



Title	Abscisic acid switches cell division modes of asymmetric cell division and symmetric cell division in stem cells of protonemal filaments in the moss <i>Physcomitrium patens</i>
Author(s)	Hiroguchi, Akihiko; Nakamura, Kohei; Fujita, Tomomichi
Citation	Plant biotechnology, 39(1), 13-17 <a href="https://doi.org/10.5511/plantbiotechnology.22.0107a">https://doi.org/10.5511/plantbiotechnology.22.0107a</a>
Issue Date	2022-03-25
Doc URL	<a href="http://hdl.handle.net/2115/86152">http://hdl.handle.net/2115/86152</a>
Type	article (author version)
File Information	Plant Biotechnol._39(1)_13-17.pdf



[Instructions for use](#)

1 **Title**

2 Abscisic acid switches cell division modes of asymmetric cell division and symmetric  
3 cell division in stem cells of protonemal filaments in the moss *Physcomitrium patens*

4

5 **Names of authors**

6 Akihiko Hiroguchi, Kohei Nakamura, Tomomichi Fujita

7

8 **Affiliation of authors**

9 Faculty of Science, Hokkaido University, Kita 10 Nishi8 Kita-ku, Sapporo, Hokkaido  
10 060-0810, Japan

11

12

13 **Corresponding author**

14 Name: Tomomichi Fujita

15 Address: Faculty of Science, Hokkaido University, 18 Kita10 Nishi8 Kita-ku,

16 Sapporo, Hokkaido 060-0810, Japan

17 Phone/Fax number: 011-706-2740

18 E-mail address: tfujita@sci.hokudai.ac.jp

19

20 **Running title**

21 Cell division modes switched by ABA in moss

22

23 **Abbreviations**

24 ABA, abscisic acid; ACD, asymmetric cell division; SCD, symmetric cell division

25

26 **Figures: 4**

27

28 **Abstract**

29 Multicellular organisms regulate cell numbers and cell fate by using asymmetric cell  
30 division (ACD) and symmetric cell division (SCD) during their development and to adapt  
31 to unfavorable environmental conditions. A stem cell self-renews and generates  
32 differentiated cells. In plants, various types of cells are produced by ACD or SCD;  
33 however, the molecular mechanisms of ACD or SCD and the cell division mode switch  
34 are largely unknown. The moss *Physcomitrium (Physcomitrella) patens* is a suitable  
35 model to study plant stem cells due to its simple anatomy. Here, we report the cell division  
36 mode switch induced by abscisic acid (ABA) in *P. patens*. ABA is synthesized in  
37 response to abiotic stresses and induces round-shape cells, called brood cells, from  
38 cylindrical protonemal cells. Although two daughter cells with distinct sizes were  
39 produced by ACD in a protonemal stem cell on ABA-free media, the sizes of two  
40 daughter cells became similar with ABA treatment. Actin microfilaments were spatially  
41 localized on the apices of apical stem cells in protonemata on ABA-free media, but the  
42 polar accumulation was lost under the condition of ABA treatment. Moreover, ABA  
43 treatment conferred an identical cell fate to the daughter cells in terms of cell division  
44 activity. Collectively, the results indicate ABA may suppress the ACD characteristics but  
45 evoke SCD in cells. We also noticed that ABA-induced brood cells not only self-renewed  
46 but regenerated protonemal cells when ABA was removed from the media, suggesting  
47 that brood cells are novel stem cells that are induced by environmental signals in *P. patens*.

48

49

50 **Key words**

- 51 Abscisic acid, Asymmetric cell division (ACD), *Physcomitrium patens*, Stem cell,
- 52 Symmetric cell division (SCD)

53 **Text**

54 Stem cells in multicellular organisms continuously produce various types of cells with  
55 diverse functions throughout their life cycle. Stem cells are relatively undifferentiated  
56 cells that have the characteristics of generation of differentiated cells and self-renewal for  
57 maintaining stem cell identity, and these characteristics are essential for the maintenance  
58 of a continuous cell population (Morrison and Kimble 2006). Asymmetric cell division  
59 (ACD) in stem cells generates two daughter cells with distinct fates of stemness and  
60 differentiation, while symmetric cell division (SCD) produces two daughter cells with  
61 equivalent or similar shapes, sizes or fates (Tajbakhsh et al. 2009). During ACD, stem  
62 cells establish an intracellular asymmetry of organelles and cellular structures including  
63 cytoskeletons. The asymmetric inheritance of cell context generates the distinct cell fates  
64 of two daughter cells (Sunchu and Cabernard 2020). In plants, activity of the meristem,  
65 which is composed of various types of stem cells, is regulated by developmental and  
66 environmental signals (Harris 2015; Pillitteri et al. 2016; Wybouw and Rybel 2019).  
67 Whereas the molecular mechanism of ACD in a certain stem cell, e.g., cortical-epidermal  
68 initials in root apical meristem, has been well studied (Fisher and Sozzani 2016),  
69 information on ACD or SCD in the other plant stem cells is limited. During  
70 embryogenesis or guard cell formation, ACD and SCD also play pivotal roles in  
71 specialized cell production (Petricka et al. 2009). However, we still need to learn much  
72 more; for example, how cell division modes are switched during development and how  
73 such a division mode is spatio-temporally regulated in response to differential  
74 environmental conditions.

