



**HAL**  
open science

## Phyllotaxis from a Single Apical Cell

Elsa Véron, Teva Vernoux, Yoan Coudert

► **To cite this version:**

Elsa Véron, Teva Vernoux, Yoan Coudert. Phyllotaxis from a Single Apical Cell. Trends in Plant Science, In press, 26 (2), pp.124-131. 10.1016/j.tplants.2020.09.014 . hal-03011975

**HAL Id: hal-03011975**

**<https://hal.science/hal-03011975>**

Submitted on 18 Nov 2020

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

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

# Phyllotaxis from a single apical cell

Elsa Véron<sup>1</sup>, Teva Vernoux<sup>1,\*</sup> and Yoan Coudert<sup>1,\*,@</sup>

<sup>1</sup>Laboratoire Reproduction et Développement des Plantes, Université de Lyon, ENS de Lyon, UCB Lyon 1, CNRS, INRA, INRIA, Lyon 69007, France

\*Correspondence: yoan.coudert@ens-lyon.fr (Y. Coudert), teva.vernoux@ens-lyon.fr (T. Vernoux)

@Twitter: @coudertlab

## Keywords

phyllotaxis, moss, *Arabidopsis*, *Physcomitrella*, *Physcomitrium*

## Abstract

Phyllotaxis, the geometry of leaf arrangement around stems, determines plant architecture. Molecular interactions coordinating the formation of phyllotactic patterns have mainly been studied in multicellular shoot apical meristems of flowering plants. Phyllotaxis evolved independently in the major land plant lineages. In mosses, it arises from a single apical cell, raising the question of how asymmetric divisions of a single-celled meristem create phyllotactic patterns and whether associated genetic processes are shared across lineages. We present an overview of the mechanisms governing shoot apical cell specification and activity in the model moss, *Physcomitrium patens*, and argue that similar molecular regulatory modules have been deployed repeatedly across evolution to operate at different scales and drive apical function in convergent shoot forms.

## 1 **Convergent evolution of phyllotactic patterns in plants**

2 Phyllotaxis – the regular arrangement of leaves around stems – is a primary  
3 determinant of plant architecture and amongst the most striking and elegant natural  
4 patterns [1]. Deciphering how the periodic emergence of leaves is coordinated in  
5 space and time at the apex of growing shoots constitutes an enigma that has puzzled  
6 biologists, mathematicians and physicists for centuries [2]. Besides some early  
7 studies in ferns [3], most experiments investigating the cellular and molecular  
8 mechanisms of phyllotaxis have been carried out in flowering plants, including  
9 *Arabidopsis thaliana* (thale cress), *Solanum lycopersicum* (tomato) and *Zea mays*  
10 (maize) [4]. In these plants, leaves bulge out at the periphery of the shoot apical  
11 meristem, a multicellular structure located at the shoot tip and that contains a stem  
12 cell niche at its center. The phyllotactic pattern is defined by the number of leaves  
13 forming at a given node ( $j$ ) and the divergence angle between consecutive leaf  
14 initiation events ( $\alpha$ ). Phyllotaxis is frequently spiral with a divergence angle close to  
15 the golden angle ( $j = 1, \alpha \approx 137.5^\circ$ ) as in *Arabidopsis* or tomato, but it may also be  
16 distichous ( $j = 2, \alpha = 180^\circ$ ) as in maize, or reflect a few other arrangements [4,5].  
17 Similar phyllotactic patterns are described in ferns, lycophytes, leafy liverworts or  
18 mosses but in comparison with flowering plants, they emerge from meristems with a  
19 simpler structure [6–9]. An extreme example is seen in bryophytes whose meristem is  
20 composed of a single apical stem cell (**Figure 1**). Evidence from phylogeny and the  
21 fossil record suggests that leaves (e.g. fern fronds, lycophyte microphylls) or leaf-like  
22 structures (e.g. bryophyte phyllids) evolved multiple times independently, which  
23 implies that despite compelling morphological resemblance phyllotaxis is a  
24 convergent feature of major plant groups [10]. The continuing development of the  
25 moss *Physcomitrium patens* as a model system beyond flowering plants offers  
26 unprecedented opportunities to investigate how phyllotaxis can emerge from a single  
27 apical cell and whether shared mechanisms underlie morphogenesis from unicellular  
28 and multicellular shoot apical meristems [11,12].

29

## 30 **Apical cell shape and cleavage directs phyllotaxis**

31 The production of phyllids (hereafter named leaves) in the moss gametophore (i.e.,  
32 the gametophytic leafy shoot, hereafter named leafy shoot) is directly determined by

33 the activity of a single apical cell. Each asymmetric cleavage of the apical cell  
34 produces a leaf initial and perpetuates the apical cell itself to sustain shoot growth,  
35 as evidenced by live imaging of physcomitrium shoot buds [9]. The relationship  
36 between apical cell division angle and resulting phyllotactic patterns has mainly been  
37 inferred from histological observations of fixed specimens [6,13,14]. In some moss  
38 species, the apical cell is lenticular with two alternating division planes that produce  
39 opposite segments ( $j = 1, \alpha = 180^\circ$ ), resulting in distichous phyllotaxis like in *Fissidens*  
40 **(Figure 1A, 1B)**. In most species, the apical cell is tetrahedral, resembling an inverted  
41 pyramid, and produces leaves by successive rotating divisions of three cutting faces.  
42 Divisions parallel to apical cell side walls distribute leaves at fixed circumferential  
43 positions around the stem ( $j = 1, \alpha = 120^\circ$ ), resulting in tristichous phyllotaxis like in  
44 *Fontinalis* **(Figure 1C, 1D)**. Divisions may also be oblique to the side walls and  
45 successive leaves will be positioned at a slightly broader angle ( $j = 1, 120^\circ < \alpha < 180^\circ$ ),  
46 resulting in spiral phyllotaxis like in *Physcomitrium* or the hair cap moss, *Polytrichum*  
47 **(Figure 1E, 1F)** [6,9]. Other less common scenarios have also been described, but in  
48 all cases, direct measurements of apical cell geometry, orientation of successive  
49 apical cell division planes and leaf divergence angles are still needed to quantitatively  
50 understand the functional link between cell division orientation in single-celled  
51 meristems and phyllotactic patterning [6,14,15]. To date, the molecular mechanisms  
52 controlling apical cell division patterns also remain elusive. However, the existing data  
53 suggest that understanding how the geometry and division plane orientation of the  
54 shoot apical cell are specified in the first place from the gametophore initial cell (i.e.,  
55 the leafy shoot precursor cell) holds some of the keys to decipher how moss  
56 phyllotaxis is established and perpetuated in development.

