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1 Re-examining meristems through the lens of evo-devo

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15 Abstract

16 The concept of the *meristem* was introduced in 1858 to characterize multicellular,
17 formative and proliferative tissues that give rise to the entire plant body, based on observations of
18 vascular plants. Although its original definition did not encompass bryophytes, this concept has
19 been used and continuously refined over the past 165 years to describe the diverse apices of all
20 land plants. Here, we re-examine this matter in light of recent evo-devo research and show that,
21 despite displaying high anatomical diversity, land plant meristems are unified by shared genetic
22 control. We also propose a modular view of meristem function and highlight multiple evolutionary
23 mechanisms that likely contributed to the assembly and diversification of the varied meristems
24 during the course of plant evolution.

27 Keywords

28 meristem; bryophytes; apical cell; ferns; lycophytes; *Physcomitrium*; *Marchantia*; *Anthoceros*; evo-
29 devo; *CLAVATA*; *LEAFY*; *KNOX*; *WUSCHEL*; *WOX*

30 **The evolving concept of the meristem**

31 Introductory remarks

32 In 1858, von Nägeli coined the term “meristem” based on microscopical observations of
33 vascular plants. The word “meristem”, originating from the Greek word *μερίζω* and meaning “to
34 divide into parts”, then referred to a transient, formative and dividing tissue capable of giving rise
35 to all other plant tissues. Additionally, von Nägeli proposed the concept of “primordial meristem”
36 to distinguish primary meristems found at shoot and root apices from secondary meristems, such
37 as the cambium, which fulfill more specialized functions [1,2]. His work had a profound and lasting
38 impact in the field of plant sciences, and it is now widely accepted that apical meristems underpin
39 the development of all land plants, including both vascular plants and bryophytes. However, von
40 Nägeli’s definitions originally applied to multicellular structures found in vascular plants and did not
41 include bryophytes. At the same time, Hofmeister proposed the alternative idea that a single apical
42 cell is responsible for generating all other plant tissues, notably in bryophytes [3,4]. Later,
43 cytological and functional studies have identified the features that characterize vascular plant and
44 bryophyte apices. These findings have gradually blurred the conceptual distinction between single-
45 celled and complex multicellular apices, thereby challenging the original definition of the meristem.

46 In this context, this article pursues three main objectives. First, we present a concise
47 overview of the rich historical context concerning the structure and function of meristems, with a
48 focus on the shoot to highlight the main commonalities and differences between bryophytes and
49 vascular plants. Then, we discuss how the new molecular understanding, when put into the context
50 of evolutionary and developmental studies [5], has the potential to overturn some of the classical
51 distinctions outlined earlier and lead us towards a more unified model of meristem function. Last,
52 we speculate on how recent discoveries could provide new insights into the molecular events that
53 accompanied the assembly of multicellular plant meristems from simpler ancestral structures, over
54 evolutionary times.

55

56 Multiple types of shoot apical meristems in vascular plants

57 Since its elaboration, the concept of the meristem has been subject to a continuous re-
58 appraisal. Subsequent histological and cytological studies in vascular plants have offered refined
59 insights into the complex organization of apical meristems, which facilitated the reconciliation of
60 von Nägeli and Hofmeister’s seemingly contradictory theories. For instance, works by Hanstein,
61 Schmidt and Foster have contributed to the establishment of distinct, non-mutually exclusive
62 conceptual frameworks, including the histogen theory, the tunica-carpus theory and the cyto-
63 histological zonation of meristems, respectively [6–9]. These theories have illuminated the

64 composite nature of apical meristems and supported the idea that cytological zones could coincide
65 with functional zones. Along this line, and building on a substantial collection of anatomical
66 descriptions of vascular plant apices, Newman proposed in 1961 that apical meristems are made
67 of two components: the 'initial cells' (or 'continuing meristematic residue'), which constitute a
68 source of new cells emerging continuously and at a slow pace, and the 'general meristem' described
69 as a broader region of comparatively rapid activity and short duration, where cells mature [10].
70 This view has been well reflected on substance through the more recent adoption of the concept
71 of 'stem cells' co-opted from the animal biology field [6,8,11–13]. For instance, functional studies
72 in the angiosperm *Arabidopsis thaliana* have established a model where the shoot apical meristem
73 comprises a multilayered pool of slowly dividing stem cells, called the stem cell niche, underlaid by
74 an organizing center. Stem cells have the capacity to self-renew and supply new cells to a peripheral
75 zone of active cell proliferation and differentiation where lateral organs arise [14]. More generally,
76 and in an attempt to capture the structural and functional diversity of vascular plant apical
77 meristems, Newman defined three basic types of meristems named monoplex, simplex and duplex
78 [10]. These meristem types are mainly distinguished by differences in the form, position and
79 division patterns of initial cells, but not their number that can vary greatly between species (Figure
80 1). This nomenclature has proven particularly useful to distinguish the apical organization of spore-
81 producing vascular plants, *i.e.* ferns and lycophytes, from that of seed-producing vascular plants,
82 *i.e.* gymnosperms and angiosperms. In the following sections, we outline the main meristematic
83 features of ferns and lycophytes, before discussing the similarities and differences with those of
84 bryophytes.