75 Abscisic acid (ABA) is synthesized in plant cells in response to abiotic stresses and  
76 is a well-known phytohormone that regulates various abiotic stresses, seed dormancy and

77 germination, and stomatal closure (Harris 2015; Munemasa et al. 2015; Shu et al. 2016).  
78 The response to ABA is initiated, after perception, by activation of a signaling consisting  
79 of kinase cascades to activate the downstream transcription pathway. Recent studies have  
80 revealed conservation of the core components of ABA signaling from bryophytes to  
81 angiosperms (Komatsu et al. 2020). The moss *Physcomitrium patens* is used for evo-devo  
82 study in land plants (Naramoto et al. 2021), and it consists of at least eight stem cells in  
83 its life and possesses exposed stem cells on the edge of filamentous protonemata (Kofuji  
84 and Hasebe 2014). In the presence of ABA, the protonemal cells from a variety of mosses  
85 exhibit cell-morphological changes from cylindrical shape to more spherical, thick-  
86 walled cells with segmented vacuoles, which are called brood cells (also called  
87 brachycytes, brood bodies or diaspores) (Goode et al. 1993; Mallon et al. 2006; Martinez  
88 et al. 2011; Schnepf and Reinhard 1997; Nagao et al. 2005), and indeed, some mosses use  
89 brood cells as a dispersal unit for propagation (Glime and Bisang et al. 2017). Brood cell  
90 formation is, at least partly, governed by the ABA signaling that is conserved among land  
91 plants (Komatsu et al. 2013; Mengkai et al. 2018; Saruhashi et al. 2015; Shinozawa et al.  
92 2019; Stevenson et al. 2016), and brood cells are tolerant to various abiotic stresses such  
93 as desiccation and freezing stresses (Minami et al. 2003; Stevenson et al. 2016). In the  
94 current study, we examined cell sizes, cytoskeleton and cell fate regulation during brood  
95 cell formation, and we found that pivotal features of ACD were suppressed by ABA.  
96 Hence, we propose a novel system to study switching between ACD and SCD of stem  
97 cells through ABA signaling.

98 *P. patens* (Hedw.) Bruch & Schimp subsp. *Patens* Tan strain was used as the wild type  
99 (Nishiyama et al. 2000). *P. patens* was cultured on cellophane mounting on BCDAT or  
100 BCDATG solid media with 0.8% (w/v) agar (Nacalai Tesque) and 1% (w/v) glucose

101 (FUJIFILM Wako Pure Chemical) at 25°C under continuous white light (40-50  $\mu\text{mol m}^{-2}$   
102  $\text{s}^{-1}$ ) (Nishiyama et al. 2000). To compare sizes of daughter cells, the ratio of the section  
103 area of an apical cell or a side branch initial to a basal daughter cell, which were originated  
104 from the same mother cell, was calculated. Protonemata were transferred onto BCDATG  
105 media containing 50  $\mu\text{M}$  ABA, referring to ABA concentration in the previous research  
106 (Nagao et al. 2005). Section areas of an apical cell, a side branch initial and a basal cell  
107 were measured using Image J software (<https://imagej.nih.gov/ij/>). To examine the  
108 cytoskeleton distribution in an apical stem cell in the protonema, localization of the actin  
109 marker LifeAct-Venus (Era et al. 2009) was observed. Transgenic protonemata carrying  
110 LifeAct-Venus were transferred onto BCDAT solid media containing 50  $\mu\text{M}$  ABA. The  
111 fluorescence in chloronemal cells was observed with an inverted microscope (ECLIPSE  
112 Ti-E, Nikon) equipped with a mercury fluorescence source (C-SHG1, Nikon) at 0, 3 and  
113 5 h after transferring. To examine cell division activities of protonemata with ABA  
114 treatment, cell division of daughter cells was examined. Protonemata were transferred  
115 onto BCDATG solid media containing 50  $\mu\text{M}$  ABA and daughter cells, which were  
116 produced by the cell division of a basal daughter cell, were observed at 0, 25 and 96 h  
117 after transferring. The ability to regenerate chloronemal cells from brood cells was  
118 examined. The brood cells, which were induced by 50  $\mu\text{M}$  ABA treatment for 10 d, were  
119 transferred onto ABA-free media and then chloronemal regeneration from the brood cells  
120 was observed at 0 and 24 h after transferring.

