BRI1 should restore the signaling activity to approximately normal levels.
335 As shown in figure 9 A, the model was actually able to represent the BR activity of the respective
336 growth-related phenotypes of *Arabidopsis* plants with altered BIR3 levels (*Imkampe et al., 2017*).
337 Against this background we decided to investigate the behavior of different BIR3 expression levels
338 in comparison to wild-type level in the root by analyzing the pH change 20 min after stimulation
339 with 10 nM BL. As shown in the resulting expression-response curve (Fig. 9 B), the overall response
340 decreased with increasing concentrations of BIR3 for all model parameterizations. This suggests
341 that it is possible for the plant to fine-tune the signaling output by adjusting the expression level
342 of the negative regulator BIR3. Finally, we also analyzed the dynamics of the overall pH response
343 at different BIR3 accumulation levels, namely in the absence of BIR3, the normal protein amount
344 of around 13 BIR3 molecules μm^{-2} PM and a 10- and 100-fold overaccumulation of BIR3. Here,
345 the actual time-course behavior of the acidification varies between the different model parame-
346 terizations as the span of possible values deviated from the average pH response for the BIR3
347 expression (Fig. 9 C). Depending on the parameterization, it was possible for the model to either
348 show a strong activation that tapered off or a more gradual response over the time- frame of an
349 hour. For most model parameterizations, a 10-fold overexpression of BIR3 is sufficient to inactivate
350 the BRI1 signaling module confirming the importance of the regulation by BIR3.

351 Discussion

352 BR fulfill a central role in regulating plant physiology, growth and development as well as adap-
353 tion to the environment (*Lv and Li, 2020*). A prominent example for a BR function is the rapid
354 initiation of the (epidermal) cell growth in the EZ but not in the MZ of the *Arabidopsis* root tip (*Lv*
355 *and Li, 2020*). Evidently, the hormone implements on an already existing, functional competence
356 of the root cells that, according to our experimental data, cannot be attributed to the absence
357 of the BRI1/BAK1 perception system but must have other reasons. Moreover, although the main

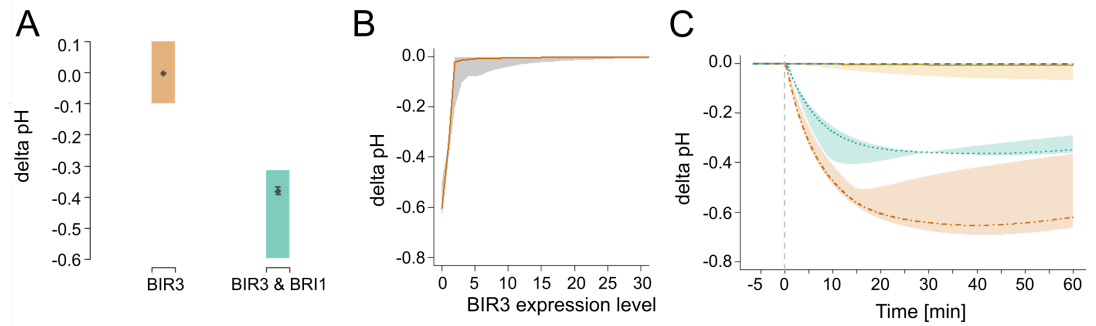


Figure 9. *In silico* analysis of the functional role of the negative regulator BIR3 on BL-regulated apoplastic acidification. A. Modelled qualitative acidification output of plants overexpressing BIR3 and BIR3&BRI1, respectively. The colored area represents the pH response targeted during parameter estimation, which was approximated by the activity of BR signaling indicated by the plant phenotypes (*Imkamp et al., 2017*). B. BIR3-Expression-response curve. Shown is the pH change 20 min after stimulation with 10 nM BL at different BIR3 expression levels ranging from 0- (loss-of-function mutant) to 30-times the normal expression level of the wild-type. The entire range of simulated responses is indicated by the shaded area, the averaged response of all models is denoted by the line. C. Exemplary time-course simulations of the pH change at 0 (loss-of-function mutant, orange), 1- (wild type expression, green), 10- (yellow) and 100-fold (blue) expression of BIR3 upon virtual application of 10 nM BL. Shown is the average pH response for the respective BIR3 expression level with the span between minimal and maximal values indicated by the colored area. The virtual addition of BL at time 0 is indicated by the vertical dashed line.

358 molecular determinants of BR perception and signaling are known, the processes leading to this
 359 competence and its realization towards, in this case, elongation were so far not well understood.
 360 To address this problem we quantitatively analyzed the dynamics of the PM-resident fast BR
 361 response pathway as a whole by a recurring combination of computational modeling and wet lab
 362 experiments. The model's predictions of the crucial constituents in the BRI1 nanocluster were
 363 experimentally verified, thereby determining the deciding and regulating elements for the signaling
 364 output. Using a detailed kinetic model on the basis of ODEs we could analyze the interplay of the
 365 signaling components and the system as a whole: We captured the dynamics of the apoplastic
 366 acidification and E_m hyperpolarization without BR and in response to the hormone. In addition, we
 367 showed that the rapidity and degree of the apoplast acidification in response to BR application is
 368 determined largely by the amount and activity of the ATPase AHA2 in the PM of the epidermal root
 369 cells. Furthermore, the model predicted that an influx of cations is required in order to explain both
 370 the pH and E_m changes of the PM simultaneously. We found that CNGC10 is the responsible cation
 371 (potassium) channel, as, besides functional evidence, it associates with BRI1, BAK1 and the proton
 372 pumps AHA2 *in vivo*. CNGC10 could therefore be another constituent of the BRI1 nanocluster in
 373 the PM of root cells. Lastly, we refined by computational modeling the putative regulatory role of
 374 BIR3 in the response pathway, as the signaling output can be in principle fine-tuned depending
 375 on the BIR3 level. Based on these results, we propose that the ongoing of the elongation growth,
 376 that involves altered gene expression later in time, is not possible if the initial rapid processes such
 377 as apoplast acidification, E_m hyperpolarization and charge compensation, which eventually lead to
 378 cell wall loosening followed by rapid wall swelling (*Elgass et al., 2009; Caesar et al., 2011*), do not
 379 occur adequately.

380 If we project the measured AHA2 amount and AHA activity, and the apoplastic pH of epidermal
381 cells along the axis of the root tip, we observe that they both increase and decrease, respectively,
382 with the begin of the EZ and strongly correlate with the competence to grow upon BL application.
383 Proposed by the computational model the AHA2s appear to be the rate-limiting factor for the cells
384 to be able to respond to BR by elongation. The BR-mediated control of the H⁺ ATPase and, thus, the
385 E_m concerns not only elongation growth. The E_m is also central for adaptive responses to a broad
386 range of abiotic cues and for developmental processes. Our observations therefore suggest that
387 the regulation of H⁺ ATPase contributes to the versatile functions of BR in all of these processes
388 (*Lv and Li, 2020; Wolf, 2020*).

389 Regardless, we are uniquely able to represent BR signaling activity *in silico* based on the cell
390 physiological parameters (apoplastic pH, E_m of the PM, cell wall swelling) in a temporal and quanti-
391 tative manner from the origin of a cell in the root apical meristem to its destination in the EZ. Most
392 interestingly, the availability of especially AHA2 for its incorporation into the BRI1 nanocluster in
393 the PM is built on the cytokinin-induced onset of AHA expression in the TZ (*Pacifici et al., 2018*). The
394 positional information for the cytokinin function is, in turn, created by an auxin gradient along the
395 root tip (*Pacifici et al., 2018*). Our results therefore show how a developmental gradient along the
396 root tip, which is generated by the interplay of auxin and cytokinin, translates into the cell-specific
397 competence for BR-regulated elongation growth.

398 As proposed recently, a further determinant of the cellularly different BR response appears to
399 be a locally different BR biosynthesis and, thus, BR amount (*Vukasinovic et al., 2020*). Whereas low
400 BR concentrations are optimal for the cellular activity in the MZ, high concentrations are required
401 for the optimal cellular activity in the EZ. However, this concept cannot explain why the cells of the
402 MZ hardly start to elongate independently of the BR concentration. As discussed above, we pro-
403 pose that either an increased proportion of AHA2 in the BRI1 nanoclusters or the increased number
404 of AHA2-containing BRI1 nanoclusters are critical for establishing the differential competence of
405 epidermal cells for (BR-regulated) growth along the root tip axis.

406 Varying the nanoclusters composition is an elegant way to achieve cell- and tissue-specific re-
407 sponses to a given cue when the number of available perception, signaling and output elements
408 is limited. This principle also seems to be realized in various BRI1-mediated function. For example,
409 the BRI1-dependent regulation of the vascular cell fate in the MZ of the root or the BRI1-mediated
410 cross-tissue control of the cell wall homeostasis require BRI1 nanoclusters that contain at least ad-
411 ditionally RLP44 (*Wolf et al., 2014; Holzwart et al., 2018*). Moreover, RLP44-containing BRI1/BAK1
412 nanoclusters are spatially distinct from for instance FLS2/BAK1 nanoclusters (*Glöckner et al., 2020*).

413 The availability of a sophisticated model also enables *in silico* genetics that simplify the under-
414 standing of complex regulatory processes and their sometimes non-intuitive effects on the func-
415 tional outputs. This is illustrated here by the example of the negative regulator BIR3 that prevents
416 the interaction of BAK1 and BRI1 in the absence of the hormone thereby suppressing BR signal-
417 ing (*Imkampe et al., 2017; Großholz et al., 2020*). Our computational model not only represents
418 and predicts the BR activity of the growth-related phenotypes of the *Arabidopsis bir3* mutant and
419 BIR3-overexpressing plants but also allows statements about the dose-dependent fine-tuning of
420 BIR3 on BR/BRI1/BAK1-related functions. Such *in silico* genetic and physiological approaches can

421 be used to determine the functional and regulatory significance of other components of the fast BR
422 response pathway as shown for AHA2 and the prediction of a cation channel for charge compensa-
423 tion. Thus, computational modeling facilitates the prioritization of the components of a perception
424 and signaling system whose function should first be tested experimentally.