57

### 58 **Geometry distinguishes shoot initials**

59 In physcomitrium, leafy shoots spring up from a network of filaments – the protonema.  
60 A small proportion of protonemal side-branches (c. 2-5%) becomes leafy shoots,  
61 while the majority gives rise to other filaments [16,17]. Although both leafy shoot and  
62 filament initial cells emerge as a localized swelling at the distal end of protonemal  
63 cells, several geometrical features allow them to be distinguished before the first  
64 division [9,18,19]. In comparison with filament initials, leafy shoot initials have a more

65 bulbous and irregular shape, a greater width and a wider division plane angle relative  
66 to the growth axis of the filament from which they emerge (**Figure 2**) [9,20]. These  
67 features might be related to distinct cellular growth modes: filamentous cells elongate  
68 by tip growth while leafy shoot initials might expand by diffuse growth [9,21]. Genes  
69 encoding cell wall remodeling proteins, including cellulose synthase (CESA),  
70 xyloglucan endotransglycosylase, expansin, cellulose synthase-like D (CSLD) and  
71 pectin methylesterase (PME) enzymes, are differentially expressed between  
72 filamentous cells and nascent shoot buds, which suggests that the different  
73 geometries and growth properties could result from different wall compositions [22].  
74 Indeed, disruption of the *PpCESA5* gene leads to severe morphological defects in the  
75 leafy shoot, but not in the filaments [23]. Although apical cell specification is not  
76 affected in *ppcesa5* knock-out mutants, leaf emergence is impaired due to cellulose  
77 deficiency and associated defects on cell expansion, growth anisotropy and  
78 cytokinesis. This reveals a shared feature with flowering plants as *CESA* genes also  
79 play a key role in shoot morphogenesis and phyllotaxis in arabidopsis. *CESA1* and  
80 *CESA3* genes are expressed throughout the shoot apical meristem, and  
81 corresponding mutants have smaller shoot apical meristems with fewer cells and  
82 increased surface curvature than wild-type, which affects the distribution of  
83 phyllotactic angles in the inflorescence [24]. Other arabidopsis cell wall related genes  
84 such as *CSLD*, *PME*, *Xyloglucan  $\alpha$ -Xylosyltransferase* or  *$\alpha$ -xylosidase* are also  
85 essential for shoot apical meristem maintenance, leaf emergence or phyllotactic  
86 patterning, but their contribution in plants with single-celled meristems has not been  
87 explored so far [25–28]. Furthermore, it remains to be tested whether controlled  
88 changes of wall composition in the side-branches of moss filaments may be sufficient  
89 to trigger specific cell growth mode and fate transition.

90

### 91 **Stem cell regulators and microtubules dictate division plane orientation**

92 The apical stem cell governing physcomitrium leafy shoot morphogenesis is  
93 established by a series of four oblique divisions from the shoot initial cell (**Figure 2**)  
94 [9]. In contrast, filament initials divide exclusively perpendicular to the growth axis,  
95 which suggests that cell geometry might subtend division plane orientation, as  
96 described in other plant tissues [29–31]. Live imaging of shoot initials has revealed

97 that the angle of the first three divisions is robust, indicating that division plane  
98 establishment is tightly controlled and crucial for phyllotaxis [9,32]. This is also  
99 suggested by mutants where apical cell formative divisions are defective and leafy  
100 shoot development cannot proceed normally [33], such as *Defective Kernel1 (DEK1)*  
101 [34,35], *No Gametophores 1 (NOG1)* [18], *Targeting Protein for Xklp2 (TPX2)* [36] or  
102 mutants in the CLAVATA (CLV) signaling pathway [37].

103 Cytoskeleton reorganization is an integral feature of plant cell division [38], and  
104 the study of the microtubule-associated protein TPX2 has provided direct evidence  
105 for a role of the cytoskeleton in cell division plane positioning and shoot  
106 morphogenesis. The hypomorphic *tpx2-5* mutant produces stunted gametophores  
107 with obvious leaf patterning defects. This phenotype is explained by the abnormal  
108 displacement of the mitotic spindle (i.e., a microtubule-based structure that guides  
109 chromosome segregation in the two daughter cells) towards the basal side of shoot  
110 initials and the consequent mispositioning of the phragmoplast (i.e., an array of  
111 microtubules arranged perpendicular to the division plane that accompanies cell plate  
112 expansion towards the plasma membrane fusion site during cytokinesis) and, hence,  
113 the oblique division plane (**Figure 2**). This mechanism is dependent on actin  
114 microfilaments, as treatment with the actin inhibitor latrunculin B suppresses spindle  
115 position defects of *tpx2-5* mutants [36]. The gametosome, a cytoplasmic microtubule  
116 organizing center that forms in prophase, also plays a key role. Transient oryzalin  
117 treatment of shoot initials causing specifically depolymerization of gametosome  
118 microtubules is sufficient to alter spindle and division plane orientation [32].  
119 Unfortunately, it has not been shown whether gametosome disruption affects  
120 development at later stages. Altogether, this shows that the transient deployment of  
121 microtubule-based structures is needed to tilt the division plane in shoot initials. In  
122 this context, it remains to be seen whether microtubule-dependent division plane  
123 orientation is prescribed by initial cell shape, mechanical forces, or both, as proposed  
124 in other plant tissues [39,40].