85

86 *Distinctive features of fern and lycophyte shoot meristems*

87 In ferns and lycophytes, the diploid sporophyte constitutes the dominant phase of the life
88 cycle. It is typically distinguished by a vegetative leafy shoot that produces leaf-like lateral organs,
89 called fronds in ferns and microphylls in lycophytes. In contrast, the haploid gametophyte
90 comprises a small, short-lived, photosynthetic thallus in its more elaborate form. A peculiarity of
91 most fern and lycophyte sporophyte meristems is the presence of at least one clearly
92 distinguishable apical initial cell of a characteristic shape, usually resembling an inverted pyramid
93 or a portion of pyramid, pointing downwards, with inclined side walls, and located at the surface
94 of the apex (Figure 1). In this case, division of the apical initial cell occurs in a direction parallel with
95 the inclined walls, ensuring growth of the shoot axis in both width and depth. Such features, which
96 define Newman's monoplex meristem type, are missing from seed plant meristems. In contrast,
97 initial cells in the latter have no easily recognizable shape and may undergo both anticlinal and

98 periclinal divisions. Nonetheless, it has also been proposed that in the lycophytes *Isoetes* and
99 *Lycopodium* the shoot apical cells may undergo both anticlinal and periclinal divisions, which results
100 in multilayered structures resembling the simplex meristems of gymnosperms (see also [15–17] for
101 a more exhaustive account on the structure and division patterns of fern and lycophyte apical cells).

102 Besides classical anatomical studies, experimental approaches have allowed to determine
103 the number of stem cells in the apical meristems of a few species. For example, clonal sector
104 analyses coupled with predictive modeling have shown that the sporophytic shoot of the fern
105 *Nephrolepis exaltata* is derived from a single apical cell [18]. In contrast, the tracking of X-ray
106 induced sectors has revealed that a minimum of two transient apical cells underpins shoot growth
107 in the lycophyte *Selaginella kraussiana*, a feature that could be shared with other ferns [19,20].
108 Similarly, the gametophyte of ferns develops by the activity of an apical cell that may be permanent
109 or short-lived. If the apical cell is short-lived, growth after apical cell termination is orchestrated by
110 a multicellular meristematic region, called the lateral or marginal meristem. However, its structure
111 and function has never been experimentally evidenced [21–24]. Although no apical cell has been
112 documented in the gametophyte of *S. kraussiana*, studies in lycophytes are too scarce to draw any
113 general conclusion about the structure of gametophyte meristems in these plants [15]. Another
114 important feature of the sporophyte meristem is the presence a multicellular zone of proliferation
115 subtending the apical cells. This proliferative domain comprises actively dividing apical cell
116 derivatives, known as merophytes, and corresponds to the ‘general meristem’ defined by Newman.
117 This domain plays an essential role by contributing to the formation of lateral organs at a distance
118 from the apical initial cells [15,16].

119

120 Concealed diversity in bryophyte meristems

121 In contrast with vascular plants, the dominant phase of the life cycle in bryophytes is the
122 haploid gametophyte. Its morphology varies across the three lineages of bryophytes, namely the
123 mosses, liverworts and hornworts [25]. In mosses and leafy liverworts, gametophyte development
124 initiates with a transient stage called protonema. This stage is characterized by a filamentous,
125 thalloid, or spherical tissue, from which leafy shoots called gametophores eventually emerge. In
126 hornworts and thalloid liverworts, the prevalent growth form is a horizontally spreading mat of
127 vegetative tissues called thallus. Regardless of their morphology, all bryophyte gametophytes
128 produce determinate, unbranched and leafless sporophytes [26,27].

129 A conspicuous feature of the apex of bryophyte gametophytes, which is shared with ferns
130 and lycophytes, is the presence of at least one morphologically distinct initial cell. In thalloid
131 liverworts and hornworts, the apical initial cells are located in marginal and invaginated regions

132 referred to as notches [27,28]. Although the precise cell division patterns in the notch meristem
133 are not fully clear yet [29], anatomical observations have suggested that the number of initial cells
134 might not be constant and could increase, at least transiently, during dichotomous branching of
135 the thallus [30]. More recently, single-cell transcriptomics in the liverwort *Marchantia polymorpha*
136 has suggested that the number of stem cells also decreases with age [31]. Recently developed tools
137 for clonal analysis based on Cre-lox and CRISPR-Cas9 methodologies have provided valuable
138 insights into the role of lineage and positional cues in the formation of dorsal organs in *M.*
139 *polymorpha* [29]. Such tools hold the potential to provide a clearer understanding of the number
140 and division patterns of apical initial cells, and their contribution to thallus development [29,32]. In
141 leafy liverworts and mosses, the development of the gametophytic shoot is likely governed by a
142 single apical initial cell. In contrast with vascular plants where lateral organs arise distantly from
143 the apical cells, each immediate derivative of the shoot apical cell directly gives rise to a phyllid,
144 the leaf-like organ of bryophytes [33–36]. This means that the phyllotactic pattern is probably a
145 direct consequence of the cleavage patterns of the apical cell [33]. While histology has shown that
146 divisions of shoot apical cell derivatives also contribute to the formation of stem tissues [37–39],
147 the only clonal analysis ever conducted in a moss has not provided a definitive understanding of
148 the specific role of merophytes to the construction of the leafy shoot [35].