121 Apical stem cells of protonemata produce daughter cells with distinct sizes and nature  
122 by ACD under normal conditions (Kofuji and Hasebe 2014; Figure. 1A). A side branch  
123 initial is also produced as a stem cell from a protonemal cell by the mode of ACD (Kofuji  
124 and Hasebe 2014; Figure. 1A). In contrast, ABA-treated protonemal cells display



125 transverse division formed almost in the middle of the mother, protonemal cells (Schnepf  
126 and Reinhard 1997), and the cell division induced by ABA seems to have similar features  
127 to those of SCD; that is, cell sizes and cell fates of two daughter cells are equal or very  
128 similar. To verify this, we first compared the sizes of two daughter cells, which were  
129 produced within 2 h since cell division of the mother cell occurred. While the ratio of  
130 section areas of the apical cells (A) to basal cells (B) was 1: 1.63 on ABA-free solid media  
131 (Figure 1B, Mock, Apical), on media containing 50  $\mu$ M ABA, the ratio became 1: 1.10  
132 (Figure 1B, 50  $\mu$ M ABA, Apical). Moreover, in the basal parts of protonemata, while the  
133 ratio of the side branch initials (A) to basal cells (B) was 1: 4.21 on ABA-free media  
134 (Figure 1 B), the ratio of the two daughter cells was 1: 1.53 on media containing 50  $\mu$ M  
135 ABA. These results suggest that ABA-induced cell division lost the characteristic of  
136 ACD; that is, two daughter cells having similar sizes were produced in protonemata,  
137 which is a feature of SCD. Another characteristic of ACD is the establishment or  
138 maintenance of intracellular asymmetry. In fact, apical stem cells of protonemata are  
139 highly polarized cells that exhibit tip growth under normal culture conditions (Rounds  
140 and Bezanilla 2013). When we observed the actin marker LifeAct-Venus, actin  
141 cytoskeleton was highly accumulated on the apex of apical stem cells on ABA-free media  
142 (Figure 2 Mock). In contrast, the polarized accumulation was lost within 3 h after 50  $\mu$ M  
143 ABA treatment (Figure 2 50  $\mu$ M ABA), suggesting that intracellular asymmetry is  
144 attenuated by ABA.

145 ACD also has the characteristic of producing two daughter cells that have distinct cell  
146 fates. A protonemal apical stem cell divides asymmetrically to produce two daughter cells,  
147 and the apical daughter cell behaves as a stem cell that can continuously divide to produce  
148 more daughter cells (Figure. 1A ACD of apical stem cell). On the other hand, the basal

149 daughter cell shows greatly reduced cell division activity and it usually divides only once  
150 again or twice at most when it produces side branch initial cells (Figure. 1A Side branch  
151 initial formation), suggesting that the two daughter cells produced by ACD of an apical  
152 stem cell have distinct cell proliferation potentials. To examine whether two daughter  
153 cells produced by ABA treatment have similar cell proliferation potentials or not, we grew  
154 protonemata on media containing 50  $\mu$ M ABA. As shown in Figure. 3, at 96 h after  
155 transferring onto media containing 50  $\mu$ M ABA, the basal side of the daughter cell, now  
156 converting to a brood cell, was able to produce daughter cells continuously as well as the  
157 apical side of the daughter cell, suggesting maintenance of cell proliferation ability even  
158 in basal daughter cells. Thus, ABA-induced brood cells lose prominent characteristics  
159 found in ACD but exhibit several SCD features and, consequently, both daughter cells  
160 have the ability to self-renew.