125 Beyond its structural role, the proper positioning of the cell division plane is  
126 likely needed to compartmentalize positional cues involved in the establishment of  
127 asymmetric cell identities. For example, the graded distribution of  
128 CLAVATA3/EMBRYO SURROUNDING REGION-RELATED (CLE) peptides may

129 provide positional information to specify distinct cell fates in arabidopsis [41,42].  
130 Interestingly, transgenic mosses in which *PpCLE* expression has been silenced rarely  
131 form shoots with more than one leaf. The first and second division plane of *Ppcle*  
132 mutant shoot initials deviate from a normally oblique orientation and are often parallel  
133 to each other, which is not seen in wild-type plants (**Figure 2**). Altered division plane  
134 orientation is also reported in *Physcomitrium clv1a1b* double mutants and *receptor-*  
135 *like protein kinase 2 (Pprpk2)* mutants and affects shoot morphogenesis at various  
136 stages. This role for CLV signaling is conserved in evolution as arabidopsis *clv1/barely*  
137 *any meristem 1 (bam1)/bam2/bam3* mutants show abnormal periclinal divisions in root  
138 tissues [37]. Components of the CLV signaling pathway have first been identified as  
139 regulators of stem cell homeostasis in the arabidopsis shoot apical meristem.  
140 Perturbation of CLV activity in flowering plants typically leads to a variety of  
141 phenotypes ranging from moderate meristem enlargement to fasciation and massive  
142 cell overproliferation, correlating with a gradual increase in stem cell number and  
143 activity [43–46]. Such increase in meristem size perturbs leaf divergence angles in  
144 arabidopsis *clv3-2* mutants that transit through decussate and whorled phyllotactic  
145 patterns over their lifetime [47]. Overproliferation of ectopic apical stem cells was  
146 observed in moss *Ppclv* mutants, and exogenous treatment with PpCLE peptides is  
147 sufficient to suppress cell proliferation in wild-type shoots. Thus, *CLV* genes exert key  
148 roles in leaf initiation and positioning in mosses and flowering plants and may operate  
149 at different scales, through regulation of stem cell homeostasis, cell identity and  
150 division plane orientation. In moss, the analysis of *PPCLV* mutants highlights the  
151 functional link between cell division orientation and shoot architecture, and further  
152 work is needed to determine how PpCLV signaling controls microtubule-dependent  
153 division plane orientation.

154

### 155 **Hormonal interactions control apical function**

156 In addition to cell geometry and the above-mentioned molecular players, the  
157 phytohormone auxin influences microtubule distribution [48] and can act on division  
158 plane orientation control, as proposed in arabidopsis [49]. Whether a similar  
159 mechanism operates in unicellular meristems is unknown, but several lines of  
160 evidence indicate that auxin-based shoot patterning is a shared characteristic of

161 mosses and flowering plants (**Figure 3**). Pharmacological perturbations of polar auxin  
162 transport in tomato and arabidopsis [50–53] and genetic studies of auxin influx and  
163 efflux carriers have established a prominent role for auxin transport in phyllotaxis  
164 control [54,55]. Computational models and 4D quantitative imaging using auxin  
165 biosensors have allowed to define further that auxin transport functions in phyllotaxis  
166 by self-organizing zones of lower auxin concentration that act as inhibitory fields  
167 around organs, a mechanism that may account for the diversity of phyllotactic  
168 patterns observed in flowering plants [56–61]. Radioactive auxin transport assays  
169 have been used to assess the conservation of polar auxin transport beyond flowering  
170 plants [62,63]. These experiments have failed to detect long-range unidirectional  
171 auxin movement in the leafy shoot of four different moss species [62], and it has been  
172 proposed that auxin could flow by a diffusion-like mechanism in *Physcomitrium* [64].  
173 Nevertheless, treatment of wild-type moss with the polar auxin transport inhibitor, *N*-  
174 1-Naphthylphthalamic Acid (NPA), and/or the synthetic auxin, 1-Naphthaleneacetic  
175 acid (NAA), causes a range of developmental defects such as stunted shoots with  
176 fewer and narrower leaves, suppression of leaf outgrowth or loss of apical cell activity  
177 [65]. Canonical *PIN-FORMED* (*PIN*) genes are conserved in *Physcomitrium*, and *PINA*  
178 and *PINB* are highly expressed in the gametophyte [65–67]. *pinA pinB* double mutants  
179 have twisted leaves and reduced leaf cell proliferation, resembling plants that  
180 accumulate auxin. They also display hypersensitivity to exogenous auxin, which  
181 causes phenotypes also seen in wild-type moss treated with both NPA and NAA, such  
182 as growth termination. This led to the proposal that PIN proteins sustain apical  
183 function and rhythmic leaf production by exporting auxin out of the apical cell and  
184 newly formed leaves [65]. Thus, both in mosses and flowering plants, PIN-mediated  
185 auxin transport regulates meristem function and leaf development.

186         Local auxin biosynthesis is also essential to shoot apical meristem activity in  
187 arabidopsis. Notably, PLETHORA (PLT) transcription factors determine lateral organ  
188 position by controlling auxin levels [68,69]. *plt3/5/7* mutant plants display abnormal  
189 phyllotaxis with an increase of successive 180° divergence angles. Transcript levels  
190 of *YUCCA1* (*YUC1*) and *YUC4* auxin biosynthetic genes are reduced in *plt3/5/7*  
191 mutants, PLT5 directly promotes *YUC4* expression, and heterozygous *yuc1 yuc4*  
192 double mutants tend to have distichous leaf arrangement [68]. It is worth stressing



193 that YUC-dependent auxin biosynthesis more likely occurs in developing primordia  
194 [60] than in the meristem center as previously proposed [68]. In *Physcomitrium*, four  
195 *APB* (for *A*INTEGUMENTA, *P*LETHORA and *B*ABY BOOM) genes are orthologous to  
196 *Arabidopsis* *PLTs*, and their expression is induced by auxin and associated with leafy  
197 shoot initials [16]. Quadruple *apb* mutants are unable to initiate leafy shoots and  
198 ectopic *APB4* expression raises shoot number, suggesting that *APBs* are necessary  
199 and sufficient for apical cell specification. *APB4* might regulate auxin biosynthesis  
200 [16], but this has not been tested at the transcriptional level.