149 Sporophyte development occurs through distinct developmental trajectories in the three
150 bryophyte lineages. In mosses, it is conducted by at least one single apical initial cell. However,
151 histology in various species has suggested that this stem cell is short-lived and ceases its activity
152 after cutting off a small number of merophytes. Further growth in the apical region of the
153 sporophyte is ensured by merophyte divisions [40,41]. The next growth stage is marked by the
154 formation and proliferation of a multicellular intercalary meristem from early merophytes, at a
155 distance below the apex, which serves to elongate the sporophyte axis until spore differentiation
156 starts [42]. Similar to the marginal meristem described earlier, the stem cell number and division
157 patterns of the intercalary meristem have not been formally determined. In contrast with mosses,
158 hornwort and liverwort sporophytes lack conspicuous apical stem cells and likely extend by the
159 activity of a multicellular basal meristem and diffuse cell expansion, respectively [27,43].

160 While all the structures described above serve a 'primordial meristem' function in the sense
161 that they give rise to all the other cells of the plant body, their diversity makes the definition of the
162 bryophyte meristem not straightforward. This diversity not only reflects variations in the number
163 of stem cells and their capacity for sustained proliferation through time (short-lived *versus*
164 indeterminate), but also in the position of multicellular proliferative zones (apical *versus* non-
165 apical).

166 The data presented above collectively suggests that Newman's concept of the monoplex
167 meristem aptly captures the features of initial cells found in bryophyte apices. However, notable
168 differences emerge when comparing them to fern and lycophyte shoot apices. First, the 'general
169 meristem' is considerably smaller in bryophytes. Then, lateral organs can either emerge directly
170 from the cleavage of the apical cell itself, like in the moss leafy shoot, or not develop at all, as
171 observed in the gametophyte of certain thalloid species or in the sporophyte of all bryophytes.
172 Moreover, the activity of apical cells can be temporally and spatially decoupled from that of
173 merophytes and multicellular proliferation zones, as exemplified with the intercalary meristem of
174 the moss sporophyte. In some cases, it even appears that no apical cell is identifiable like in the
175 sporophyte of liverworts and hornworts where growth occur through diffuse cell proliferation and
176 expansion.

177 These differences prompt the question of whether meristem function, and specifically, the
178 function of apical initial cells on one hand, and multicellular proliferation zones on the other, are
179 governed by distinct or evolutionary conserved genetic regulatory mechanisms in bryophytes and
180 vascular plants.

181

182 **Conserved genetic regulators operate in various land plant meristems**

183 In the past few decades, genetic screens conducted in angiosperms such as *A. thaliana*, *Zea*
184 *mays* (maize) and *Antirrhinum majus* (snapdragon) have successfully identified essential regulators
185 of meristem function, including *KNOTTED1-LIKE HOMEODOMAIN (KNOX)* [44], *LEAFY/FLORICAULA*
186 (*LFY/FLO*) [45,46], *WUSCHEL (WUS)* [47,48] and *CLAVATA (CLV)* [49,50] genes. In recent years,
187 advancements in genomic resources and genetic transformation methods in bryophytes [51–54],
188 ferns and lycophytes [55,56] have offered unparalleled opportunities to investigate the
189 conservation, expression pattern and role of these genes. Below, we explore the diverse
190 contributions of *KNOX*, *LFY/FLO*, *CLV* and *WUS* homologues across different plant lineages, offering
191 a molecular perspective on the evolution of meristems (Figure 2).

192

193 *KNOX I genes underpin multicellular proliferation in sporophyte meristems*

194 In angiosperms, class I *KNOX (KNOX I)* genes encode transcription factors responsible for
195 maintaining cells in an undifferentiated state at the apex and are necessary and sufficient for shoot
196 apical meristem specification (Figure 2A). For example, in *A. thaliana*, the *KNOX I* gene *SHOOT*
197 *MERISTEMLESS (STM)* is expressed throughout the shoot apical meristem. *stm* mutants lack a shoot
198 apical meristem and undergo growth arrest after germination [57], while *STM*-overexpressing
199 plants develop ectopic meristems on their leaves [58]. The presence of *KNOX I* expression in shoot

200 apical meristem cells of the gymnosperm *Welwitschia mirabilis* suggests that this meristematic
201 function may be conserved across seed plants [59]. *KNOX I* genes originated before the emergence
202 of land plants and were likely lost in hornworts, but their function has been studied in other
203 bryophytes [60].