161 Stem cells are cells that have the potential to self-renew and can differentiate into  
162 different types of cells. We next examined whether brood cells have the ability to generate  
163 a different type of cell. To this end, brood cells were induced by 50  $\mu$ M ABA for 10 d  
164 and transferred onto ABA-free media. After 10 days of ABA treatment, we noticed that  
165 the connection between the cells was weakened and the brood cells were easily loosened  
166 into pieces as small clumps of cells (Figure. 4A, 0 h). Then at 24 h after transfer, some  
167 brood cells showed protrusions, likely resuming tip growth, and others regenerated  
168 chloronemal cells by ACD (Figure. 4A, 24 h), suggesting that brood cells maintain the  
169 ability to generate a distinct type of cell. Collectively, the results indicate that brood cells  
170 have characteristics of stem cells, which possess the abilities to self-renew and to generate  
171 distinct cell types.

172 Although many studies have been performed to try to determine the regulatory

173 mechanism of stem cells in plant meristems in angiosperms, it is still difficult to track a  
174 single stem cell behavior and uncover the regulatory mechanism at a single cell level. *P.*  
175 *patens* possesses a single stem cell on each of the edges of protonemal filaments, a  
176 chloronema apical stem cell, a caulonema apical stem cell or a gametophore apical stem  
177 cell (Kofuji and Hasebe, 2014). Because these apical stem cells are exposed outside of  
178 the tissue, the single stem cells can be observed continuously with a microscope. In the  
179 present study, we found that different modes of cell division, ACD and SCD, are used in  
180 *P. patens* protonemal tissue depending on the growth environment (Figure. 4B). Under  
181 normal growth conditions, protonema apical stem cells continue to self-renew and to  
182 produce differentiated protonemal cells with distinct cell proliferative activity by ACD to  
183 expand their growth niche. On the other hand, under stress conditions, ABA induces  
184 stress-tolerant stem cells, brood cells, with the number of stress-tolerant cells being  
185 increased by SCD, thus increasing their own survival potential. When the stress  
186 conditions have disappeared, the brood cells differentiate into protonemal cells again by  
187 ACD and continue growing to expand their growth range. Hence, brood cells are novel  
188 stem cells that temporarily appear in response to environmental stress conditions.

189       How is the switching between ACD and SCD controlled? We speculate that ABA  
190 induces a mechanism that suppresses ACD under stress conditions. Rho of plants (ROPs),  
191 which are small GTPases, are essential for cell polarity establishment and maintenance,  
192 which are also important for asymmetric organization of the cytoskeletal distribution  
193 (Feiguelman et al. 2018). The activity of ROPs is positively regulated by ROP guanine  
194 nucleotide exchange factors (RopGEFs). In the absence of ABA, RopGEFs negatively  
195 regulate the downstream factors of the ABA signal transduction by directly interacting  
196 with type 2C protein phosphatases (PP2Cs), inhibitory phosphatases of ABA signaling

197 (Li and Liu 2012; Yu et al. 2012). On the other hand, in the presence of ABA, i.e., under  
198 abiotic stress conditions, ABA induces the degradation of RopGEFs to facilitate ABA  
199 signal transduction by releasing the interaction between PP2Cs and RopGEFs, suggesting  
200 the significance of the PP2C-RopGEF-ROP circuit loop to control critical cellular  
201 processes via ABA signal transduction during growth of *Arabidopsis thaliana* (Li et al.  
202 2016). While PP2C-RopGEF interaction has not been shown in *P. patens*, considering  
203 that PP2C, RopGEFs and ROPs are evolutionarily conserved in *P. patens* (Ito et al. 2014;  
204 Komatsu et al. 2020), we postulate that the change in the cell division mode from ACD  
205 to SCD under stress conditions might be mediated through the degradation of RopGEFs  
206 induced by ABA in *P. patens*. In *A. thaliana*, stomatal lineage is initiated from  
207 meristemoid mother cells, which undergo ACD to generate meristemoids. ABA represses  
208 meristemoid formation (Tanaka et al. 2013), suggesting that ABA suppresses ACD of  
209 meristemoid mother cells. It is tempting to speculate that ABA switches cell division  
210 modes by suppressing cell polarity signaling and this mechanism might be conserved in  
211 land plants.

212

### 213 **Acknowledgments**

214 We are grateful to Takashi Ueda and Mitsuyasu Hasebe (National Institute for Basic  
215 Biology) for their kind gifts of pENTR-LifeAct-Venus plasmid and transgenic *P. patens*,  
216 respectively. We thank Satoshi Naramoto (Hokkaido University) for helpful discussions.

217

### 218 **Author contributions**

219 AH performed experiments, wrote the manuscript and contributed to Figures 1, 2 and 4.  
220 KN performed experiments and contributed to Figures 1, 3, and 4. TF conceptualized the

221 study and supervised the writing of the manuscript.