201 Cytokinin promotes leafy shoot initiation in moss (**Figure 3**), probably by  
202 converting receptive cells to shoot apical cells, and this effect is enhanced by auxin  
203 [16,64,70,71]. Although *APB* expression is not regulated by cytokinin, *abp* quadruple  
204 mutants fail to respond to the synthetic cytokinin BAP, suggesting that auxin-induced  
205 *APBs* are required for cytokinin-dependent shoot initiation in moss [16]. Studies of  
206 *Arabidopsis histidine phosphotransfer protein 6* (*ahp6*) and *abphyl1* (*abph1*)  
207 phyllotactic mutants have highlighted the importance of auxin-cytokinin crosstalk in  
208 flowering plant shoot development [72,73]. *AHP6* is activated by  
209 MONOPTEROS/AUXIN RESPONSE FACTOR5 and hence acts downstream of auxin  
210 to set the pace of organ production [72]. Unlike wild-type maize plants, *abph1* mutants  
211 display decussate phyllotaxis ( $j = 2$ ,  $\alpha = 90^\circ$ ), and this is caused by the disruption of  
212 a cytokinin-inducible type A response regulator (ARR) [74]. NPA treatment restricts  
213 *ABPH1* expression in the shoot apical meristem, *ZmPIN1a* expression and auxin  
214 levels are reduced in *abph1* mutants, and cytokinin promotes *ZmPIN1a* expression in  
215 an *ABPH1*-dependent manner. These results emphasize the importance and possible  
216 conservation of auxin-cytokinin interactions at the shoot apex of land plants and  
217 support a probable involvement in the regulation of moss phyllotaxis (**Figure 3**) [73].

218

### 219 **Concluding remarks and future perspectives**

220 Although shoot architecture diversified independently in the gametophyte and  
221 sporophyte generations of the plant life cycle [75], analogous shoot forms and  
222 phyllotactic patterns arose by convergence in both generations and their  
223 development is underpinned by a series of deep homologies (defined in [76] as “*the*  
224 *sharing of the genetic regulatory apparatus that is used to build morphologically and*

225 *phylogenetically disparate (...) features*”), as illustrated throughout this article (**Figure**  
226 **3** and references therein). Further work is now needed to elucidate how conserved  
227 hormonal regulatory modules pattern phyllotaxis from single apical cells and how  
228 polarity cues are set at the single-cell level and translated into asymmetric divisions  
229 (see **Outstanding questions**). The concept of inhibitory fields introduced above  
230 comes notably from surgical experiments on fern meristems [3]. It is thus of particular  
231 interest to question whether auxin-based inhibitory fields exist in other spore-  
232 producing plant species, possibly at the scale of one or a few cells. Beyond mosses,  
233 these questions have started to be addressed in *Selaginella kraussiana* in which NPA  
234 treatment perturbs phyllotaxis and causes shoot apex termination [63], but the lack  
235 of a lycophyte transformation method hinders functional genomic analyses. Recently,  
236 an efficient genetic transformation procedure has been established for the fern  
237 *Ceratopteris richardii*, opening the possibility to bridge a huge gap in our  
238 understanding of plant form evolution [77]. The development of more spore-producing  
239 plant models will be pivotal to explore the mechanisms of phyllotaxis control in a  
240 broader evolutionary context and in meristems with increasing complexities [78].

241

## 242 **Acknowledgements**

243 We thank Fabrice Besnard, Joe Cammarata and Adrienne Roeder for commenting on  
244 the manuscript, two anonymous reviewers for their constructive suggestions and  
245 other laboratory members for stimulating discussions.

246

## 247 **References**

- 248 1 Stevens, P.S. (1976) *Patterns in Nature*, Penguin Books.
- 249 2 Adler, I. *et al.* (1997) A History of the Study of Phyllotaxis. *Annals of Botany* 80,  
250 231–244
- 251 3 Wardlaw, C.W. (1949) Phyllotaxis and Organogenesis in Ferns. *Nature* 164, 167–  
252 169
- 253 4 Kuhlemeier, C. (2017) Phyllotaxis. *Current Biology* 27, R882–R887
- 254 5 Besnard, F. *et al.* (2018) Quand les plantes font des maths. *Pour La Science*
- 255 6 Gola, E.M. and Banasiak, A. (2016) Diversity of phyllotaxis in land plants in  
256 reference to the shoot apical meristem structure. *Acta Soc Bot Pol* 85, 1–21
- 257 7 Harrison, C.J. *et al.* (2007) Growth from two transient apical initials in the  
258 meristem of *Selaginella kraussiana*. *Development* 134, 881–889
- 259 8 Sanders, H.L. *et al.* (2011) Sector analysis and predictive modelling reveal iterative  
260 shoot-like development in fern fronds. *Development* 138, 2925–2934