204 In the moss *Physcomitrium patens*, there are three *KNOX I* genes, named *MOSS KNOX2*
205 (*MKN2*), *MKN4* and *MKN5*. Among these, two are expressed in the sporophyte apical stem cell, but
206 phenotypes of corresponding mutants suggest that these genes are not needed for its function [61].
207 *MOSS KNOX I* genes are not required either for gametophyte apical cell function, even though their
208 expression has been reported in the apex of initiating *P. patens* gametophytic leafy shoots [61,62].
209 In contrast, all three genes are active in the middle region of the developing sporophyte. In
210 particular, deletion of the *MKN2* gene prevents elongation of the sporophyte axis. Conversely,
211 constitutive or inducible *MKN2* overexpression promotes its extension. Whilst this suggests that
212 *MKN2* is essential and capable of modulating intercalary meristem activity, protracting *MKN2*
213 expression in elongated sporophytes does not result in a persisting, indeterminate meristem [42].
214 In the liverwort *Marchantia polymorpha*, the only *KNOX I* gene identified, *MpKNOX1*, is expressed
215 in the egg cell and throughout the developing sporophyte. Mutant sporophytes with *Mpknox1*
216 deficiency are arrested in development soon after fertilization, suggesting a role for *MpKNOX1* in
217 the haploid-to-diploid phase transition without ruling out a possible function in cell division beyond
218 this stage [63,64]. In the lycophyte *Selaginella kraussiana*, *SkKNOX1* is expressed in a multicellular
219 region subtending apical cells, and in the fern *Ceratopteris richardii*, the *KNOX I* genes *CrKNOX1* and
220 *CrKNOX2* are expressed in and around the apical cell of the sporophytic shoot but are not detected
221 in gametophytic tissues [65,66].

222 While ARP proteins play a crucial role in restraining *KNOX* activity in vascular plant
223 meristems, the evolutionary significance of the *KNOX-ARP* regulatory module in bryophytes
224 remains unclear [66]. Notably, *ARP* genes are absent from sequenced hornwort and moss genomes
225 [51,52,67]. In the case of *M. polymorpha*, only one *ARP* homologue has been identified in its
226 genome, yet its precise developmental function remains to be elucidated [53].

227 Thus, *KNOX I* genes are broadly associated with meristem function, both in apical and non-
228 apical regions. *KNOX I* genes can be expressed in apical cells, merophytes and distal multicellular
229 proliferative regions and are conserved regulators of sporophyte development.

230

231 *LEAFY controls meristem function in vascular plants and cell division in a moss*

232 The transcription factor LFY/FLO (referred to as LFY hereafter) was first identified in
233 snapdragon mutants that were unable to produce flowers and is primarily recognized for its

234 essential role in flower development (Figure 2B) [45,46]. However, it appears that *LFY* may also be
235 required for vegetative meristem function, as demonstrated in the angiosperm *Cucumis sativus*,
236 where a reduction of *LFY* expression leads to shoot apical meristem abortion, and other species
237 even if this role may be hidden by other genetic regulators [68,69]. This dual function in vegetative
238 and reproductive meristems is likely conserved in lycophytes like *Isoetes* and *Selaginella*, the
239 horsetail *Equisetum* and the fern *Adiantum*, where *LFY* expression is detected in various
240 reproductive and vegetative tissues of the sporophyte [70]. A study in *C. richardii* where *CrLFY1* and
241 *CrLFY2* have been knocked-down by RNA interference has revealed that *LFY* is essential to sustain
242 stem cell division at the apex of the sporophyte body and also plays a critical role in gametophyte
243 meristematic cells [71].

244 Although the role of *LFY* in bryophytes remains poorly understood, a pioneering study has
245 shown that the two *LFY* homologues of the moss *P. patens* are broadly expressed in the sporophyte.
246 Impairment of *LFY* function blocks the first zygotic division after fertilization, which prevents the
247 establishment of the apical cell. *P. patens LFY* genes are also expressed in the apex of the
248 gametophytic leafy shoot, but the lack of phenotypic differences between *PpLfy* mutants and wild-
249 type plants indicate that these genes are not needed for its function [72]. Transcriptome analyses
250 in the hornwort *A. agrestis* suggest that its single *LFY* gene copy is expressed in both phases of the
251 life cycle but predominantly active in the gametophyte, which points towards a putative role in
252 vegetative tissues or an early function in the reproductive transition [51]. *LFY* in the liverwort *M.*
253 *polymorpha* is highly transcribed in male sexual organs, but its developmental role remains
254 unknown [73].

255 Thus, *LFY* expression marks vegetative meristematic tissues in the gametophyte of
256 bryophytes and spore-producing vascular plants. *LFY* also has essential roles in both vegetative
257 meristems and reproductive structures of the sporophyte of vascular plants, and controls cell
258 division in the sporophyte of a moss. Beyond *LFY*'s well-characterized function in floral
259 development, these data suggest a possible conserved function in the regulation of cell
260 proliferation in land plants [74].