425 In summary, the recurrent application of computational modeling and subsequent wet lab ex-
426 periments provided a novel in-depth and quantitative view of the initial cell physiological processes,
427 regulatory networks and information processing leading to a minimal molecular and biochemical
428 framework of the onset of BR-regulated elongation growth along the axis of the root tip. This ap-
429 proach can in principle be applied for the analysis of every signal perception and transduction
430 process as long as a minimal set of elements and quantitative data are available or experimentally
431 accessible.

432 The ongoing challenge will now be to establish a model of elongation growth across all tissues
433 of the root tip. At the cellular level, the further aim is to integrate into the model the data of the
434 potentially BR-modified composition, assembly and dynamics of the BRI1 nanocluster in the PM
435 obtained by sophisticated super-resolution microscopy and *in vivo* FRET studies (*Glöckner et al.,*
436 *2020*).

437 **Methods and Materials**

438 **Experimental Methods**

439 **Plant Material**

440 Seeds of the *Arabidopsis* mutants and lines expressing the different fusion proteins were surfaced
441 sterilized and placed on ½ Murashige and Skoog (MS) medium plates with 1 % phytoagar and 1 %
442 sucrose followed by stratification at 4° C in the dark for 2 days. Afterwards the plants were grown
443 in growth chambers at 20° C under long day conditions (16 h light/8 h dark) for 5 days. The trans-
444 genic *Arabidopsis* lines (Col-0 ecotype) contained either a *pBRI1:BRI1-GFP* (wild type background;
445 *(Friedrichsen, 2000)*), a *pAHA2:AHA2-GFP* (*aha2-4* mutant background; *(Fuglsang et al., 2014)*) or a
446 *pBIR3:BIR3-GFP* construct (*bir3-2* background; *(Imkampe et al., 2017)*). The *Arabidopsis bri1-301* mu-
447 tant (Col-0) was described in detail previously *(Lv et al. (2018); Zhang et al. (2018)* and references
448 therein).

449 **Microelectrode ion flux estimation (MIFE) measurement**

450 For MIFE measurements, 5-days-old seedlings were grown as described but in continuous light.
451 Experiments were performed as described by *Fuglsang et al. (2014)*. The seedlings were equili-
452 brated in bath medium (0.1 mM CaCl₂, 0.5 mM KCl, pH 5.8) for 2 h before the measurements. Only
453 seedlings without proton oscillations were used. At time point 0, 1 nM BL was added. The bathing
454 solution was mixed two times by carefully pipetting up and down after addition of BL. The proximal
455 position of the electrode (near the root) and the distal position (far from the root) were swapped
456 compared to the previous study *(Fuglsang et al., 2014)*. Consequently, a decrease in values repre-
457 sents proton efflux and an increase represents proton influx in our measurements.

458 8-Hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) measurement

459 For root apoplastic pH measurements, plates containing ½ MS agar media pH 5.7 without buffer,
460 1 mM HPTS dye, and the respective treatments were used. 5 days old *Arabidopsis* seedlings were
461 transferred onto the media and treated for 60 min with HPTS prior to imaging. For shorter treat-
462 ments, seedlings were prestained with HPTS and subsequently treated according to the indications.
463 For imaging, the plants on the media were flipped into a nunc imaging chamber (Ibidi 80286), the
464 roots being close to the chamber bottom and covered by the media. Ratiometric imaging was con-
465 ducted at an inverted Zeiss LSM880 confocal scanning microscope. The 405nm and 458nm laser
466 were used at 0.2% and 100% intensity respectively, a PMT detector range from 495 to 535 nm was
467 used and line sequential scans were performed. The detector gain was set at 1200. For imaging,
468 a 40x water immersion objective was used. The evaluation of ratio in the resulting images was
469 determined following the workflow described by *Barbez et al. (2017)*. For calibration curve mea-
470 surements, ½MS agar media supplemented with 10 mM MES were adjusted to the desired pH and
471 roots of 5 days old seedlings were analyzed as described above.

472 Mating-based split-ubiquitin system (mbSUS) measurements

473 For the mbSUS the coding sequences of *CNGC10*, *AHA2*, *BAK1* and *BRI1* were either fused to the se-
474 quences coding for the C-terminal part of ubiquitin (Cub) or the N-terminal part of ubiquitin (Nub).
475 Namely, the plasmids pMetYC (Cub) and pXNubA22 (Nub) were used (*Grefen et al., 2009*). pNubWt-
476 Xgate (*Obrdlik et al., 2004*) and the empty pXNubA22 vector served as positive and negative control,
477 respectively. The experiments were performed as described by *Grefen (2014)* with some modifica-
478 tions: After dropping the mated yeasts on yeast extract peptone dextrose (YPD) plates they were
479 scratched off with pipette tips, resuspended in 100 µl H₂O and 5 µl were transferred to complete
480 supplement mixture (CSM)-Leu -Trp -Ura -Met plates. The growth assay was performed with ad-
481 justed optical density of the yeast cultures in one dilution. Here, vector selective plates (CSM-Leu
482 -Trp -Ura -Met) or interaction selective plates (CSM-Leu -Trp -Ura -Met, -Ade, -His) with 5 µM and
483 500 µM methionine were used. The growth of the yeast was documented after 72 h of incubation
484 at 28 °C.

485 FRET-FLIM analysis

486 For FRET-FLIM analysis, the coding sequences were expressed as C-terminal fluorophore fusions,
487 using pH7FWG2 (GFP), pB7RWG2 (RFP) or pABind-mCherry (*Karimi et al., 2002; Bleckmann et al.,*
488 *2010*). These binary vectors and *p19* as gene silencing suppressor were transformed into *Agrobac-*
489 *terium tumefaciens* strain GV3101 and infiltrated into *Nicotiana benthamiana* leaves. The measure-
490 ments were performed 2 to 3 days after infiltration using a SP8 laser scanning microscope (Leica
491 Microsystems GmbH) with LAS AF and SymPhoTime (PicoQuant) software as described (*Veerabagu*
492 *et al., 2012*). Before performing the FRET-FLIM measurement, the presence of the fluorophores
493 was imaged by using 488 nm or 561 nm lasers for GFP or RFP excitation, respectively. The fluore-
494 scence lifetime τ [ns] of either the donor only expressing cells or the cells expressing the indicated
495 combinations was measured with a pulsed laser as an excitation light source with 470 nm and a
496 repetition rate of 40 MHz (PicoQuant Sepia Multichannel Picosecond Diode Laser, PicoQuant Time-

497 harp 260 TCSPC Module and Picosecond Event Timer). The acquisition was performed until 500
498 photons in the brightest pixel were reached. To obtain the GFP fluorescence lifetime, data pro-
499 cessing was performed with SymPhoTime software and bi-exponential curve fitting and correction
500 for the instrument response function.

501 Statistics

502 If not otherwise indicated, for calculation of average, standard error (SE) and standard deviation
503 (SD) Excel v1809 or SAS JMP 14 were used. For small sample numbers the 2-sample t-test was
504 chosen (*De Winter, 2013*).

505 pH time-course measurements

506 As the time-course measurements of the BL response were normalized to the control measure-
507 ments, the standard deviations of treatment and control were added quadratically for each time
508 point and replicate before averaging to calculate the combined standard deviation of all replicates,
509 which is used to compute the standard error.

510 Computational Methods

511 Model Setup

512 The model consisting of ordinary differential equations was constructed in COPASI (*Hoops et al.,*
513 *2006; Mendes et al., 2009*) 4.30, build 240, running on a 64-bit machine with Windows 8. Reactions
514 were defined as mass action or Michaelis Menten kinetics where appropriate (see Appendix 1 Ta-
515 ble 3). Compartment sizes and parameters were defined based on experimental data if possible
516 (Appendix 1 Tables 1 & 3). Unknown parameters were determined by parameter estimation. The
517 schematic of the model was drawn using VANTED (*Junker et al., 2006*) and adheres to the Systems
518 Biology Standard of Graphical Notation (SBGN) (*Novère et al., 2009*).

519

520 Parametrization

521 All unknown model parameters, where no or only a range of experimental information were avail-
522 able, were estimated. To account for parameter non-identifiabilities we generated 10 independent
523 parameter sets by randomly sampling the starting parameter values before running the parame-
524 ter estimation. Each parameter estimation run was set up using the particle swarm algorithm as
525 implemented in COPASI 4.30 (*Hoops et al., 2006*), using 5,000 generations with a swarm size of
526 50 individual parameter combinations. The parameter estimation was repeated until the resulting
527 solution had a χ^2 around 10.45.

528

529 Model Analyses

530 The time-course simulations were run deterministically using the LSODA algorithm as implemented
531 in COPASI. The impact of different BIR3 concentrations was analyzed using the parameter scan task
532 in COPASI to simulate the time course of the pH over the time frame of 20 min. The scaled sensitivi-
533 ties of the extracellular pH change in response to changes in model parameters were calculated as

534 $scaled\ sensitivity = \frac{\ln(\Delta pH)}{\ln(P_i)}$ at 5 min and 60 min. Results were plotted using R (*R Core Team, 2020*).