261 9 Harrison, C.J. *et al.* (2009) Local cues and asymmetric cell divisions underpin  
262 body plan transitions in the moss *Physcomitrella patens*. *Curr Biol* 19, 461–471  
263 10 Tomescu, A.M.F. (2009) Megaphylls, microphylls and the evolution of leaf  
264 development. *Trends Plant Sci* 14, 5–12  
265 11 Rensing, S.A. *et al.* (2020) The Moss *Physcomitrium* (*Physcomitrella*) *patens*:  
266 A Model Organism for Non-Seed Plants. *The Plant Cell* DOI:  
267 10.1105/tpc.19.00828  
268 12 Medina, R. *et al.* (2019) Phylogenomic delineation of *Physcomitrium*  
269 (Bryophyta: Funariaceae) based on targeted sequencing of nuclear exons and  
270 their flanking regions rejects the retention of *Physcomitrella*, *Physcomitridium* and  
271 *Aphanorrhagma*. *Journal of Systematics and Evolution* 57, 404–417  
272 13 Zagórska Marek, B. *et al.* (2018) Chiral events in developing gametophores of  
273 *Physcomitrella patens* and other moss species are driven by an unknown,  
274 universal direction-sensing mechanism. *Am J Bot* 138, 49–9  
275 14 Crum, H.A. (2001) *Structural Diversity of Bryophytes*, Univ of Michigan  
276 Herbarium.  
277 15 Wijk, R.V.D. (1957) Distichous and Pseudodistichous Mosses. *Acta Botanica*  
278 *Neerlandica* 6, 386–392  
279 16 Aoyama, T. *et al.* (2012) AP2-type transcription factors determine stem cell  
280 identity in the moss *Physcomitrella patens*. *Development* 139, 3120–3129  
281 17 Coudert, Y. *et al.* (2019) Design Principles of Branching Morphogenesis in  
282 Filamentous Organisms. *Current Biology* 29, R1149–R1162  
283 18 Moody, L.A. *et al.* (2018) Genetic Regulation of the 2D to 3D Growth  
284 Transition in the Moss *Physcomitrella patens*. *Current Biology* DOI:  
285 10.1016/j.cub.2017.12.052  
286 19 Bergmann, D.C. (2018) Taking Development to Three Dimensions. *Dev Cell*  
287 47, 678–679  
288 20 Tang, H. *et al.* (2020) Geometric cues forecast the switch from two- to three-  
289 dimensional growth in *Physcomitrella patens*. *New Phytologist* 225, 1945–1955  
290 21 Menand, B. *et al.* (2007) Both chloronemal and caulonemal cells expand by  
291 tip growth in the moss *Physcomitrella patens*. *J. Exp. Bot.* 58, 1843–1849  
292 22 Frank, M.H. and Scanlon, M.J. (2015) Cell-specific transcriptomic analyses of  
293 three-dimensional shoot development in the moss *Physcomitrella patens*. *The*  
294 *Plant Journal* 83, 743–751  
295 23 Goss, C.A. *et al.* (2012) A CELLULOSE SYNTHASE (CESA) gene essential for  
296 gametophore morphogenesis in the moss *Physcomitrella patens*. *Planta* 235,  
297 1355–1367  
298 24 Sampathkumar, A. *et al.* (2019) Primary wall cellulose synthase regulates  
299 shoot apical meristem mechanics and growth. *Development* 146,  
300 25 Yang, W. *et al.* (2016) Regulation of Meristem Morphogenesis by Cell Wall  
301 Synthases in Arabidopsis. *Current Biology* 26, 1404–1415  
302 26 Peaucelle, A. *et al.* (2008) Arabidopsis phyllotaxis is controlled by the methyl-  
303 esterification status of cell-wall pectins. *Curr. Biol.* 18, 1943–1948  
304 27 Peaucelle, A. *et al.* (2011) Pectin-Induced Changes in Cell Wall Mechanics  
305 Underlie Organ Initiation in Arabidopsis. *Current Biology* 21, 1720–1726  
306 28 Zhao, F. *et al.* (2019) Xyloglucans and Microtubules Synergistically Maintain  
307 Meristem Geometry and Phyllotaxis. *Plant Physiology* 181, 1191–1206

308 29 Besson, S. and Dumais, J. (2011) Universal rule for the symmetric division of  
309 plant cells. *Proc Natl Acad Sci USA* 108, 6294–6299

310 30 Louveaux, M. *et al.* (2016) Cell division plane orientation based on tensile  
311 stress in *Arabidopsis thaliana*. *Proc Natl Acad Sci USA* 113, E4294–303

312 31 Moukhtar, J. *et al.* (2019) Cell geometry determines symmetric and  
313 asymmetric division plane selection in *Arabidopsis* early embryos. *PLOS*  
314 *Computational Biology* 15, e1006771

315 32 Kosetsu, K. *et al.* (2017) Cytoplasmic MTOCs control spindle orientation for  
316 asymmetric cell division in plants. *Proceedings of the National Academy of*  
317 *Sciences* 114, E8847–E8854

318 33 Moody, L.A. (2019) The 2D to 3D growth transition in the moss *Physcomitrella*  
319 *patens*. *Current Opinion in Plant Biology* 47, 88–95

320 34 Perroud, P.-F. *et al.* (2014) Defective Kernel 1 (DEK1) is required for three-  
321 dimensional growth in *Physcomitrella patens*. *New Phytol* 203, 794–804

322 35 Demko, V. *et al.* (2014) Genetic analysis of DEFECTIVE KERNEL1 loop  
323 function in three-dimensional body patterning in *Physcomitrella patens*. *Plant*  
324 *Physiol* 166, 903–919

325 36 Kozgunova, E. *et al.* (2020) *Spindle position dictates division site during*  
326 *asymmetric cell division in moss*, *Plant Biology*.

327 37 Whitewoods, C.D. *et al.* (2018) CLAVATA Was a Genetic Novelty for the  
328 Morphological Innovation of 3D Growth in Land Plants. *Current Biology* DOI:  
329 10.1016/j.cub.2018.05.068

330 38 Livanos, P. and Müller, S. (2019) Division Plane Establishment and  
331 Cytokinesis. *Annual Review of Plant Biology* 70, 239–267

332 39 Chakraborty, B. *et al.* (2018) A Plausible Microtubule-Based Mechanism for  
333 Cell Division Orientation in Plant Embryogenesis. *Curr. Biol.* 28, 3031–3043.e2

334 40 Sablowski, R. (2016) Coordination of plant cell growth and division: collective  
335 control or mutual agreement? *Current Opinion in Plant Biology* 34, 54–60

336 41 Fletcher, J.C. (2020) Recent Advances in *Arabidopsis* CLE Peptide Signaling.  
337 *Trends in Plant Science* 0,

338 42 Etchells, J.P. and Turner, S.R. (2010) The PXY-CLE41 receptor ligand pair  
339 defines a multifunctional pathway that controls the rate and orientation of vascular  
340 cell division. *Development* 137, 767–774

341 43 Fletcher, J.C. *et al.* (1999) Signaling of Cell Fate Decisions by CLAVATA3 in  
342 *Arabidopsis* Shoot Meristems. *Science* 283, 1911–1914

343 44 Clark, S.E. *et al.* (1993) CLAVATA1, a regulator of meristem and flower  
344 development in *Arabidopsis*. *Development* 119, 397–418