261

262 *WOX genes are likely not conserved regulators of meristem function in land plants*

263 Identification of *WUS* revealed its essential role in controlling stem cell fate in both
264 reproductive and vegetative shoot meristems of *A. thaliana* (Figure 2C) [47,48]. *WUS* gene is
265 transcribed in the organizing center of the shoot apical meristem, underneath the stem cells, and
266 the *WUS* protein moves apically to modulate their proliferation and differentiation [75]. *WUS*
267 belongs to the *WUS* homeobox (*WOX*) transcription factor family that is conserved in land plants,

268 but *WUS* orthologues are missing from sequenced bryophyte and lycophyte genomes. Phylogenetic
269 analyses of *WOX* gene family members in leptosporangiate ferns suggest that a single *WUS*
270 orthologue emerged in the most recent common ancestor of ferns and seed plants [76–78]. Data
271 from multiple gymnosperms and angiosperms indicate that the association of *WUS* homologues
272 with reproductive structures is broadly conserved among seed plants [77]. However, few *WUS*
273 orthologues share a similar expression pattern with *AtWUS* in the vegetative shoot apical meristem,
274 which favors the hypothesis of a sub-functionalized function within the angiosperms [77,79].

275 In the fern *C. richardii*, the *WUS* orthologue, *CrWUL*, and *WOX* homologues, *CrWOXA*,
276 *CrWOXB* and *CrWOX13A*, are expressed in both gametophyte and sporophyte tissues. *In situ*
277 hybridization experiments of *CrWUL*, *CrWOXA* and *CrWOXB* transcripts have produced contrasting
278 results. Transcripts were detected in spatially restricted domains corresponding to apical cells or
279 their derivatives in the sporophyte root [76] or, concerning *CrWOXB*, in broader regions of cell
280 proliferation of the gametophyte and sporophyte [80]. *CrWUL* developmental function has not
281 been elucidated yet. However, a reduction of *CrWOXB* expression by RNA interference delays
282 gametophyte development, and this correlates with a diminution of cell number. More striking
283 phenotypes are observed in the sporophytic shoot, which produces fewer and less complex fronds
284 and fewer and less branched roots, supporting the role of *CrWOXB* in promoting cell proliferation
285 and meristematic activity. In *Selaginella moellendorffii*, *SmWOX*, that is not a *WUS* orthologue, is
286 expressed in the proliferative zone of the sporophytic shoot apical meristem and initiating lateral
287 organs, but its function has not been reported yet [81].

288 In bryophytes, all the identified *WOX* genes belong to the *WOX13* phylogenetic clade,
289 whose function is not associated with apical meristems in *A. thaliana* [82,83]. *Anthoceros* genomes
290 contain up to six *WOX13-like* (*WOX13L*) homologues expressed in one or both phases of the life
291 cycle [51,52]. Disruption of the single *M. polymorpha* *MpWOX* gene by genome editing results in
292 mutant plants with reduced gametophyte body size but does not alter the size of the stem cell zone
293 in the meristematic notch, indicating that *MpWOX* modulates cell proliferation outside the apical
294 zone [84]. Among the three *PpWOXL13* genes identified in *P. patens*, *PpWOXLA* and *PpWOXLB* are
295 broadly expressed in gametophyte and sporophyte tissues, but *PpWOXLC* activity is not detected.
296 Fluorescently tagged *PpWOXLA* and *PpWOXLB* proteins exhibit strong signals in the zygote egg cell
297 and regenerating protonemal cells from detached phyllids, consistent with their identified function
298 in cell growth initiation during protonemal stem cell regeneration and zygote formation. Whether
299 defects observed in *Ppwox13l* mutant sporophytes result from a specific function in stem cell
300 maintenance or mirror a broader role in cell proliferation or growth control has still to be clarified.

301 Importantly, *P. patens* *WOX13L* genes are not essential for apical growth in the gametophytic leafy
302 shoot [85].

303 Thus, the association of *WUS* with shoot apical meristem function seems to be a derived
304 trait in angiosperms. Functional data in the fern *C. richardii* indicate that *WOX* genes, non-
305 orthologous to *WUS*, may be involved in the control of shoot apical meristem activity. Genetic
306 studies in the moss *P. patens* and the liverwort *M. polymorpha* suggest that *WOX13-L* genes are not
307 required for meristem function in the bryophyte gametophyte.

308

309 *CLAVATA signaling is a conserved regulatory pathway of stem cell homeostasis*

310 *CLAVATA* (*CLV*) mutants have been identified in *A. thaliana* due to their enlarged apices
311 caused by meristem cell overproliferation, in contrast with *Atwus* mutants that fail to maintain
312 actively dividing apical meristems (Figure 2D) [50,86]. *CLV* signaling involves multiple interacting
313 partners including CLE (*CLAVATA3/Endosperm surrounding region-related*) peptides and receptor-
314 like kinases, such as *CLV1*, and operates in a negative feedback loop with *WUS* to balance stem cell
315 division and differentiation at the apex [87]. Studies of the *CLV* pathway in monocots like maize and
316 dicots like tomato have demonstrated its conserved and essential role in the control of stem cell
317 homeostasis in angiosperms [88]. *CLV* genes are conserved throughout land plants and likely
318 originated in their last common ancestor [89]. Although the *CLV* pathway remains poorly studied in
319 the context of gymnosperm, fern and lycophyte meristems, functional investigations in bryophytes
320 have revealed shared roles with angiosperms [81,90].