535

536 Acknowledgments

537 The research in our laboratories is supported by the German Research Foundation (DFG) with
538 grants to K.H., K.S. and U.K. (CRC 1101-A02/D02) and grants for scientific equipment (INST 37/819-
539 1 FUGG, INST 37/965-1 FUGG, INST 37/991-1 FUGG, INST 37/992-1 FUGG). We also would like to
540 thank the Schmeil Foundation and the Joachim Herz Stiftung for their support of R.G. In addition,
541 we are grateful for the support by Tom Denyer, ZMBP - University of Tübingen, for his help in the
542 interpretation of the scRNA-Seq data.

543 References

- 544 **Allen HR**, Ptashnyk M. Mathematical modelling and analysis of the brassinosteroid and gibberellin signalling
545 pathways and their interactions. *Journal of Theoretical Biology*. 2017 nov; 432:109–131. <https://www.sciencedirect.com/science/article/pii/S002251931730379X?via=ihub>, doi: 10.1016/J.JTBI.2017.08.013.
- 546
- 547 **Baekgaard L**, Fuglsang AT, Palmgren MG. Regulation of plant plasma membrane H⁺- and Ca²⁺-ATPases
548 by terminal domains. *J Bioenerg Biomembr*. 2005 dec; 37(6):369–374. <http://link.springer.com/10.1007/s10863-005-9473-0>
549 <http://www.ncbi.nlm.nih.gov/pubmed/16691467>, doi: 10.1007/s10863-005-9473-0.
- 550 **Bairoch A**, Apweiler R, Wu CH, Barker WC, Boeckmann B, Ferro S, Gasteiger E, Huang H, Lopez R, Magrane M,
551 et al. The universal protein resource (UniProt). *Nucleic acids research*. 2005; 33(suppl_1):D154–D159.
- 552 **Band LR**, Wells DM, Fozard JA, Ghetiu T, French AP, Pound MP, Wilson MH, Yu L, Li W, Hijazi HI, Oh J, Pearce
553 SP, Perez-Amador MA, Yun J, Kramer E, Alonso JM, Godin C, Vernoux T, Hodgman TC, Pridmore TP, et al.
554 Systems analysis of auxin transport in the Arabidopsis root apex. *The Plant cell*. 2014 mar; 26(3):862–
555 75. <http://www.ncbi.nlm.nih.gov/pubmed/24632533>
556 <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4001398>, doi: 10.1105/tpc.113.119495.
- 557 **Barbez E**, Dünser K, Gaidora A, Lendl T, Busch W. Auxin steers root cell expansion via apoplastic pH regulation
558 in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences*. 2017; 114(24):E4884–E4893.
- 559 **Bleckmann A**, Weidtkamp-Peters S, Seidel CAM, Simon R. Stem Cell Signaling in Arabidopsis Re-
560 quires CRN to Localize CLV2 to the Plasma Membrane. *PLANT PHYSIOLOGY*. 2010 jan; 152(1):166–
561 176. <http://www.ncbi.nlm.nih.gov/pubmed/19933383>
562 <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2799354>
<http://www.plantphysiol.org/cgi/doi/10.1104/pp.109.149930>, doi: 10.1104/pp.109.149930.
- 563 **Borsics T**, Webb D, Andeme-Ondzighi C, Staehelin LA, Christopher DA. The cyclic nucleotide-gated calmodulin-
564 binding channel AtCNGC10 localizes to the plasma membrane and influences numerous growth responses
565 and starch accumulation in Arabidopsis thaliana. *Planta*. 2007; 225(3):563–573.
- 566 **Caesar K**, Elgass K, Chen Z, Huppenberger P, Witthöft J, Schleifenbaum F, Blatt MR, Oecking C, Harter K. A fast
567 brassinolide-regulated response pathway in the plasma membrane of Arabidopsis thaliana. *The Plant jour-
568 nal : for cell and molecular biology*. 2011 may; 66(3):528–40. <http://www.ncbi.nlm.nih.gov/pubmed/21255166>,
569 doi: 10.1111/j.1365-313X.2011.04510.x.
- 570 **Christopher DA**, Borsics T, Yuen CY, Ullmer W, Andème-Ondzighi C, Andres MA, Kang BH, Staehelin LA. The
571 cyclic nucleotide gated cation channel AtCNGC 10 traffics from the ER via Golgi vesicles to the plasma mem-
572 brane of Arabidopsis root and leaf cells. *BMC Plant Biology*. 2007; 7:1–13. doi: 10.1186/1471-2229-7-48.

- 573 **Clouse SD.** Brassinosteroid signal transduction: clarifying the pathway from ligand perception to gene expres-
574 sion. *Molecular cell.* 2002 nov; 10(5):973–82. <http://www.ncbi.nlm.nih.gov/pubmed/12453407>.
- 575 **Clouse SD.** Brassinosteroid signal transduction: from receptor kinase activation to transcriptional networks
576 regulating plant development. *The Plant cell.* 2011 apr; 23(4):1219–30. [http://www.pubmedcentral.nih.gov/
577 articlerender.fcgi?artid=3101532&tool=pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3101532&tool=pmcentrez&rendertype=abstract), doi: 10.1105/tpc.111.084475.
- 578 **De Winter JC.** Using the Student's t-test with extremely small sample sizes. *Practical Assessment, Research,
579 and Evaluation.* 2013; 18(1):10.
- 580 **Degtyarenko K,** De Matos P, Ennis M, Hastings J, Zbinden M, McNaught A, Alcántara R, Darsow M, Guedj M,
581 Ashburner M. ChEBI: a database and ontology for chemical entities of biological interest. *Nucleic acids
582 research.* 2007; 36(suppl_1):D344–D350.
- 583 **Duszyn M,** Świeżawska B, Szmidt-Jaworska A, Jaworski K. Cyclic nucleotide gated channels (CNGCs) in plant
584 signalling—Current knowledge and perspectives. *Journal of Plant Physiology.* 2019; 241:153035. [https://www.
585 sciencedirect.com/science/article/pii/S017616171930152X](https://www.sciencedirect.com/science/article/pii/S017616171930152X), doi: <https://doi.org/10.1016/j.jplph.2019.153035>.
- 586 **Elgass K,** Caesar K, Schleifenbaum F, Stierhof YD, Meixner AJ, Harter K. Novel Application of Fluorescence
587 Lifetime and Fluorescence Microscopy Enables Quantitative Access to Subcellular Dynamics in Plant Cells.
588 *PLoS ONE.* 2009 may; 4(5):e5716. <http://dx.plos.org/10.1371/journal.pone.0005716>, doi: 10.1371/jour-
589 nal.pone.0005716.
- 590 **van Esse GW,** Harter K, de Vries SC. Computational modelling of the BRI1 receptor system. *Plant, cell & envi-
591 ronment.* 2013 sep; 36(9):1728–37. <http://www.ncbi.nlm.nih.gov/pubmed/23421559>, doi: 10.1111/pce.12077.
- 592 **van Esse GW,** van Mourik S, Stigter H, ten Hove Ca, Molenaar J, de Vries SC. A mathematical model for
593 BRASSINOSTEROID INSENSITIVE1-mediated signaling in root growth and hypocotyl elongation. *Plant physi-
594 ology.* 2012 sep; 160(1):523–32. [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3440225&tool=
595 pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3440225&tool=pmcentrez&rendertype=abstract), doi: 10.1104/pp.112.200105.
- 596 **van Esse G,** Westphal AH, Surendran RP, Albrecht C, van Veen B, Borst JW, de Vries SC. Quantification of
597 the brassinosteroid insensitive1 receptor in planta. *Plant physiology.* 2011 aug; 156(4):1691–700. [http://
598 www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3149942&tool=pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3149942&tool=pmcentrez&rendertype=abstract), doi:
599 10.1104/pp.111.179309.
- 600 **van Esse W,** van Mourik S, Albrecht C, van Leeuwen J, de Vries S. A mathematical model for the corecep-
601 tors SOMATIC EMBRYOGENESIS RECEPTOR-LIKE KINASE1 and SOMATIC EMBRYOGENESIS RECEPTOR-LIKE
602 KINASE3 in BRASSINOSTEROID INSENSITIVE1-mediated signaling. *Plant physiology.* 2013 nov; 163(3):1472–
603 81. <http://www.ncbi.nlm.nih.gov/pubmed/24072582>, doi: 10.1104/pp.113.222034.
- 604 **Fasano JM,** Swanson SJ, Blancaflor EB, Dowd PE, Kao Th, Gilroy S. Changes in Root Cap pH Are Required for
605 the Gravity Response of the Arabidopsis Root. *The Plant Cell.* 2001; 13(4):907–921. [http://www.plantcell.org/
606 content/13/4/907](http://www.plantcell.org/content/13/4/907), doi: 10.1105/tpc.13.4.907.
- 607 **Friedrichsen DM.** Brassinosteroid-Insensitive-1 Is a Ubiquitously Expressed Leucine-Rich Repeat Receptor
608 Serine/Threonine Kinase. *PLANT PHYSIOLOGY.* 2000 aug; 123(4):1247–1256. [http://www.plantphysiol.org/
609 content/123/4/1247.long](http://www.plantphysiol.org/content/123/4/1247.long), doi: 10.1104/pp.123.4.1247.
- 610 **Fuglsang AT,** Kristensen A, Cuin TA, Schulze WX, Persson J, Thuesen KH, Ytting CK, Oehlenschläger CB, Mah-
611 mood K, Sondergaard TE, et al. Receptor kinase-mediated control of primary active proton pumping at the
612 plasma membrane. *The Plant Journal.* 2014; 80(6):951–964.
- 613 **Glöckner N,** Oven-Krockhaus Sz, Rohr L, Wackenhut F, Burmeister M, Wanke F, Holzwardt E, Meixner AJ, Wolf
614 S, Harter K. Three-fluorophore FRET enables the analysis of ternary protein association in living plant cells.
615 *bioRxiv.* 2020; <https://www.biorxiv.org/content/early/2020/10/26/722124>, doi: 10.1101/722124.