222

## 223 **References**

- 224 Era A, Tominaga M, Ebine K, Awai C, Saito C, Ishizaki K, Yamato KT, Kohchi T, Nakano  
225 A, Ueda T (2009) Application of lifeact reveals F-actin dynamics in *Arabidopsis*  
226 *thaliana* and the liverwort, *Marchantia polymorpha*. *Plant Cell Physiol* 50: 1041–  
227 1048
- 228 Feiguelman G, Fu Y, Yalovsky S (2018) ROP GTPases structure-function and signaling  
229 pathways. *Plant Physiol* 176: 57–79
- 230 Fisher AP, Sozzani R (2016) Uncovering the networks involved in stem cell maintenance  
231 and asymmetric cell division in the *Arabidopsis* root. *Curr Opin Plant Biol* 29: 38–  
232 43
- 233 Glime JM and Bisang I (2017) Sexual strategies. *Bryophyte Eco.* 1:  
234 <https://digitalcommons.mtu.edu/bryophyte-ecology1/2>
- 235 Goode JA, Stead AD, Duckett JG (1993) Redifferentiation of moss protonemata: an  
236 experimental and immunofluorescence study of brood cell formation. *Can J Bot* 71:  
237 1510–1519
- 238 Harris JM (2015) Abscisic acid: Hidden architect of root system structure. *Plants* 4: 548–  
239 572
- 240 Ito K, Ren J, Fujita T (2014) Conserved function of Rho-related Rop/RAC GTPase  
241 signaling in regulation of cell polarity in *Physcomitrella patens*. *Gene* 544: 241–247
- 242 Kofuji R, Hasebe M (2014) Eight types of stem cells in the life cycle of the moss  
243 *Physcomitrella patens*. *Curr Opin Plant Biol* 17: 13–21
- 244 Komatsu K, Suzuki N, Kuwamura M, Nishikawa Y, Nakatani M, Ohtawa H, Takezawa

245 D, Seki M, Tanaka M, Taji T, et al. (2013) Group A PP2Cs evolved in land plants as  
246 key regulators of intrinsic desiccation tolerance. *Nat Commun* 4: 2219

247 Komatsu K, Takezawa D, Sakata Y (2020) Decoding ABA and osmostress signalling in  
248 plants from an evolutionary point of view. *Plant Cell Environ* 43: 2894–2911

249 Li Z, Liu D (2012) ROPGEF1 and ROPGEF4 are functional regulators of ROP11 GTPase  
250 in ABA-mediated stomatal closure in Arabidopsis. *FEBS Lett* 586: 1253–1258

251 Li Z, Waadt R, Schroeder JI (2016) Release of GTP Exchange Factor Mediated Down-  
252 Regulation of Abscisic Acid Signal Transduction through ABA-Induced Rapid  
253 Degradation of RopGEFs. *PLoS Biol* 14: 1002461

254 Mallón R, Rodríguez-Oubiña J, González M (2006) In vitro development of vegetative  
255 propagules in *Splachnum ampullaceum*: brood cells and chloronematal bulbils.  
256 *Bryologist* 109: 215-223

257 Martinez K, Price M (2011) Brood Cells in the Rare, Epiphytic Moss *Tayloria*  
258 *rudolphiana* (Garov.) Bruch et Schimp. (Splachnaceae). *Cryptogamie. Bryol.* 32: 3-  
259 12

260 Mengkai Z, Qilong L, Zhenhua C, Qiang L, Fang B, Xiaoqin W, Yikun H (2018)  
261 Regulatory mechanism of ABA and ABI3 on vegetative development in the moss  
262 *Physcomitrella patens*. *Int J Mol Sci* 19: 2728

263 Minami A, Nagao M, Arakawa K, Fujikawa S, Takezawa D (2003) Abscisic acid-induced  
264 freezing tolerance in the moss *Physcomitrella patens* is accompanied by increased  
265 expression of stress-related genes. *J Plant Physiol* 160: 475–483

266 Morrison SJ, Kimble J (2006) Asymmetric and symmetric stem-cell divisions in  
267 development and cancer. *Nature* 441: 1068–1074

268 Munemasa S, Hauser F, Park J, Waadt R, Brandt B, Schroeder JI (2015) Mechanisms of

269 abscisic acid-mediated control of stomatal aperture. *Curr Opin Plant Biol* 28: 154–  
270 162