345 45 Clark, S.E. *et al.* (1995) CLAVATA3 is a specific regulator of shoot and floral  
346 meristem development affecting the same processes as CLAVATA1.  
347 *Development* 121, 2057–2067

348 46 Somssich, M. *et al.* (2016) CLAVATA-WUSCHEL signaling in the shoot  
349 meristem. *Development* 143, 3238–3248

350 47 Mandel, T. *et al.* (2016) Differential regulation of meristem size, morphology  
351 and organization by the ERECTA, CLAVATA and class III HD-ZIP pathways.  
352 *Development* 143, 1612–1622

353 48 Vineyard, L. *et al.* (2013) Progressive Transverse Microtubule Array  
354 Organization in Hormone-Induced *Arabidopsis* Hypocotyl Cells. *The Plant Cell* 25,  
355 662–676

356 49 Yoshida, S. *et al.* (2014) Genetic control of plant development by overriding a  
357 geometric division rule. *Dev Cell* 29, 75–87

358 50 Reinhardt, D. *et al.* (2000) Auxin Regulates the Initiation and Radial Position of  
359 Plant Lateral Organs. *The Plant Cell* 12, 507–518

360 51 Reinhardt, D. *et al.* (2003) Regulation of phyllotaxis by polar auxin transport.  
361 *Nature* 426, 255–260

362 52 Guenot, B. *et al.* (2012) Pin1-independent leaf initiation in Arabidopsis. *Plant*  
363 *Physiol* 159, 1501–1510

364 53 Kierzkowski, D. *et al.* (2013) Interaction between meristem tissue layers  
365 controls phyllotaxis. *Dev Cell* 26, 616–628

366 54 Bainbridge, K. *et al.* (2008) Auxin influx carriers stabilize phyllotactic  
367 patterning. *Genes Dev* 22, 810–823

368 55 Martinez, C.C. *et al.* (2016) A sister of PIN1 gene in tomato (*Solanum*  
369 *lycopersicum*) defines leaf and flower organ initiation patterns by maintaining  
370 epidermal auxin flux. *Developmental Biology* 419, 85–98

371 56 Smith, R.S. *et al.* (2006) A plausible model of phyllotaxis. *Proceedings of the*  
372 *National Academy of Sciences* 103, 1301–1306

373 57 Jönsson, H. *et al.* (2006) An auxin-driven polarized transport model for  
374 phyllotaxis. *Proc Natl Acad Sci USA* 103, 1633–1638

375 58 de Reuille, P.B. *et al.* (2006) Computer simulations reveal properties of the  
376 cell-cell signaling network at the shoot apex in Arabidopsis. *Proceedings of the*  
377 *National Academy of Sciences* 103, 1627–1632

378 59 Refahi, Y. *et al.* (2016) A stochastic multicellular model identifies biological  
379 watermarks from disorders in self-organized patterns of phyllotaxis. *eLife* 5,  
380 e14093

381 60 Galvan-Ampudia, C.S. *et al.* (2020) Temporal integration of auxin information  
382 for the regulation of patterning. *eLife* 9, e55832

383 61 Brunoud, G. *et al.* (2012) A novel sensor to map auxin response and  
384 distribution at high spatio-temporal resolution. *Nature* 482, 103–106

385 62 Fujita, T. *et al.* (2008) Convergent evolution of shoots in land plants: lack of  
386 auxin polar transport in moss shoots. *Evol Dev* 10, 176–186

387 63 Sanders, H.L. and Langdale, J.A. (2013) Conserved transport mechanisms  
388 but distinct auxin responses govern shoot patterning in *Selaginella kraussiana*.  
389 *New Phytol* DOI: 10.1111/nph.12183

390 64 Coudert, Y. *et al.* (2015) Three ancient hormonal cues co-ordinate shoot  
391 branching in a moss. *eLife* 4, e06808

392 65 Bennett, T.A. *et al.* (2014) Plasma Membrane-Targeted PIN Proteins Drive  
393 Shoot Development in a Moss. *Curr. Biol.* 24, 2776–2785

394 66 Bennett, T. *et al.* (2014) Paralogous radiations of PIN proteins with multiple  
395 origins of noncanonical PIN structure. *Mol Biol Evol* 31, 2042–2060

396 67 Viaene, T. *et al.* (2014) Directional Auxin Transport Mechanisms in Early  
397 Diverging Land Plants. *Curr Biol* 24, 2786–2791

398 68 Pinon, V. *et al.* (2013) Local auxin biosynthesis regulation by PLETHORA  
399 transcription factors controls phyllotaxis in Arabidopsis. *PNAS* 110, 1107–1112

400 69 Prasad, K. *et al.* (2011) Arabidopsis PLETHORA transcription factors control  
401 phyllotaxis. *Current Biology* 21, 1123–1128

402 70 Coudert, Y. *et al.* (2019) A KNOX-Cytokinin Regulatory Module Predates the  
403 Origin of Indeterminate Vascular Plants. *Current Biology* 29, 2743–2750.e5

404 71 Ashton, N.W. *et al.* (1979) Analysis of gametophytic development in the moss,  
405 *Physcomitrella patens*, using auxin and cytokinin resistant mutants. *Planta* 144,  
406 427–435

407 72 Besnard, F. *et al.* (2014) Cytokinin signalling inhibitory fields provide  
408 robustness to phyllotaxis. *Nature* 505, 417–421

409 73 Lee, B. *et al.* (2009) Studies of aberrant phyllotaxy1 mutants of maize indicate  
410 complex interactions between auxin and cytokinin signaling in the shoot apical  
411 meristem. *Plant Physiol* 150, 205–216

412 74 Giulini, A. *et al.* (2004) Control of phyllotaxy by the cytokinin-inducible  
413 response regulator homologue ABPHYL1. *Nature* 430, 1031–1034

414 75 Coudert, Y. *et al.* (2017) Multiple innovations underpinned branching form  
415 diversification in mosses. *New Phytol* 22, 810

416 76 Shubin, N. *et al.* (2009) Deep homology and the origins of evolutionary  
417 novelty. *Nature* 457, 818–823