- 616 **Grefen C.** The split-ubiquitin system for the analysis of three-component interactions. In: *Arabidopsis Protocols*
617 Springer; 2014.p. 659–678.
- 618 **Grefen C, Obrdlík P, Harter K.** The determination of protein-protein interactions by the mating-based split-
619 ubiquitin system (mbSUS). In: *Plant Signal Transduction* Springer; 2009.p. 217–233.
- 620 **Großholz R, Feldman-Salit A, Wanke F, Schulze S, Glöckner N, Kemmerling B, Harter K, Kummer U.** Specifying
621 the role of BAK1-interacting receptor-like kinase 3 in brassinosteroid signaling. *Journal of Integrative Plant*
622 *Biology*. 2020; 62(4). doi: [10.1111/jipb.12803](https://doi.org/10.1111/jipb.12803).
- 623 **Hacham Y, Holland N, Butterfield C, Ubeda-Tomas S, Bennett MJ, Chory J, Savaldi-Goldstein S.** Brassinosteroid
624 perception in the epidermis controls root meristem size. *Development*. 2011; 138(5):839–848.
- 625 **Higinbotham N.** Electropotentials of plant cells. *Annual Reviews of Plant Physiology*. 1973; .
- 626 **Hohmann U, Santiago J, Nicolet J, Olsson V, Spiga FM, Hothorn LA, Butenko MA, Hothorn M.** Mech-
627 anistic basis for the activation of plant membrane receptor kinases by SERK-family corecep-
628 tors. *Proceedings of the National Academy of Sciences*. 2018 mar; 115(13):201714972. [http:](http://www.pnas.org/lookup/doi/10.1073/pnas.1714972115)
629 [://www.pnas.org/lookup/doi/10.1073/pnas.1714972115](http://www.pnas.org/lookup/doi/10.1073/pnas.1714972115)<http://www.ncbi.nlm.nih.gov/pubmed/29531026><http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5879659>, doi: [10.1073/pnas.1714972115](https://doi.org/10.1073/pnas.1714972115).
- 631 **Holzheu P, Großholz R, Kummer U.** Impact of explicit area scaling on kinetic models involving multiple com-
632 partments. *BMC bioinformatics*. 2021; 22(1):1–12.
- 633 **Holzheu P, Kummer U.** Computational systems biology of cellular processes in *Arabidopsis thaliana*:
634 an overview. *Cellular and Molecular Life Sciences*. 2020; 77(3):433–440. [https://doi.org/10.1007/](https://doi.org/10.1007/s00018-019-03379-9)
635 [s00018-019-03379-9](https://doi.org/10.1007/s00018-019-03379-9), doi: [10.1007/s00018-019-03379-9](https://doi.org/10.1007/s00018-019-03379-9).
- 636 **Holzwardt E, Huerta AI, Glöckner N, Gómez BG, Wanke F, Augustin S, Askani JC, Schürholz AK, Harter K, Wolf**
637 **S.** BRI1 controls vascular cell fate in the *Arabidopsis* root through RLP44 and phyto-sulfonine signaling. *Pro-*
638 *ceedings of the National Academy of Sciences*. 2018; 115(46):11838–11843.
- 639 **Hoops S, Sahle S, Gauges R, Lee C, Pahle J, Simus N, Singhal M, Xu L, Mendes P, Kummer U.** COPASI—a COMplex
640 PATHway Simulator. *Bioinformatics (Oxford, England)*. 2006 dec; 22(24):3067–74. [http://www.ncbi.nlm.nih.](http://www.ncbi.nlm.nih.gov/pubmed/17032683)
641 [gov/pubmed/17032683](http://www.ncbi.nlm.nih.gov/pubmed/17032683), doi: [10.1093/bioinformatics/btl485](https://doi.org/10.1093/bioinformatics/btl485).
- 642 **Hübner K, Sahle S, Kummer U.** Applications and trends in systems biology in biochemistry. *The FEBS*
643 *journal*. 2011 aug; 278(16):2767–857. <http://www.ncbi.nlm.nih.gov/pubmed/21707921>, doi: [10.1111/j.1742-](https://doi.org/10.1111/j.1742-4658.2011.08217.x)
644 [4658.2011.08217.x](https://doi.org/10.1111/j.1742-4658.2011.08217.x).
- 645 **Imkampe J, Halter T, Huang S, Schulze S, Mazzotta S, Schmidt N, Manstretta R, Postel S, Wierzbna M, Yang Y, Van-**
646 **Dongen WM, Stahl M, Zipfel C, Goshe MB, Clouse S, de Vries SC, Tax F, Wang X, Kemmerling B.** The *Arabidopsis*
647 Leucine-rich Repeat Receptor Kinase BIR3 Negatively Regulates BAK1 Receptor Complex Formation and Sta-
648 bilizes BAK1. *The Plant Cell*. 2017 aug; p. tpc.00376.2017. <http://www.ncbi.nlm.nih.gov/pubmed/28842532><http://www.plantcell.org/lookup/doi/10.1105/tpc.17.00376>, doi: [10.1105/tpc.17.00376](https://doi.org/10.1105/tpc.17.00376).
- 650 **Jin Y, Jing W, Zhang Q, Zhang W.** Cyclic nucleotide gated channel 10 negatively regulates salt tolerance by
651 mediating Na⁺ transport in *Arabidopsis*. *Journal of plant research*. 2015; 128(1):211–220.
- 652 **Junker BH, Klukas C, Schreiber F.** VANTED: A system for advanced data analysis and visualization in
653 the context of biological networks. *BMC Bioinformatics*. 2006; 7(1):109. [http://bmcbioinformatics.](http://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-7-109)
654 [biomedcentral.com/articles/10.1186/1471-2105-7-109](http://bmcbioinformatics.biomedcentral.com/articles/10.1186/1471-2105-7-109)[http://www.pubmedcentral.nih.gov/articlerender.](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1413562&tool=pmcentrez&rendertype=abstract)
655 [fcgi?artid=1413562&tool=pmcentrez&rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1413562&tool=pmcentrez&rendertype=abstract){%}5Cn[http://www.biomedcentral.com/](http://www.biomedcentral.com/1471-2105/7/109/abstract)
656 [1471-2105/7/109/abstract](http://www.biomedcentral.com/1471-2105/7/109/abstract){%}5Cn[internal-pdf://160/109](http://www.biomedcentral.com/1471-2105/7/109/abstract), doi: [10.1186/1471-2105-7-109](https://doi.org/10.1186/1471-2105-7-109).

- 657 **Karimi M**, Inzé D, Depicker A. GATEWAY vectors for Agrobacterium-mediated plant transformation. Trends in
658 plant science. 2002 may; 7(5):193–5. <http://www.ncbi.nlm.nih.gov/pubmed/11992820>.
- 659 **Kinoshita T**, Caño-Delgado A, Seto H. Binding of brassinosteroids to the extracellular domain of plant recep-
660 tor kinase BRI1. Nature. 2005; 433(13):167–171. [http://www.nature.com/nature/journal/v433/n7022/abs/](http://www.nature.com/nature/journal/v433/n7022/abs/nature03227.html)
661 [nature03227.html](http://www.nature.com/nature/journal/v433/n7022/abs/nature03227.html), doi: 10.1038/nature03188.Published.
- 662 **Ladwig F**, Dahlke RI, Stührwohldt N, Hartmann J, Harter K, Sauter M. Phytosulfokine Regulates
663 Growth in Arabidopsis through a Response Module at the Plasma Membrane That Includes CYCLIC
664 NUCLEOTIDE-GATED CHANNEL17, H⁺ -ATPase, and BAK1. The Plant Cell. 2015 jun; 27(6):1718–
665 1729. <http://www.ncbi.nlm.nih.gov/pubmed/26071421>[http://www.pubmedcentral.nih.gov/articlerender.fcgi?](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4498212)
666 [artid=PMC4498212](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC4498212)<http://www.plantcell.org/lookup/doi/10.1105/tpc.15.00306>, doi: 10.1105/tpc.15.00306.
- 667 **Lv M**, Li J. Molecular Mechanisms of Brassinosteroid-Mediated Responses to Changing Environments in Ara-
668 bidopsis. International Journal of Molecular Sciences. 2020 Apr; 21(8):2737. [http://dx.doi.org/10.3390/](http://dx.doi.org/10.3390/ijms21082737)
669 [ijms21082737](http://dx.doi.org/10.3390/ijms21082737), doi: 10.3390/ijms21082737.
- 670 **Lv M**, Li M, Chen W, Wang Y, Sun C, Yin H, He K, Li J. Thermal-enhanced bri1-301 instability reveals a plasma
671 membrane protein quality control system in plants. Frontiers in plant science. 2018; 9:1620.
- 672 **Ma X**, Denyer T, Timmermans MC. PscB: A Browser to Explore Plant Single Cell RNA-Sequencing Datasets. Plant
673 Physiology. 2020; 183(2):464–467.
- 674 **Maathuis FJ**, Sanders D. Energization of potassium uptake in Arabidopsis thaliana. Planta. 1993; 191(3):302–
675 307.
- 676 **Mendes P**, Hoops S, Sahle S, Gauges R, Dada J, Kummer U. Computational modeling of biochemical networks
677 using COPASI. In: *Systems Biology* Springer; 2009.p. 17–59.
- 678 **Merlot S**, Leonhardt N, Fenzi F, Valon C, Costa M, Piette L, Vavasseur A, Genty B, Boivin K, Müller A, et al. Consti-
679 tutive activation of a plasma membrane H⁺-ATPase prevents abscisic acid-mediated stomatal closure. The
680 EMBO journal. 2007; 26(13):3216–3226.
- 681 **Minami A**, Takahashi K, Inoue Si, Tada Y, Kinoshita T. Brassinosteroid induces phosphorylation of the plasma
682 membrane H⁺-ATPase during hypocotyl elongation in Arabidopsis thaliana. Plant and Cell Physiology. 2019;
683 60(5):935–944.
- 684 **Müssig C**, Fischer S, Altmann T. Brassinosteroid-regulated gene expression. Plant physiology. 2002
685 jul; 129(3):1241–51. <http://www.ncbi.nlm.nih.gov/pubmed/12114578>[http://www.pubmedcentral.nih.](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC166518)
686 [gov/articlerender.fcgi?artid=PMC166518](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC166518)<http://www.ncbi.nlm.nih.gov/pubmed/12114578>{%}5Cn[http:](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC166518)
687 [//www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC166518](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC166518), doi: 10.1104/pp.011003.
- 688 **Newman I**. Ion transport in roots: measurement of fluxes using ion-selective microelectrodes to characterize
689 transporter function. Plant, cell & environment. 2001; 24(1):1–14.
- 690 **Novère NL**, Hucka M, Mi H, Moodie S, Schreiber F, Sorokin A, Demir E, Wegner K, Aladjem MI, Wimalaratne
691 SM, Bergman FT, Gauges R, Ghazal P, Kawaji H, Li L, Matsuoka Y, Villéger A, Boyd SE, Calzone L, Courtot
692 M, et al. The Systems Biology Graphical Notation. Nature Biotechnology. 2009 aug; 27(8):735–741. [http:](http://www.nature.com/doi/10.1038/nbt.1558)
693 [//www.nature.com/doi/10.1038/nbt.1558](http://www.nature.com/doi/10.1038/nbt.1558), doi: 10.1038/nbt.1558.
- 694 **Obrdlik P**, El-Bakkoury M, Hamacher T, Cappellaro C, Vilarino C, Fleischer C, Ellerbrok H, Kamuzinzi R, Ledent
695 V, Blaudez D, et al. K⁺ channel interactions detected by a genetic system optimized for systematic studies
696 of membrane protein interactions. Proceedings of the National Academy of Sciences. 2004; 101(33):12242–
697 12247.