271 Nagao M, Minami A, Arakawa K, Fujikawa S, Takezawa D (2005) Rapid degradation of  
272 starch in chloroplasts and concomitant accumulation of soluble sugars associated  
273 with ABA-induced freezing tolerance in the moss *Physcomitrella patens*. *J Plant*  
274 *Physiol* 162: 169–180

275 Naramoto S, Hata Y, Fujita T, Kyojuka J (2021) The bryophytes *Physcomitrium patens*  
276 and *Marchantia polymorpha* as model systems for studying evolutionary cell and  
277 developmental biology in plants. *Plant Cell* 0: 1-19

278 Nishiyama T, Hiwatashi Y, Sakakibara K, Kato M, Mitsuyasu Hasebe (2000) Tagged  
279 Mutagenesis and Gene-trap in the Moss, *Physcomitrella patens* by Shuttle  
280 Mutagenesis. *DNA Res* 28: 9–17

281 Petricka JJ, Van Norman JM, Benfey PN (2009) Symmetry breaking in plants: molecular  
282 mechanisms regulating asymmetric cell divisions in Arabidopsis. *Cold Spring Harb*  
283 *Perspect Biol* 1: a000497

284 Pillitteri LJ, Guo X, Dong J (2016) Asymmetric cell division in plants: mechanisms of  
285 symmetry breaking and cell fate determination. *Cell Mol Life Sci* 73: 4213–4229

286 Rounds CM, Bezanilla M (2013) Growth mechanisms in tip-growing plant cells. *Annu*  
287 *Rev Plant Biol* 64: 243–265

288 Saruhashi M, Ghosh TK, Arai K, Ishizaki Y, Hagiwara K, Komatsu K, Shiwa Y,  
289 Izumikawa K, Yoshikawa H, Umezawa T, et al. (2015) Plant Raf-like kinase  
290 integrates abscisic acid and hyperosmotic stress signaling upstream of SNF1-related  
291 protein kinase2. *Proc Natl Acad Sci USA* 112: 6388-6396

292 Schnepf E, Reinhard C (1997) Brachycytes in *Funaria* Protonemate: Induction by

293 Abscisic Acid and Fine Structure. *J Plant Physiol* 151: 166–175

294 Shinozawa A, Otake R, Takezawa D, Umezawa T, Komatsu K, Tanaka K, Amagai A,  
295 Ishikawa S, Hara Y, Kamisugi Y, et al. (2019) SnRK2 protein kinases represent an  
296 ancient system in plants for adaption to a terrestrial environment. *Commun Biol* 2:  
297 30

298 Shu K, Liu XD, Xie Q, He ZH (2016) Two Faces of One Seed: Hormonal Regulation of  
299 Dormancy and Germination. *Mol Plant* 9: 34–45

300 Stevenson SR, Kamisugi Y, Trinh CH, Schmutz J, Jenkins JW, Grimwood J, Muchero W,  
301 Tuskan GA, Rensing SA, Lang D, et al. (2016) Genetic analysis of *Physcomitrella*  
302 *patens* identifies ABSCISIC ACID NON-RESPONSIVE, a regulator of ABA  
303 responses unique to basal land plants and required for desiccation tolerance. *Plant*  
304 *Cell* 28: 1310–1327

305 Sunchu B, Cabernard C (2020) Principles and mechanisms of asymmetric cell division.  
306 *Development* 147: dev167650

307 Tajbakhsh S, Rocheteau P, Le Roux I (2009) Asymmetric cell divisions and asymmetric  
308 cell fates. *Annu Rev Cell Dev Biol* 25: 671-699

309 Tanaka Y, Nose T, Jikumaru Y, Kamiya Y (2013) ABA inhibits entry into stomatal-lineage  
310 development in Arabidopsis leaves. *Plant J* 74: 448–457

311 Wybouw B, de Rybel B (2019) Cytokinin – A Developing Story. *Trends Plant Sci* 24:  
312 177–185

313 Yu F, Qian L, Nibau C, Duan Q, Kita D, Levasseur K, Li X, Lu C, Li H, Hou C, et al.  
314 (2012) FERONIA receptor kinase pathway suppresses abscisic acid signaling in  
315 Arabidopsis by activating ABI2 phosphatase. *Proc Natl Acad Sci USA* 109: 14693–  
316 14698



