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Phyllotaxis from a single apical cell

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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 (α). 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 α -Xylosyltransferase* or *α -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 *abphl1* (*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

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246

247 **References**

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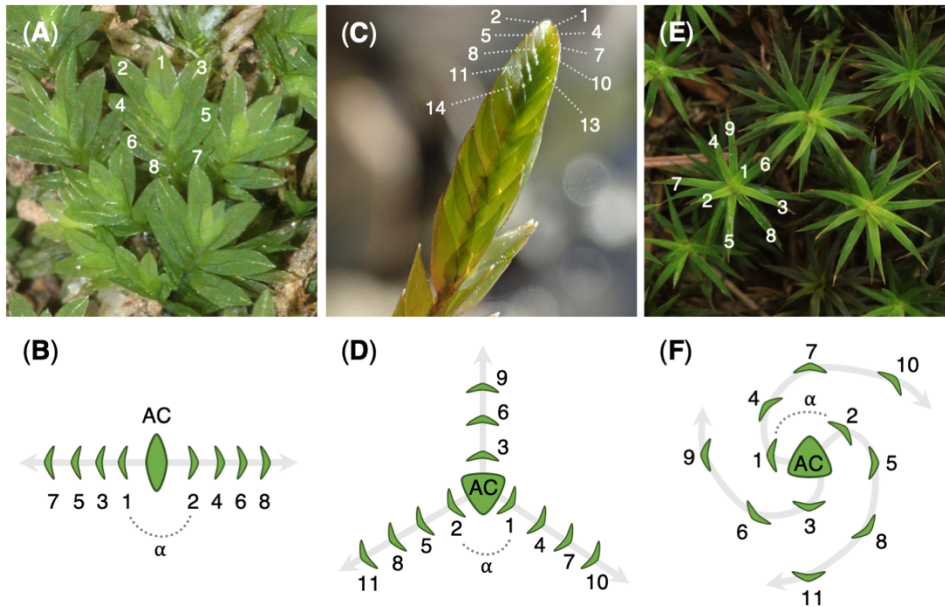
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438 **Figures**

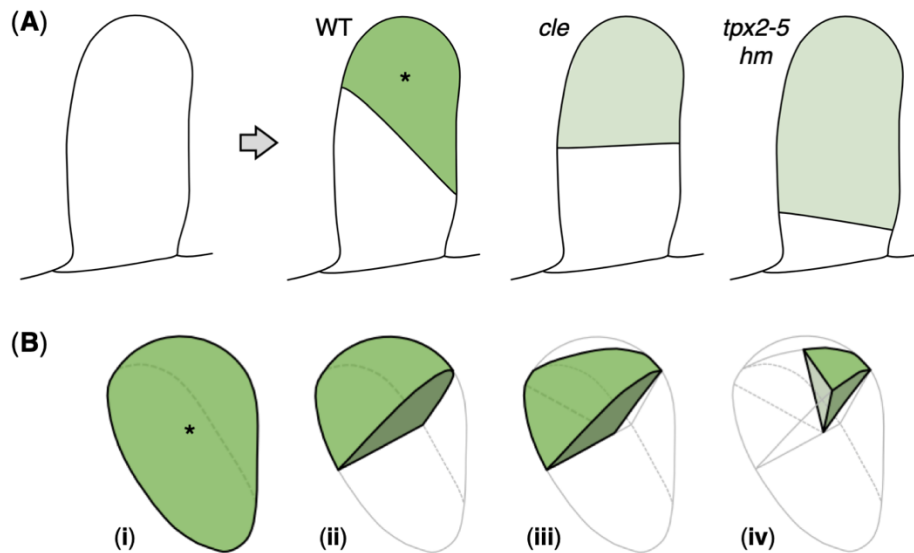
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440

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

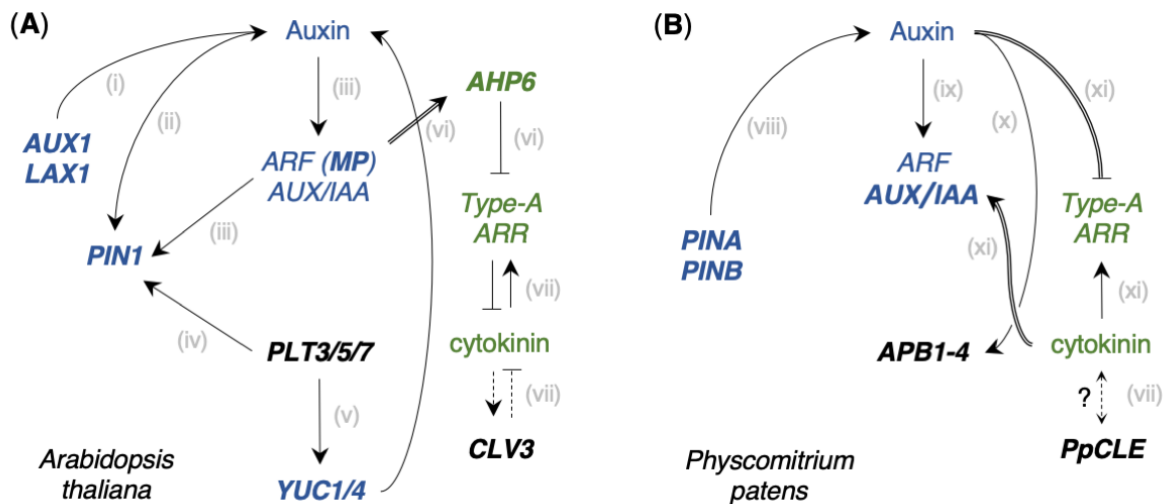
442 (A, B) A single lenticular apical cell produces distichous phyllotaxis ($j = 1$, $\alpha = 180^\circ$) in
 443 *Fissidens taxifolius*. (C, D) A single tetrahedral apical cell produces tristichous
 444 phyllotaxis ($j = 1$, $\alpha = 120^\circ$) in *Fontinalis antipyretica*. (E, F) A single tetrahedral 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 α 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.