- 698 **Oh MH**, Wang X, Clouse SD, Huber SC. Deactivation of the Arabidopsis BRASSINOSTEROID INSENSITIVE 1 (BRI1)
699 receptor kinase by autophosphorylation within the glycine-rich loop. *Proceedings of the National Academy*
700 *of Sciences of the United States of America*. 2012 jan; 109(1):327–32. [http://www.pubmedcentral.nih.gov/](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3252896&tool=pmcentrez&rendertype=abstract)
701 [articlerender.fcgi?artid=3252896{&}tool=pmcentrez{&}rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3252896&tool=pmcentrez&rendertype=abstract), doi: 10.1073/pnas.1108321109.
- 702 **Pacifici E**, Di Mambro R, Dello Ioio R, Costantino P, Sabatini S. Acidic cell elongation drives cell differentiation
703 in the Arabidopsis root. *The EMBO journal*. 2018; 37(16):e99134.
- 704 **Palmgren MG**, Sommarin M, Serrano R, Larsson C. Identification of an autoinhibitory domain in the C-terminal
705 region of the plant plasma membrane H⁺-ATPase. *Journal of Biological Chemistry*. 1991; 266(30):20470–
706 20475.
- 707 **Phyo P**, Gu Y, Hong M. Impact of acidic pH on plant cell wall polysaccharide structure and dynamics: insights
708 into the mechanism of acid growth in plants from solid-state NMR. *Cellulose*. 2019; 26(1):291–304. <https://doi.org/10.1007/s10570-018-2094-7>, doi: 10.1007/s10570-018-2094-7.
- 710 **R Core Team**. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing,
711 Vienna, Austria; 2020, <https://www.R-project.org/>.
- 712 **Regenberg B**, Villalba JM, Lanfermeijer FC, Palmgren MG. C-terminal deletion analysis of plant plasma
713 membrane H⁽⁺⁾-ATPase: yeast as a model system for solute transport across the plant plasma mem-
714 brane. *The Plant cell*. 1995 oct; 7(10):1655–1666. <http://www.ncbi.nlm.nih.gov/pubmed/7580256>[http://www.](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC161027)
715 [pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC161027](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC161027), doi: 10.1105/tpc.7.10.1655.
- 716 **Sankar M**, Osmont KS, Rolcik J, Gujas B, Tarkowska D, Strnad M, Xenarios I, Hardtke CS. A qualitative contin-
717 uous model of cellular auxin and brassinosteroid signaling and their crosstalk. *Bioinformatics*. 2011 may;
718 27(10):1404–1412. <https://academic.oup.com/bioinformatics/article-lookup/doi/10.1093/bioinformatics/btr158>,
719 doi: 10.1093/bioinformatics/btr158.
- 720 **Staal M**, De Cnodder T, Simon D, Vandenbussche F, Van Der Straeten D, Verbelen JP, Elzenga T, Vissenberg K.
721 Apoplastic alkalization is instrumental for the inhibition of cell elongation in the Arabidopsis root by the
722 ethylene precursor 1-aminocyclopropane-1-carboxylic acid. *Plant Physiology*. 2011; 155(4):2049–2055.
- 723 **Sullivan A**, Purohit PK, Freese NH, Pasha A, Esteban E, Waese J, Wu A, Chen M, Chin CY, Song R, et al. An 'eFP-Seq
724 Browser' for visualizing and exploring RNA sequencing data. *The Plant Journal*. 2019; 100(3):641–654.
- 725 **Veerabagu M**, Elgass K, Kirchler T, Huppenberger P, Harter K, Chaban C, Mira-Rodado V. The Arabidop-
726 sis B-type response regulator 18 homomerizes and positively regulates cytokinin responses. *Plant Jour-*
727 *nal*. 2012 dec; 72(5):721–731. <http://doi.wiley.com/10.1111/j.1365-313X.2012.05101.x>, doi: 10.1111/j.1365-
728 313X.2012.05101.x.
- 729 **Verbelen JP**, Cnodder TD, Le J, Vissenberg K, Baluška F. The root apex of Arabidopsis thaliana consists of
730 four distinct zones of growth activities: meristematic zone, transition zone, fast elongation zone and growth
731 terminating zone. *Plant signaling & behavior*. 2006; 1(6):296–304.
- 732 **Vernoux T**, Brunoud G, Farcot E, Morin V, Van den Daele H, Legrand J, Oliva M, Das P, Larrieu A, Wells D, Gué-
733 don Y, Armitage L, Picard F, Guyomarc'h S, Cellier C, Parry G, Koumproglou R, Doonan JH, Estelle M, Godin
734 C, et al. The auxin signalling network translates dynamic input into robust patterning at the shoot apex.
735 *Molecular systems biology*. 2011 jan; 7(508):508. [http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3167386&tool=pmcentrez&rendertype=abstract)
736 [3167386{&}tool=pmcentrez{&}rendertype=abstract](http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3167386&tool=pmcentrez&rendertype=abstract), doi: 10.1038/msb.2011.39.
- 737 **Vukasinovic N**, Wang Y, Vanhoutte I, Fendrych M, Guo B, Kvasnica M, Jiroutova P, Oklestkova J, Strnad M, Russi-
738 nova E. Local brassinosteroid biosynthesis enables optimal root growth. *bioRxiv*. 2020; .