321 In the moss *P. patens*, the gametophore apical cell forms through a series of stereotypical,
322 oblique divisions from a precursor cell located on the protonema [35]. In *P. patens* *Ppclv1a1b* and
323 *Pprpk2* mutants, and *Ppcle* knock-down plants, the failure to properly orient cell division planes at
324 an early developmental stage leads to the production of abnormally shaped or aborted
325 gametophores [89]. Similar defects have been observed in others transgenic lines carrying
326 unrelated mutations, like *Ppdek1* or *Pptpx2*, but also unable of controlling cell division plane
327 orientation, which suggests that such perturbations likely impede the formation of a functional
328 apical cell [91,92]. Cell division orientation defects observed in the root of *A. thaliana*
329 *Atclv1/Atbam1/Atbam2/Atbam3* quadruple mutants indicate that this regulatory function of *CLV*
330 signaling is shared with angiosperms [89]. Moreover, in the rare cases where *P. patens* *Ppclv1a1b*
331 or *Pprpk2* mutants grow beyond the bud stage, gametophores produce ectopic stem cells at their
332 base and eventually terminate growth in a mass of proliferating apical cells [89,93]. By contrast, the
333 application of synthetic analogs of PpCLE peptides to gametophores restricts overall growth and
334 suppresses cell division in leaves. Treatment of *A. thaliana* roots either with AtCLV3 or PpCLE

335 peptides inhibits meristem activity, which suggests that *P. patens* CLEs can operate through a
336 molecular mechanism that is conserved with *A. thaliana* [89]. Thus, *CLV* signaling in *P. patens*
337 regulates cell division orientation and controls gametophore meristem homeostasis by repressing
338 stem cell identity.

339 Treating *M. polymorpha* with a synthetic form of MpCLE2, its sole AtCLV3 peptide
340 orthologue, leads to an expansion of the apical notch and a promotion of stem cell activity. In
341 contrast, disrupting *MpCLE2* or *MpCLV1* gene function reduces stem cell number. This suggests that
342 *CLV* signaling has a positive effect on stem cell activity in *M. polymorpha* gametophyte, which is the
343 opposite of what has been observed in *A. thaliana* shoot apical meristem [84]. These contrasting
344 behaviors mirror antagonistic *AtCLV3* and *MpCLE2* expression patterns in apical stem cells and the
345 stem cell-surrounding proliferative zone, respectively. Together with another study on MpCLE1
346 peptide, these results have led to a model where the zonation of meristems evolved by *CLE* gene
347 duplication (see [94,95] for further details).

348 Thus, *CLV* signalling is a shared regulator of stem cell activity in bryophyte gametophytes
349 and angiosperm sporophytes. However, it remains to be formally tested whether *CLV* regulates
350 stem cell function in bryophyte sporophytes.

351

352 The aforementioned data collectively suggests that, despite exhibiting great anatomical
353 diversity, meristems in bryophytes and vascular plants function, at least partially, through
354 evolutionarily conserved gene families. *KNOX I* gene activity is associated with the multicellular
355 proliferative zones of apical and non-apical meristems in the sporophyte. *CLV* signaling regulates
356 stem cell identity and proliferation in both the gametophyte and sporophyte. The function of *CLV*
357 can be either promoting or inhibitory, depending on the species and developmental context. It is
358 noteworthy that these genes are not exclusive regulators of primary meristem function and may
359 operate in other developmental contexts [88,96]. *LFY* homologues are broadly expressed in the
360 vegetative and reproductive meristems of vascular plants and have demonstrated roles in the
361 regulation of meristem activity and fate in multiple angiosperms and a fern. Although *LFY*
362 homologues control the first division of the moss zygote, hence affecting the formation of the
363 sporophyte apical cell, their precise contribution to meristem function has not been conclusively
364 demonstrated. Similarly, *WUS* has a recognized function in controlling stem cell homeostasis in
365 angiosperms, but related members of the *WOX13L* clade have no identified role in meristem
366 function in bryophytes yet.

367 Furthermore, recent studies have unveiled the involvement of Arabidopsis LSH1 and *Oryza*
368 G1 (ALOG) proteins in lateral organ formation in *M. polymorpha* [97], and GIBBERELLIC ACID

369 INSENSITIVE, REPRESSOR OF GA and SCARECROW (GRAS) [98,99] and APETALA2 (AP2)-type [100]
370 transcription factors in stem cell formation and homeostasis in *P. patens*. Additional layers of
371 hormonal and epigenetic control also contribute to the intricate mechanisms governing meristem
372 function both in bryophytes and vascular plants [26,33,101,102]. Recent works have notably
373 evidenced the conserved contribution of auxin and cytokinin and addressed how these hormones
374 may interact with each other and with *CLV* signaling to regulate stem cell proliferation [33,93,102–
375 107]. Based on current molecular knowledge, we therefore conclude that shared genetic control
376 stands as a unifying feature of the varied land plant meristems.