317

## 318 **Figure legends**

### 319 **Figure 1. Ratios of section areas of apical stem cells or side branch initials to basal** 320 **cells**

321 (A) A schematic model of cell division patterns in protonemal cells on ABA-free media.  
322 Apical stem cells divide asymmetrically to produce daughter cells, an apical daughter cell  
323 and a basal daughter cell. While the apical daughter cell maintains cell division activity,  
324 the basal daughter cell, becoming a basal cell, shows reduced cell division activity and it  
325 divides up to two times to produce two more daughter cells. (B) Protonemata were  
326 transferred onto media containing 50  $\mu$ M ABA. The section areas of apical cells (A), side  
327 branch initials (A) and basal cells (B), which were just produced by cell division of the  
328 mother cells, were measured and the ratios of the section areas (B/A) were calculated.  
329 Values are means  $\pm$  SD of 11-20 independent protonemal filaments. Asterisks indicate  
330 significant difference between mock and ABA treatments ( $***P<0.001$ ; Student's *t*-test).  
331 Scale bars =50  $\mu$ m. Daughter cells with similar sizes were produced under the condition  
332 of ABA treatment.

333

### 334 **Figure 2. Distribution of an actin marker in apical stem cells of protonemata**

335 Protonemata were transferred onto solid media containing 50  $\mu$ M ABA. Fluorescence of  
336 the actin marker LifeAct-Venus in 3-8 independent chloronemal cells was observed with  
337 an inverted microscope at 0, 3 and 5 h after transferring. The same cells were not  
338 sequentially observed after transferring but representative images are shown at each time  
339 point. Arrowheads indicate accumulation of LifeAct-Venus at the apices of chloronemal  
340 cells. Scale bars = 50  $\mu$ m. The polarized accumulation of LifeAct-Venus, which was

341 highly accumulated on the apices of apical stem cells on ABA-free media, was lost within  
342 3 h after 50  $\mu$ M ABA treatment.

343

344 **Figure 3. Cell division activity of basal daughter cells after ABA treatment**

345 Protonemata were transferred onto solid media containing 50  $\mu$ M ABA and cell division  
346 was observed at 0, 25 and 96 h. Red dots indicate cells that originated from the basal  
347 daughter cell (at 25 and 96 h). Black arrowheads indicate septum of basal daughter cells.  
348 Scale bars =50  $\mu$ m. The basal daughter cell at 0 h (shown by an open red circle)  
349 continuously divided to produce 3 daughter cells, indicating maintenance of cell division  
350 activity in the basal daughter cells as well as in the apical daughter cells.

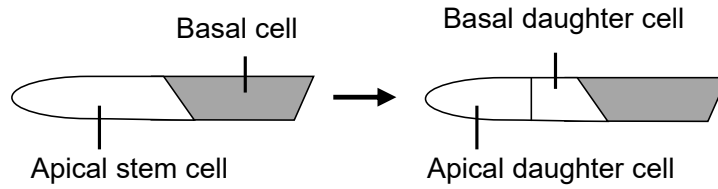
351

352 **Figure 4. Chloronemal regeneration from brood cells**

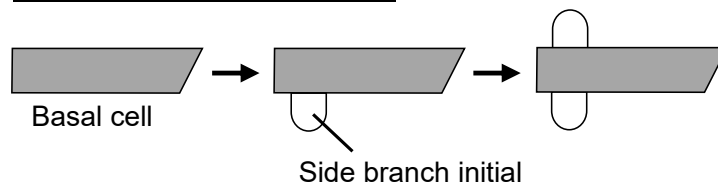
353 Brood cells, which were induced by 50  $\mu$ M ABA treatment for 10 d, were transferred  
354 onto ABA-free media. Chloronemal regeneration from the brood cells was observed at  
355 24 h after transferring. Scale bars =50  $\mu$ m. A schematic model of the cell division mode  
356 switching induced by ABA. Protonemata undergo ACD under normal conditions but  
357 undergo SCD under stress conditions.

**A**

**ACD of an apical stem cell**



**Side branch initial formation**



**B**

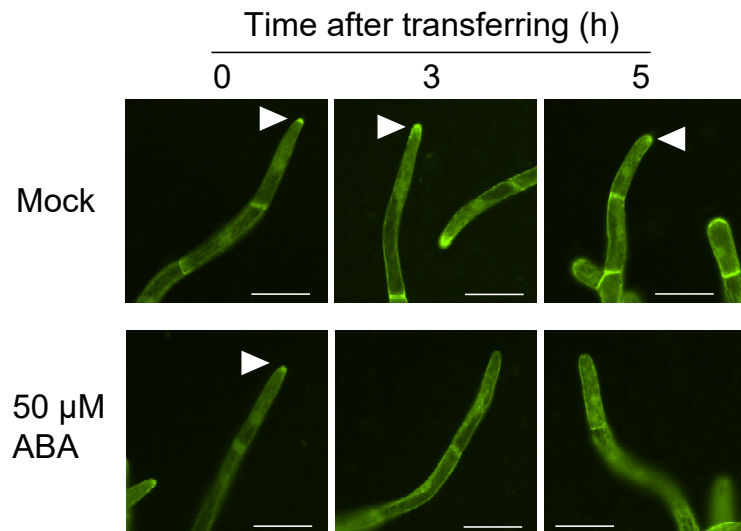
	Mock		50 $\mu$ M ABA	
	Apical	Basal	Apical	Basal
Area ratio (A:B)	1:1.63 $\pm$ 0.3	1:4.21 $\pm$ 1.1	1:1.10 $\pm$ 0.3***	1:1.53 $\pm$ 1.3***