- 739 **Wang J**, Jiang J, Wang J, Chen L, Fan Sl, Wu Jw, Wang X, Wang Zx. Structural insights into the negative regulation
740 of BRI1 signaling by BRI1-interacting protein BKI1. *Cell research*. 2014; 24(11):1–14. <http://dx.doi.org/10.1038/cr.2014.132><http://www.ncbi.nlm.nih.gov/pubmed/25331450>, doi: 10.1038/cr.2014.132.
- 742 **Wang Zy**, Seto H, Fujioka S, Yoshida S, Chory J. BRI1 is a critical component of a plasma-membrane receptor
743 for plant steroids. *Nature*. 2001; 410(MARCH):380–383.
- 744 **White PJ**, Biskup B, ElzengaJTM, Homann U, Thiel G, Wissing F, Maathuis FJM. Advanced patch-clamp techniques
745 and single-channel analysis. *Journal of Experimental Botany*. 1999 jun; 50(Special):1037–1054. <http://jxb.oxfordjournals.org/content/50/Special{ }Issue/1037.short>, doi: 10.1093/jxb/50.Special_Issue.1037.
- 747 **Winter D**, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ. An "electronic fluorescent pictograph" Browser
748 for exploring and analyzing large-scale biological data sets. *PLoS ONE*. 2007 aug; 2(8):e718. <http://dx.plos.org/10.1371/journal.pone.0000718>, doi: 10.1371/journal.pone.0000718.
- 750 **Witthöft J**, Caesar K, Elgass K, Huppenberger P, Kilian J, Schleifenbaum F, Oecking C, Harter K. The activation
751 of the Arabidopsis P-ATPase 1 by the brassinosteroid receptor BRI1 is independent of threonine 948 phos-
752 phorylation. *Plant Signaling & Behavior*. 2011 jul; 6(7):1063–1066. <http://www.landesbioscience.com/journals/psb/article/15650/>, doi: 10.4161/psb.6.7.15650.
- 754 **Witthöft J**, Harter K. Latest news on Arabidopsis brassinosteroid perception and signaling. *Frontiers in plant*
755 *science*. 2011 jan; 2(October):58. <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3355717{ }& }tool=pmcentrez{ }& }rendertype=abstract>, doi: 10.3389/fpls.2011.00058.
- 757 **Wolf S**. Deviating from the Beaten Track: New Twists in Brassinosteroid Receptor Function. *Internation-*
758 *Journal of Molecular Sciences*. 2020 Feb; 21(5):1561. <http://dx.doi.org/10.3390/ijms21051561>, doi:
759 10.3390/ijms21051561.
- 760 **Wolf S**, Van Der Does D, Ladwig F, Sticht C, Kolbeck A, Schürholz AK, Augustin S, Keinath N, Rausch T, Greiner
761 S, et al. A receptor-like protein mediates the response to pectin modification by activating brassinosteroid
762 signaling. *Proceedings of the National Academy of Sciences*. 2014; 111(42):15261–15266.
- 763 **Yuan W**, Li Y, Li L, Siao W, Zhang Q, Zhang Y, Liu J, Xu W, Miao R. BR-INSENSITIVE1 regulates hydrotropic re-
764 sponse by interacting with plasma membrane H⁺-ATPases in Arabidopsis. *Plant Signaling & Behavior*. 2018;
765 13(8):e1486147. <https://doi.org/10.1080/15592324.2018.1486147>, doi: 10.1080/15592324.2018.1486147,
766 pMID: 30067914.
- 767 **Zhang X**, Zhou L, Qin Y, Chen Y, Liu X, Wang M, Mao J, Zhang J, He Z, Liu L, et al. A temperature-sensitive misfolded
768 bri1-301 receptor requires its kinase activity to promote growth. *Plant Physiology*. 2018; 178(4):1704–1719.

769 **Appendix 1**

770 **Model Information**

771 **Compartments**

772 **Appendix 1 Table 1.** Overview of model compartments and sizes for both MZ and early EZ. ^a
 773 calculated by multiplying the membrane area with the cell wall thickness (*van Esse et al., 2011; Caesar*
 774 *et al., 2011*). ^b estimated volume based on cell dimensions and cellular volume (*van Esse et al., 2011*).
 775 ^c estimated surface area, included as scaling factor in the global quantities.

Root zone	Compartment	Size
Meristematic zone	cytosol	$8.47 \times 10^{-13} \text{ dm}^3$
	membrane	$7.67 \times 10^{-8} \text{ dm}^2$
	cell wall ^a	$3.03 \times 10^{-13} \text{ dm}^3$
	vacuole	NA
	vacuolar surface	NA
Early elongation zone	cytosol	$2.271 \times 10^{-12} \text{ dm}^3$
	membrane	$2.098 \times 10^{-7} \text{ dm}^2$
	cell wall ^a	$8.2871 \times 10^{-13} \text{ dm}^3$
	vacuole ^b	$2.352 \times 10^{-12} \text{ dm}^3$
	vacuolar surface ^c	$1.087 \times 10^{-7} \text{ dm}^2$

779 **Ordinary Differential Equations**

780 **Compartment Sizes**

781 **Cell Wall Volume**

782

$$V_{cell\ wall}(t) = A_{cell\ surface} \cdot cell\ wall\ thickness(t)$$

784

785 **Model species**

786 **BRI1**

$$\begin{aligned} \frac{d([BRI1] \cdot A_{cell\ surface})}{dt} = & - A_{cell\ surface} \cdot (k_{on} \cdot [BL] \cdot [BRI1] - k_{off} \cdot [BRI1\ BL]) \\ & - A_{cell\ surface} \cdot (k \cdot [BRI1] \cdot [BKI1] - k \cdot K_D \cdot [BRI1\ BKI1]) \\ & + A_{cell\ surface} \cdot k_{off1} \cdot [BRI1p\ BL] \\ & + A_{cell\ surface} \cdot k_{off2} \cdot [BAK1\ BRI1p\ BL] \\ & + A_{cell\ surface} \cdot k_{off2} \cdot [BAK1p\ BRI1pp\ BL] \\ & + A_{cell\ surface} \cdot k_{off3} \cdot [BAK1p\ BRI1pppS891\ BL] \end{aligned}$$

787

788

789

790

BL

$$\begin{aligned} \frac{d([BL] \cdot A_{cell\ surface})}{dt} = & - A_{cell\ surface} \cdot (k_{on} \cdot [BL] \cdot [BRI1] - k_{off} \cdot [BRI1\ BL]) \\ & + A_{cell\ surface} \cdot k_{off} \cdot [BRI1p\ BL] \\ & + A_{cell\ surface} \cdot k_{off2} \cdot [BAK1\ BRI1p\ BL] \\ & + A_{cell\ surface} \cdot k_{off2} \cdot [BAK1p\ BRI1pp\ BL] \\ & + A_{cell\ surface} \cdot k_{off3} \cdot [BAK1p\ BRI1pppS891\ BL] \\ & + V_{cell\ wall} \cdot (k \cdot dose \cdot stimulation - k \cdot [BL]) \end{aligned}$$

791

792

793

794

BKI1pY211

$$\begin{aligned} \frac{d([BK11pY211] \cdot V_{cytosol})}{dt} = & + A_{cell\ surface} \cdot k \cdot [BRI1\ BL] \\ & * \frac{[BK11]}{(K_{i,BK11} + [BK11]) * (1 + \frac{[BK11]}{K_{i,BK11}}) * (1 + \frac{[BK11]}{K_{i,BK11}})} \\ & - A_{cell\ surface} \cdot k \cdot [BK11pY211] \end{aligned}$$

795

796

797

798

BKI1

$$\begin{aligned} \frac{d([BK11] \cdot A_{cell\ surface})}{dt} = & - A_{cell\ surface} \cdot k \cdot [BRI1\ BL] \\ & * \frac{[BK11]}{(K_{i,BK11} + [BK11]) * (1 + \frac{[BK11]}{K_{i,BK11}}) * (1 + \frac{[BK11]}{K_{i,BK11}})} \\ & + A_{cell\ surface} \cdot k \cdot [BK11pY211] \end{aligned}$$

799

800

801

802

phosphorylated AHA C-terminus

$$\begin{aligned} \frac{d([AHA\ CTp] \cdot A_{cell\ surface})}{dt} = & - A_{cell\ surface} \cdot k \cdot [AHA\ CTp] \\ & + A_{cell\ surface} \cdot k \cdot [BAK1p\ BRI1pp\ BL] \cdot \frac{[AHA\ CT]}{[AHA\ CT] + K} \end{aligned}$$

803

804

805

806

AHA C-terminus

$$\begin{aligned} \frac{d([AHA\ CT] \cdot A_{cell\ surface})}{dt} = & + A_{cell\ surface} \cdot k \cdot [AHA\ CTp] \\ & - A_{cell\ surface} \cdot k \cdot [BAK1p\ BRI1pp\ BL] \cdot \frac{[AHA\ CT]}{[AHA\ CT] + K} \end{aligned}$$

807

808

809

810

BAK1

$$\begin{aligned} \frac{d([BAK1] \cdot A_{cell\ surface})}{dt} = & - A_{cell\ surface} \cdot (k \cdot [BAK1] \cdot [BIR3] - k \cdot K_D \cdot [BIR3 \ BAK1]) \\ & - A_{cell\ surface} \cdot (k \cdot [BAK1] \cdot [BRI1p \ BL] - k \cdot K_D \cdot [BAK1 \ BRI1p \ BL]) \\ & + A_{cell\ surface} \cdot k_{off2} \cdot [BAK1 \ BRI1p \ BL] \\ & + A_{cell\ surface} \cdot k_{off2} \cdot [BAK1p \ BRI1pp \ BL] \\ & + A_{cell\ surface} \cdot k_{off3} \cdot [BAK1p \ BRI1pppS891 \ BL] \end{aligned}$$

811

812

813

814

BAK1 BRI1p BL

$$\begin{aligned} \frac{d([BAK1 \ BRI1p \ BL] \cdot A_{cell\ surface})}{dt} = & + A_{cell\ surface} \cdot (k \cdot [BRI1p \ BL] \cdot [BAK1] \\ & - k_{off} \cdot [BAK1 \ BRI1p \ BL]) \\ & - A_{cell\ surface} \cdot k_{off2} \cdot [BAK1 \ BRI1p \ BL] \\ & - A_{cell\ surface} \cdot k \cdot [BAK1 \ BRI1p \ BL] \end{aligned}$$

815

816

817

818

BAK1p BRI1pp BL

$$\begin{aligned} \frac{d([BAK1p \ BRI1pp \ BL] \cdot A_{cell\ surface})}{dt} = & + A_{cell\ surface} \cdot k \cdot [BAK1 \ BRI1p \ BL] \\ & - A_{cell\ surface} \cdot k_{off2} \cdot [BAK1p \ BRI1pp \ BL] \\ & - A_{cell\ surface} \cdot k \cdot [BAK1p \ BRI1pp \ BL] \cdot \frac{[BAK1p \ BRI1pp \ BL]}{[BAK1p \ BRI1pp \ BL] + K_M} \end{aligned}$$

819

820

821

822

BAK1p BRI1pppS891 BL

$$\begin{aligned} \frac{d([BAK1p \ BRI1pppS891 \ BL] \cdot A_{cell\ surface})}{dt} = & + A_{cell\ surface} \cdot k \cdot [BAK1p \ BRI1pp \ BL] \\ & \cdot \frac{[BAK1p \ BRI1pp \ BL]}{[BAK1p \ BRI1pp \ BL] + K_M} \\ & - A_{cell\ surface} \cdot k_{off3} \cdot [BAK1p \ BRI1pppS891 \ BL] \end{aligned}$$