377

378 We note that research in bryophytes has primarily concentrated on testing the biological
379 function of homologues of known regulators identified in angiosperms. In the years to come, a key
380 challenge will be to ascertain the extent of conservation in the genetic control of meristems in
381 vascular plants and bryophytes since their divergence from their more recent common ancestor,
382 and beyond known regulators. This could be accomplished through various means. The
383 development of forward genetic screens in *P. patens*, *M. polymorpha* and *A. agrestis* should
384 facilitate the identification of novel genetic components crucial to meristem function in bryophytes.
385 By applying this knowledge to vascular plants, we may gain a deeper comprehension of meristem
386 control within this lineage and illuminate the mechanism controlling meristem function in the
387 earliest land plants, much like the recent accomplishments with the mechanism underpinning the
388 development of tip-growing rooting cells. Moreover, we anticipate that the application of single-
389 cell RNA sequencing methodologies to bryophyte meristems will enable us to gain a more profound
390 understanding of the diversity of cell identities in these tissues but also identify new molecular
391 signatures and gene expression domains shared with vascular plants.

392

393 **Multiple evolutionary mechanisms underpin meristem diversification**

394 Recent phylogenomic studies have proposed that bryophytes and vascular plants form two
395 monophyletic lineages, diverging from a common ancestor approximately half a billion years ago
396 [108,109]. Following this divergence, bryophytes likely experienced substantial gene loss and body
397 organization simplification [110,111]. However, due to the scarcity of fossils, the fundamental
398 nature and precise morphology of this putative ancestor remains hypothetical [112]. Moreover, the
399 cellular architecture of meristems from early diverging and intermediary, extinct land plant lineages
400 is largely under-documented or inaccessible [113]. Consequently, establishing homology at the
401 anatomical level between extant bryophyte and vascular plant meristems poses challenges, and
402 our understanding of the gradual assembly of extant meristems is very limited. Nevertheless, we

403 have shown that meristem function in distantly related species is underpinned by multiple
404 conserved molecular regulators. Such regulators often act within modules, *i.e.* sub-units of a
405 molecular network consisting of two or more interacting partners. Through cross-species
406 comparisons of module function and expression domains, we can infer homology at the genetic
407 level and speculate about the mechanisms that might have led to the emergence of novel meristem
408 features during evolution.

409

410 Gene duplication and neofunctionalization

411 Gene duplication and neofunctionalization may underpin the elaboration of new modules,
412 as exemplified hereafter with *CLV* and *WUS* (see also [95]). The interaction between CLE peptides
413 and *CLV* receptors defines a minimal regulatory module that coordinates intercellular
414 communication to ensure stem cell division and homeostasis. This module operates in bryophyte
415 gametophytes and angiosperm sporophytes, but its function in bryophyte sporophytes has not yet
416 been demonstrated [84,89]. Under the hypothesis that the morphology of extant bryophytes
417 partially reflects that of the earliest land plants, this could imply a potential co-option of the *CLV*
418 module from the gametophyte to the sporophyte generation during the emergence of vascular
419 plants. The origin of *CLV* coincided with plant terrestrialization, whereas *WUS* evolved more
420 recently as a result of gene duplication and neo-functionalization in the *WOX* lineage [76,89] (Figure
421 3A). Typically, following gene duplication, one of the two duplicates retains the ancestral function,
422 while the other undergoes neofunctionalization, acquiring a new molecular function. The
423 recruitment of *WUS* to regulate meristem function in interaction with *CLV* therefore likely
424 represents a synapomorphy of ferns and seed plants, *i.e.* a shared characteristic gained by their last
425 common ancestor and conserved to this day.

426 In contrast, experiments reported in the previous section show that perturbations of *CLV*
427 and *WOX13L* gene function in the gametophyte of *P. patens* and *M. polymorpha* lead to different
428 and unrelated developmental phenotypes [84,85]. Moreover, *Mpwox* mutant thalli remain
429 sensitive to *MpCLE2* peptide treatment [84]. This suggests that *CLV* and *WOX13L* gene pathways
430 operate independently from each other in the gametophyte of bryophytes. Nevertheless, based on
431 current data, we cannot definitively rule out the possibility of an interaction between these
432 pathways in the sporophyte. To investigate the relationship between *CLV* and *WOX13L*, the first
433 step would be to determine the phenotype of corresponding mutant sporophytes. Subsequently,
434 interactions between both pathways could be examined by combining mutations through genetic
435 crosses.

436 Based on the expression domain of *WUS* orthologues in ferns and angiosperms, we
437 speculate that the evolution of CLV-WUS interaction enabled cross-talk between apical stem cells
438 and more distal proliferation zones, potentially increasing meristem size and resilience against
439 harmful mutations. Functional studies in relevant fern and gymnosperm models would be needed
440 to test the contribution of putative *CLV-WOX* regulatory modules in the evolution of meristem
441 function and size.