**Figure number:** Figure 1

**Author name:** Akihiko Hiroguchi

Kohei Nakamura

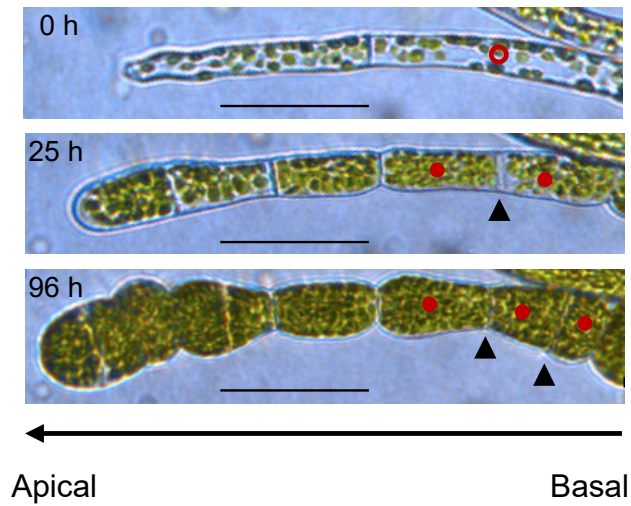
Tomomichi Fujita



**Figure number:** Figure 2

**Author name:** Akihiko Hiroguchi  
Kohei Nakamura  
Tomomichi Fujita

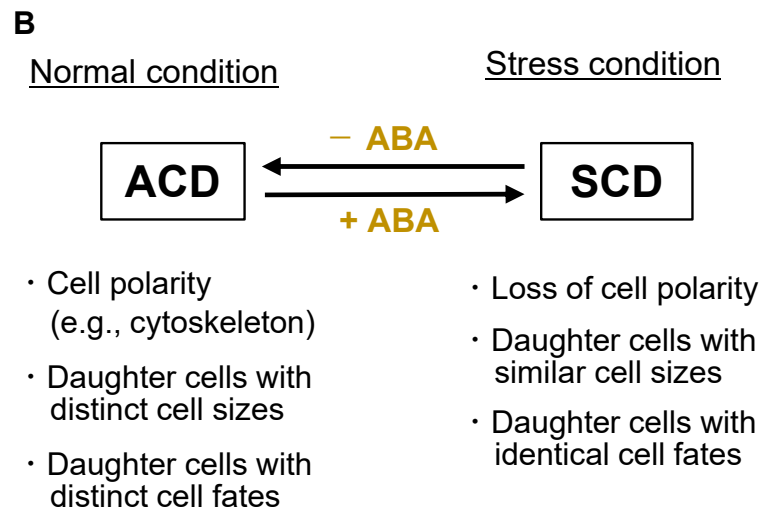
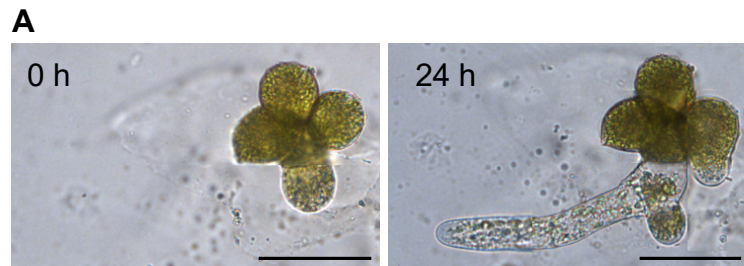
**Top**



**Figure number:** Figure 3

**Author name:** Akihiko Hiroguchi  
Kohei Nakamura  
Tomomichi Fujita

**Bottom**



**Figure number:** Figure 4

**Author name:** Akihiko Hiroguchi  
Kohei Nakamura  
Tomomichi Fujita