823

824

825

826

BIK1

$$\begin{aligned} \frac{d([BIK1] \cdot A_{cell\ surface})}{dt} = & - A_{cell\ surface} \cdot k \cdot [BRI1 \ BL] \\ & \cdot \frac{[BIK1]}{(K_{i,BIK1} + [BIK1]) \cdot (1 + \frac{[BK11]}{K_{i,BK1}}) \cdot (1 + \frac{[BIK1]}{K_{i,BIK1}})} \\ & + A_{cell\ surface} \cdot k \cdot [BIK1p] \end{aligned}$$

827

828

829

830

BIK1p

$$\begin{aligned} \frac{d([BIK1p] \cdot A_{cell\ surface})}{dt} &= + A_{cell\ surface} \cdot k \cdot [BRI1\ BL] \\ &\cdot \frac{[BIK1]}{(K_{i,BIK1} + [BIK1]) \cdot (1 + \frac{[BK11]}{K_{i,BK11}}) \cdot (1 + \frac{[BIK1]}{K_{i,BIK1}})} \\ &- A_{cell\ surface} \cdot k \cdot [BIK1p] \end{aligned}$$

831

832

833

834

BRI1 BL

$$\begin{aligned} \frac{d([BRI1\ BL] \cdot A_{cell\ surface})}{dt} &= - A_{cell\ surface} \cdot k \cdot [BRI1\ BL] \\ &\cdot \frac{[1]}{(1 + \frac{[BK11]}{K_{i,BK11}}) \cdot (1 + \frac{[BIK1]}{K_{i,BIK1}})} \\ &+ A_{cell\ surface} \cdot (k \cdot stimulation \cdot [BL] \cdot [BRI1] - k_{off} \cdot [BRI1\ BL]) \end{aligned}$$

835

836

837

838

BRI1p BL

$$\begin{aligned} \frac{d([BRI1p\ BL] \cdot A_{cell\ surface})}{dt} &= + A_{cell\ surface} \cdot k \cdot [BRI1\ BL] \\ &\cdot \frac{[1]}{(1 + \frac{[BK11]}{K_{i,BK11}}) \cdot (1 + \frac{[BIK1]}{K_{i,BIK1}})} \\ &- A_{cell\ surface} \cdot (k \cdot [BAK1] \cdot [BRI1p\ BL] - k_{off} \cdot [BAK1\ BRI1p\ BL]) \\ &- A_{cell\ surface} \cdot k_{off} \cdot [BRI1p\ BL] \end{aligned}$$

839

840

841

842

BIR3

$$\begin{aligned} \frac{d([BIR3] \cdot A_{cell\ surface})}{dt} &= - A_{cell\ surface} \cdot (k \cdot [BIR3] \cdot [BAK1] - k \cdot K_D \cdot [BIR3\ BAK1]) \\ &- A_{cell\ surface} \cdot (k \cdot [BIR3] \cdot [BRI1] - k \cdot K_D \cdot [BIR3\ BRI1]) \end{aligned}$$

843

844

845

846

BIR3 BAK1

$$\frac{d([BIR3\ BAK1] \cdot A_{cell\ surface})}{dt} = - A_{cell\ surface} \cdot (k \cdot [BIR3] \cdot [BAK1] - k \cdot K_D \cdot [BIR3\ BAK1])$$

847

848

849

850

BIR3 BRI1

$$\frac{d([BIR3 BRI1] \cdot A_{cell\ surface})}{dt} = - A_{cell\ surface} \cdot (k \cdot [BIR3] \cdot [BRI1] - k \cdot K_D \cdot [BIR3 BRI1])$$

851

852

853

854

Extracellular Proton Concentration

$$\begin{aligned} \frac{d([H_{out}^+] \cdot V_{cell\ wall})}{dt} = & + A_{cell\ surface} \cdot k \cdot [AHA] \cdot [H_{in}^+] \cdot \frac{[AHA]}{[AHA] + Inhibition_{AHACT} \cdot [AHACT]} \\ & - A_{cell\ surface} \cdot k \cdot ([H_{out}^+] - [H_{in}^+]) \end{aligned}$$

855

856

857

858

CNGC10 open

$$\begin{aligned} \frac{d([CNGC10_{open}] \cdot A_{cell\ surface})}{dt} = & + A_{cell\ surface} \\ & \cdot \left(k_1 \cdot [BAK1p BRI1pp BL] \cdot \frac{[CNGC10_{closed}]}{K_M + [CNGC10_{closed}]} - k_2 \cdot [CNGC10_{open}] \right) \end{aligned}$$

859

860

861

862

CNGC10 closed

$$\begin{aligned} \frac{d([CNGC10_{closed}] \cdot A_{cell\ surface})}{dt} = & - A_{cell\ surface} \\ & \cdot \left(k_1 \cdot [BAK1p BRI1pp BL] \cdot \frac{[CNGC10_{closed}]}{K_M + [CNGC10_{closed}]} - k_2 \cdot [CNGC10_{open}] \right) \end{aligned}$$

863

864

865

866

Intracellular Potassium Concentration

$$\begin{aligned} \frac{d([K_{in}^+] \cdot V_{cell})}{dt} = & + k \cdot A_{cell\ surface} \cdot [CNGC10_{open}] \cdot \frac{[K_{out}^+]}{K_d} \cdot \left(\frac{E_m}{-0.59} - 1 \right) \\ & + A_{cell\ surface} \cdot k \cdot [K_{in}^+] - k \cdot K_{eq} \cdot [K_{ex}^+] \\ & - A_{vacuole} \cdot k \cdot (K_{in}^+ - K_{vac}^+) \end{aligned}$$

867

868

869

870

871

Vacuolar Potassium Concentration

$$\frac{d([K_{vac}^+] \cdot V_{vacuole})}{dt} = + A_{vacuole} \cdot k \cdot ([K_{in}^+] - [K_{vac}^+])$$

872

873

876

877

874

Global Quantities

878

875

Net charge change

879

880

881

$$\Delta Q = (([K_{in}^+] - [K_{in,0}^+]) \cdot V_{cell} - ([H_{out}^+] - [H_{out,0}^+]) \cdot V_{cell\ wall}) \cdot Faraday\ constant\ F \cdot factor_{pmol\ to\ mol}$$

$$with : F = 96485.33212C\ mol^{-1}$$

883
882
884
885
887
886
888
889
891
890
892
893
894
895
896
897
898
899
900
901
902
903

Membrane potential change

$$\Delta E_m = \frac{\text{net charge distribution change } \Delta Q}{\text{specific capacitance} * \text{membrane area}}$$

Cell wall instability

$$\text{cell wall instability}(t) = \frac{1}{1 + e^{-0.001([H_{out}^+] - 1.2 \cdot \text{proton readout})}} \cdot \text{stimulation} \cdot \left(1 - \frac{1}{1 + e^{-10^7 \cdot (\text{cell wall}(t) - 5.0 \cdot 10^{-6})}} \right)$$

Cell wall thickness

$$\frac{d(\text{cell wall thickness})}{dt} = 0.00015 \cdot \text{stimulation} \cdot \text{cell wall thickness}(t)$$

factor BIR3

Factor representing the expression level of BIR3. 1 represents the normal expression level, 100 represents the overexpression level.

factor BRI1

Factor representing the expression level of BRI1. 1 represents the normal expression level, 130 represents the overexpression level.

Events

Stimulation

Trigger: *Model Time* > 86400 s

Target: Global quantity *stimulation transient value* is set to 1 from the initial value of 0.

904
905
906
907
908
910
911

Overview of model components

Appendix 1 Table 2. Protein are specified by the Uniprot identifier (*Bairoch et al., 2005*) and the corresponding gene ID. For ions and chemical compounds, the ChEBI (Chemical Entities of Biological Interest (*Degtyarenko et al., 2007*)) identifier is used instead. The initial concentrations of all un-phosphorylated species and complexes between proteins were set to 0 pM.

Species	Uniprot ID / ChEBI ID	Gene ID	Initial Concentration	Source
BRI1	O22476	At4g39400	0.182 633 pM	(<i>van Esse et al., 2011</i>)
BAK1	Q94F62	At4g33430	0.099 632 pM	(<i>van Esse et al., 2011</i>)
BIR3			0.237 423 11 pM	this study
AHA			0.232 442 pM	<i>AHA1 + AHA2</i>
AHA1	P20649	At2g18960	0.116 221 pM	assumption: $\frac{AHA1}{AHA2} \approx \frac{1}{1}$ mRNA data (eFP Browser) (<i>Winter et al., 2007</i>)
AHA2	P19456	At4g30190	0.116 221 pM	this study
AHA C-terminus			0.232 442 pM	<i>AHA1 + AHA2</i>
BKI1	Q9FMZ0	At5g42750	0.219 16 pM	assumption: $1.2 * [BRI1]_{t=0}$
BIK1	O48814	At2g39660	0.219 16 pM	assumption: $1.2 * [BRI1]_{t=0}$
CNGC10 _{closed}	Q9LNJ0	At1g01340	0.1 pM	
H ⁺ _{in}	24636	-	63 000 pM	
H ⁺ _{out}	24636	-	fitted to data	
K ⁺ _{out}	29103	-	9.8425×10^9 pM	½ MS medium
K ⁺ _{in}	29103	-	8.4×10^{10} pM	(<i>Maathuis and Sanders, 1993</i>)
K ⁺ _{vac}	29103	-	8.4×10^{10} pM	assumed to be identical to K ⁺ _{in}
BL	28277	-	<i>dose</i>	see experimental setup

912
913
914
916

Experimentally determined parameters

Appendix 1 Table 3. Model parameter that were either experimentally determined or where a range or estimate of experimentally determined values was available. Rate law abbreviations: MA - mass action kinetics, MM - Michaelis-Menten kinetics, CF - constant flux.