418 77 Plackett, A.R.G. *et al.* (2015) Ferns: the missing link in shoot evolution and  
419 development. *Front. Plant Sci.* 6, 5328–19

420 78 Rensing, S.A. (2017) Why we need more non-seed plant models. *New Phytol*  
421 DOI: 10.1111/nph.14464

422 79 Bhatia, N. *et al.* (2016) Auxin Acts through MONOPTEROS to Regulate Plant  
423 Cell Polarity and Pattern Phyllotaxis. *Current Biology* DOI:  
424 10.1016/j.cub.2016.09.044

425 80 Cammarata, J. *et al.* (2019) Cytokinin and CLE signaling are highly intertwined  
426 developmental regulators across tissues and species. *Current Opinion in Plant*  
427 *Biology* 51, 96–104

428 81 Lavy, M. *et al.* (2016) Constitutive auxin response in *Physcomitrella* reveals  
429 complex interactions between Aux/IAA and ARF proteins. *eLife Sciences* 5,  
430 e13325

431 82 Prigge, M.J. *et al.* (2010) *Physcomitrella patens* Auxin-Resistant Mutants  
432 Affect Conserved Elements of an Auxin-Signaling Pathway. *Curr Biol* DOI:  
433 10.1016/j.cub.2010.08.050

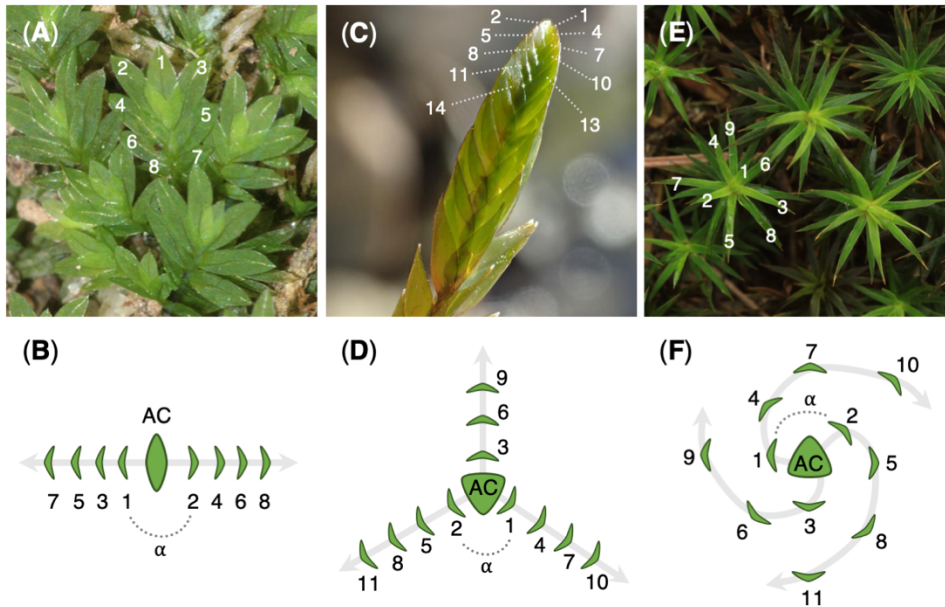
434 83 Mähönen, A.P. *et al.* (2014) PLETHORA gradient formation mechanism  
435 separates auxin responses. *Nature* 515, 125–129

436

437

438 **Figures**

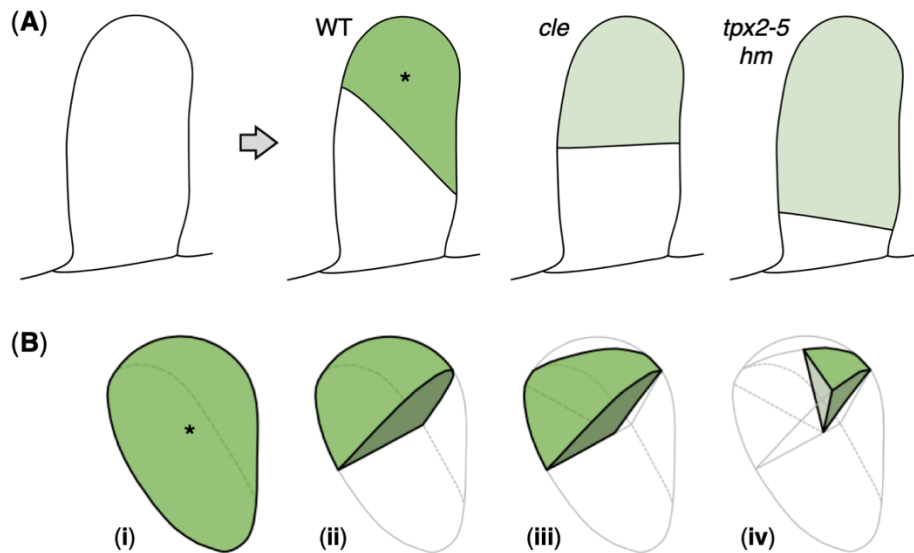
439



440

441 **Figure 1. Phyllotactic patterns in mosses**

442 (A, B) A single lenticular apical cell produces distichous phyllotaxis ( $j = 1, \alpha = 180^\circ$ ) in  
 443 *Fissidens taxifolius*. (C, D) A single tetrahedral apical cell produces tristichous  
 444 phyllotaxis ( $j = 1, \alpha = 120^\circ$ ) in *Fontinalis antipyretica*. (E, F) A single tetrahedral apical  
 445 cell produces spiral phyllotaxis ( $j = 1, 120^\circ < \alpha < 180^\circ$ ) in *Polytrichum commune*. Spiral  
 446 phyllotaxis is also typical of the model moss *Physcomitrium patens*. (A, C, E) Numbers  
 447 indicate the sequence of leaf emergence, from the youngest “1” to the oldest visible  
 448 leaf. In (C), the leaves numbered “3”, “6”, “9” and “12” are not visible. (B, D, F) “AC”  
 449 marks the apical cell and numbers indicate the relative order and position of leaf  
 450 emergence, “1” being the youngest leaf. Arrows indicate parastichies,  $j$  corresponds  
 451 to the leaf number per node and  $\alpha$  is the divergence angle. Image credits : (A, E)  
 452 Hermann Schachner, <https://commons.wikimedia.org>, (C) Hugues Tinguy,  
 453 <https://inpn.mnhn.fr>.