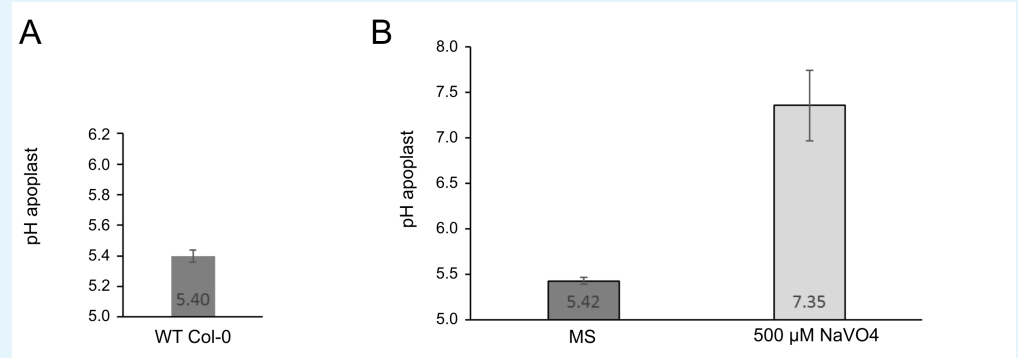
ID	Rate Law	Parameter	Value	Source
r_{01}	modified MA	K_i	up to 7.7 fold for AHA2	(Regenberg et al., 1995)
r_{02}	modified MA	k	$0.84 \times 10^{-9} \text{ dm s}^{-1}$ to $1.25 \times 10^{-9} \text{ dm s}^{-1}$	this study Appendix 1 Fig. 1
r_{03}	modified MA			
r_{04}	MA			
r_{05}	MA			
r_{06}	MA			
r_{07}	MA			
r_{08}	CF, MA	<i>dose</i>	0 nM, 1×10^4 pM, 5×10^4 pM, 1×10^5 pM	(Caesar et al., 2011) this study
r_{09}	modified MA	K_d	7.4×10^3 pM to 5.5×10^4 pM	(Clouse, 2002) (Hohmann et al., 2018) (Kinoshita et al., 2005) (Wang et al., 2001)
		k_{on}	$9.49 \times 10^{-7} \text{ pMol}^{-1} \text{ s}^{-1}$	(Hohmann et al., 2018)
r_{10}	modified MM	k	0.97 s^{-1}	(Wang et al., 2014)
r_{11}	modified MM			
r_{12}	modified MA			
r_{13}	MA			
r_{14}	MA			
r_{15}	MM			
r_{16}	MM			
r_{17}	MM	<i>time scale</i>	slow increase over 12 h	(Oh et al., 2012)
r_{d1}	MA			
r_{d2}	MA	max. k_d	$1.05 \times 10^{-2} \text{ s}^{-1}$	(Hohmann et al., 2018)
r_{d3}	MA	max. k_d	$1.05 \times 10^{-2} \text{ s}^{-1}$	(Hohmann et al., 2018)
r_{d4}	MA	<i>time scale</i>	residual P_i after 5 d	(Oh et al., 2012)
r_{d5}	MA			
r_{d6}	MA			
r_{d7}	MA			

917
918

919
920
921
922
923

pH measurements using pHusion

A. thaliana seedlings stably expressing SYP122-pHusion were treated with with 500 μM ortho-vanadate and the pH was measured after 30 min and 60 min based on the fluorescent ratio of mRFP and eGFP in the EZ. The measurements were conducted for $n = 30$ seedlings. Plants treated with MS medium were taken as control, outliers were set to pH 8.



924
925
926
927
928
929

Appendix 1 Figure 1. Measurement of the proton leak flux from the cell wall using SYP122-pHusion. A. Resting pH in the EZ of the WT Col-0. Error bars represent SD ($n = 3$). B. pH after 1 h of treatment with 500 μM ortho-Vanadate compared to control (MS). Error bars represent SD ($n = 30$). The proton leak was estimated based on the pH difference and the average size of an epidermis cell in the mid EZ (*van Esse et al., 2011*).

931
933
932
934
936
935
937
938
940
939
941

Example calculation of E_m and pH change

pH 5.4 \rightarrow 5.0

$$\Delta[H^+] : 10^{-5.0} M - 10^{-5.4} M = 1 * 10^{-5} - 3.16 * 10^{-6} M = 6.019 * 10^{-6} M$$

$$\Delta nH^+ : 6.019 * 10^{-6} M * 8.2892 * 10^{-13} l = 4.99 * 10^{-18} mol$$

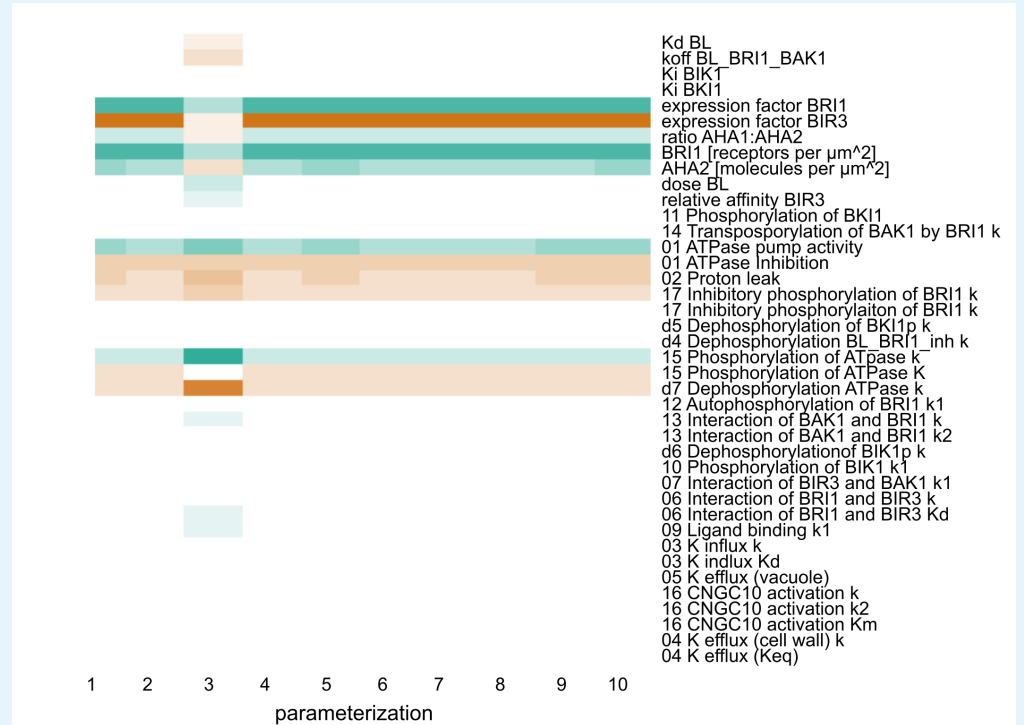
$$\Delta Q : 4.99 * 10^{-18} mol * 96485.33212 \frac{C}{mol} = 4.81 * 10^{-13} C$$

$$\Delta E_m : \frac{4.81 * 10^{-13} C}{0.0081 * 2.098 * 10^{-9} m^2} = 2.83 * 10^{-2} V = 28.3 mV$$

942
944
943
945
946
947

948

Supporting Figures



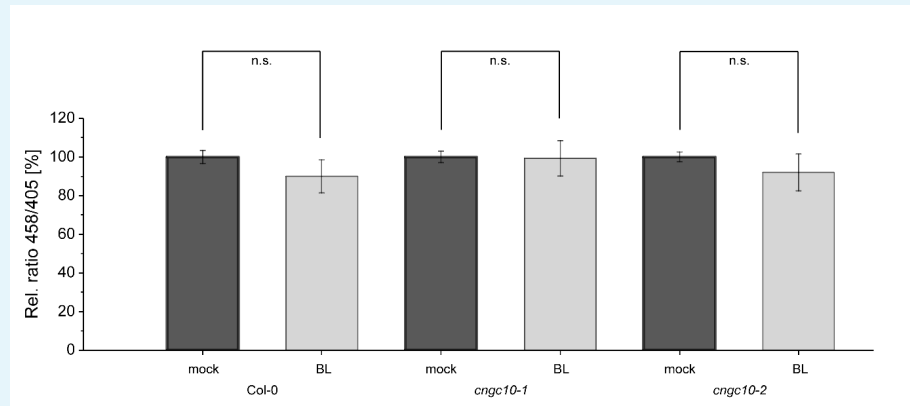
949

950

951

953

Appendix 1 Figure 2. Scaled sensitivities of the pH change 60 min after stimulation with 10 nM BL in response to changes in the parameter and global quantities values. Color code: red - negative control, white - no influence, green - positive control. Color saturation indicates strength of the influence.



954

955

956

957

958

960

Appendix 1 Figure 3. Comparison of the relative apoplastic pH of epidermal root cells in the MZ of wild type and two independent *cngc10* mutant plants after 60 min of BL (10 nM) or mock treatment, visualized by HPTS staining. The data derived from the mock treatments of the respective line were set to 100. Error bars represent SE ($n \geq 8$). Statistical evaluations were performed as described in Fig. 7C; n.s.: not significant.