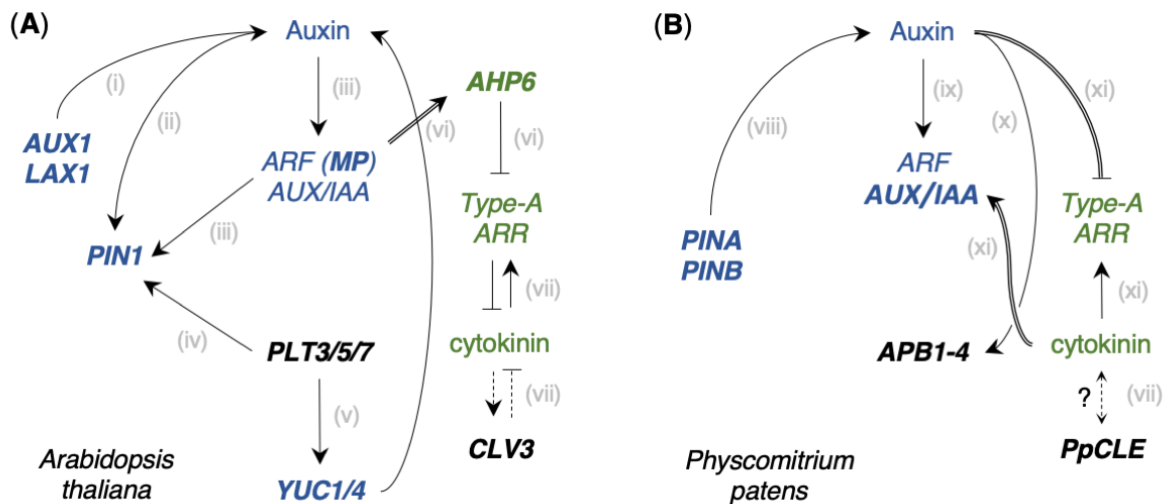


454

455 **Figure 2. Asymmetric divisions underpin tetrahedral apical cell specification and**  
 456 **proliferation in the moss *Physcomitrium patens***

457 (A) In *physcomitrium*, the gametophore initial cell (i.e., the leafy shoot precursor cell)  
 458 develops from the protonema (i.e., the filamentous tissue produced by spore  
 459 germination) and is characterized by an irregular and oblong shape (drawing to the  
 460 left of the arrow). In wild-type (WT), the first division of the leafy shoot initial is oblique  
 461 and produces apical (marked by an asterisk) and basal domains with asymmetric  
 462 identities. In abnormal situations this division may be symmetrical and perpendicular  
 463 to the growth axis (*Pp cle* mutants) or skewed toward the basal side of the initial (*tpx2-*  
 464 *5 hm* mutant), which may affect or prevent leafy shoot apical cell specification or lead  
 465 to conspicuous leaf patterning defects. (B) In WT, following the first division of the  
 466 shoot initial (i), the apical domain (marked by an asterisk) undergoes a series of  
 467 oblique divisions (ii-iv) leading to the formation of the tetrahedral apical stem cell  
 468 (coloured in green) at the origin of spiral phyllotaxis. (A) adapted from [9,36,37], (B)  
 469 redrawn from [9] with permission from corresponding author.





470

471 **Figure 3. Hormonal cues control apical function in moss and flowering plant leafy**  
 472 **shoots**

473 (A) Interacting auxin and cytokinin-dependent regulatory modules control shoot apical  
 474 meristem function and phyllotactic patterning in *Arabidopsis thaliana*. Mutated forms  
 475 of genes in bold are associated with phyllotaxis defects. (B) Gametophore apical cell  
 476 specification and activity are regulated by at least partly similar regulatory modules in  
 477 *Physcomitrium patens*. Mutants in genes shown in bold are unable to form  
 478 gametophores or have apical cell and leaf developmental defects. Molecular  
 479 interactions are inferred from the following references : (i), [54]; (ii), [50,51,56–58]; (iii),  
 480 [79]; (iv), [69]; (v), [68]; (vi), [72]; (vii), [37,47,80]; (viii), [65]; (ix), [81]; (x), [16]; (xi), [82]  
 481 and involve the following genes : *AUXIN RESISTANT 1 (AUX1)*, *LIKE AUXIN*  
 482 *RESISTANT 1 (LAX1)*, *PIN-FORMED (PIN)*, *AUXIN RESPONSE FACTOR (ARF)*,  
 483 *MONOPTEROS (MP)*, *PLETHORA (PLT)*, *YUCCA (YUC)*, *ARABIDOPSIS HISTIDINE*  
 484 *PHOSPHOTRANSFER PROTEIN (AHP6)*, *ARABIDOPSIS RESPONSE REGULATOR*  
 485 *(ARR)*, *CLAVATA3 (CLV3)*, *AINTEGUMENTA*, *PLETHORA and BABY BOOM (APB)*,  
 486 *CLAVATA3/EMBRYO SURROUNDING REGION-RELATED (CLE)*. Molecular  
 487 interactions occurring outside the shoot are not mentioned in the figure, e.g. auxin  
 488 regulation of PLTs in the arabidopsis root [83]. Dashed lines in (A) indicate the indirect  
 489 regulation of *CLV3* expression by cytokinin, via *Type-B ARR* and *WUSCHEL (WUS)*,  
 490 and of cytokinin signaling by *CLV3* via *CLAVATA1* and *WUS*. In moss shoots (B),  
 491 PpCLE and cytokinin have antagonistic developmental effects, but evidence for a  
 492 direct or indirect interaction between PpCLE and cytokinin is lacking. Auxin  
 493 biosynthesis, signaling and transport components are shown in blue, and cytokinin

494 signaling components are shown in green. Double lines indicate auxin-cytokinin  
495 signaling crosstalks.