442

443 Molecular heterotopy

444 Molecular heterotopy refers to a spatial change in a gene's expression domain. It can lead
445 to new interactions between pre-existing, but spatially isolated modules, as exemplified by *KNOX*
446 genes. For instance, the class I *KNOX* gene *STM* acts through the *ISOPENTENYL TRANSFERASE 7*
447 (*IPT7*) gene to promote cytokinin biosynthesis and maintain *A. thaliana* shoot apical meristem
448 indeterminacy [114]. Although the moss sporophyte is a determinate structure, the class I *KNOX*
449 homolog *MKN2* also acts through *IPT*-dependent cytokinin biosynthesis to promote *P. patens*
450 intercalary meristem function and sporophyte axis elongation. This indicates a commonality
451 between moss and angiosperm sporophytes through a *KNOX*-cytokinin module, suggesting a deep
452 homology between non-apical and apical multicellular proliferative zones [42]. Looking at *KNOX*
453 expression patterns in lycophyte and fern shoot apices (Figure 2), we speculate that the
454 repositioning of a *KNOX*-cytokinin module to an apical position might have contributed to the
455 emergence of indeterminate vascular plant meristems (Figure 3B). Moreover, this could have been
456 facilitated by novel interactions with pre-existing modules. Recent research in *A. thaliana* supports
457 this modular view by demonstrating that *STM* and *WUS* proteins cooperate through
458 heterodimerization and bind to the *CLV3* promoter to control its expression and sustain stem cell
459 production [115]. Further investigations are now required to understand when and how the spatial
460 and temporal interaction between *KNOX*-cytokinin and *CLV* (or *CLV-WUS*) modules arose in
461 evolution, and their functional consequences during the diversification of meristems. For example,
462 it would be interesting to manipulate *KNOX* expression domain in the moss sporophyte, aiming to
463 sustain its activity in a more apical position throughout its development. This experiment could
464 provide valuable insights into how such an alteration might influence the activity of other apically
465 expressed genes and modulate stem cell determinacy.

466

467 Gene co-option

468 Gene co-option serves as a possible mechanism contributing to the diversification of
469 meristem identity, as exemplified by *LFY*. The *LFY* transcription factor plays a crucial role in the floral

470 development of angiosperms. However, data gathered from a moss, a fern and various angiosperms
471 suggest that *LFY* has a conserved and ancestral role in controlling cell division and/or vegetative
472 meristems. It is plausible that *LFY* was co-opted into a floral role once it gained the capacity to
473 regulate gene regulatory networks associated with reproduction (Figure 3C). Unlike the *WOX*
474 lineage, *LFY* appears as a single orthologue in most angiosperm genomes, indicating that the same
475 molecule fulfills both ancestral and derived functions. However, the precise mechanism by which
476 *LFY* achieves this dual function within a single species remains unclear [116]. More generally, how
477 *LFY* was co-opted into floral development remains largely elusive, although evidence suggests that
478 its connection to MADS-box gene targets, central to flower development, likely predates the origin
479 of flowers [117]. Additionally, it remains unknown whether *LFY* acts across all land plants by
480 targeting conserved or distinct genes. Deciphering the repertoire of *LFY* targets, as well as the role
481 of its transcriptional cofactor *UFO*, beyond *A. thaliana* should help solve this question [118–120].
482 Nevertheless, a study in the angiosperm *C. sativus* has shown that *LFY* interacts with *WUS* protein
483 to regulate *WUS* expression and maintain vegetative growth. This provides initial evidence that *LFY*
484 gene regulatory network has diverged in evolution, as *WUS* is absent in bryophytes [68]. This result
485 further supports the idea that the evolution of meristems involved the rewiring of interactions
486 between pre-existing molecular modules.

487

488 **Concluding remarks**

489 Recent evo-devo studies have shed light on the molecular function of meristems in both
490 bryophytes and vascular plants, revealing the role of deeply conserved genetic regulators like *CLV*,
491 *KNOX I*, and *LFY*. The emergence of complex, apical multicellular meristems in vascular plants likely
492 required the spatial rearrangement of ancient genetic regulatory modules, as well as the evolution
493 of new factors such as *WUS*, alongside the rewiring of pre-existing molecular interactions. Many
494 gene families involved in meristematic control are conserved in streptophyte algae, which are the
495 closest relatives to land plants. This suggests that meristem-associated genes existed in the algal
496 ancestor of land plants, thus predating the origin of multicellular meristems (Figure 2) [121–123].

497 A major challenge now is to decipher the ancestral function of meristem-associated genes
498 and understand how they were integrated into the development of land plant meristems,
499 ultimately enabling the evolution of multicellular apical growth [124]. A first step towards
500 answering this question comes from a functional study of *Gsm1*, a *KNOX* orthologue in the
501 unicellular green alga *Chlamydomonas reinhardtii*, which has provided evidence of its role in the
502 alternation of generations [125]. In this context, the development of streptophyte algal models will

503 be pivotal to gain a deeper understanding of this fundamental problem in a broader evolutionary
504 context [126].

505

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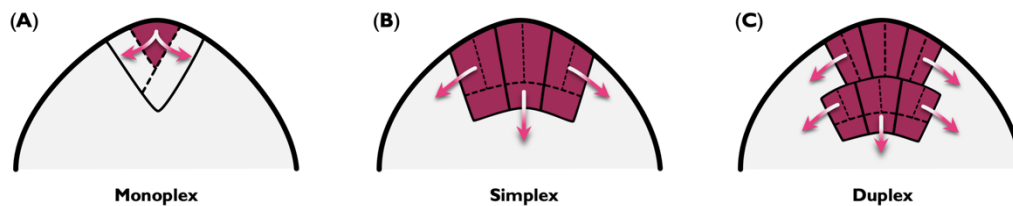
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516 **Figure legends**

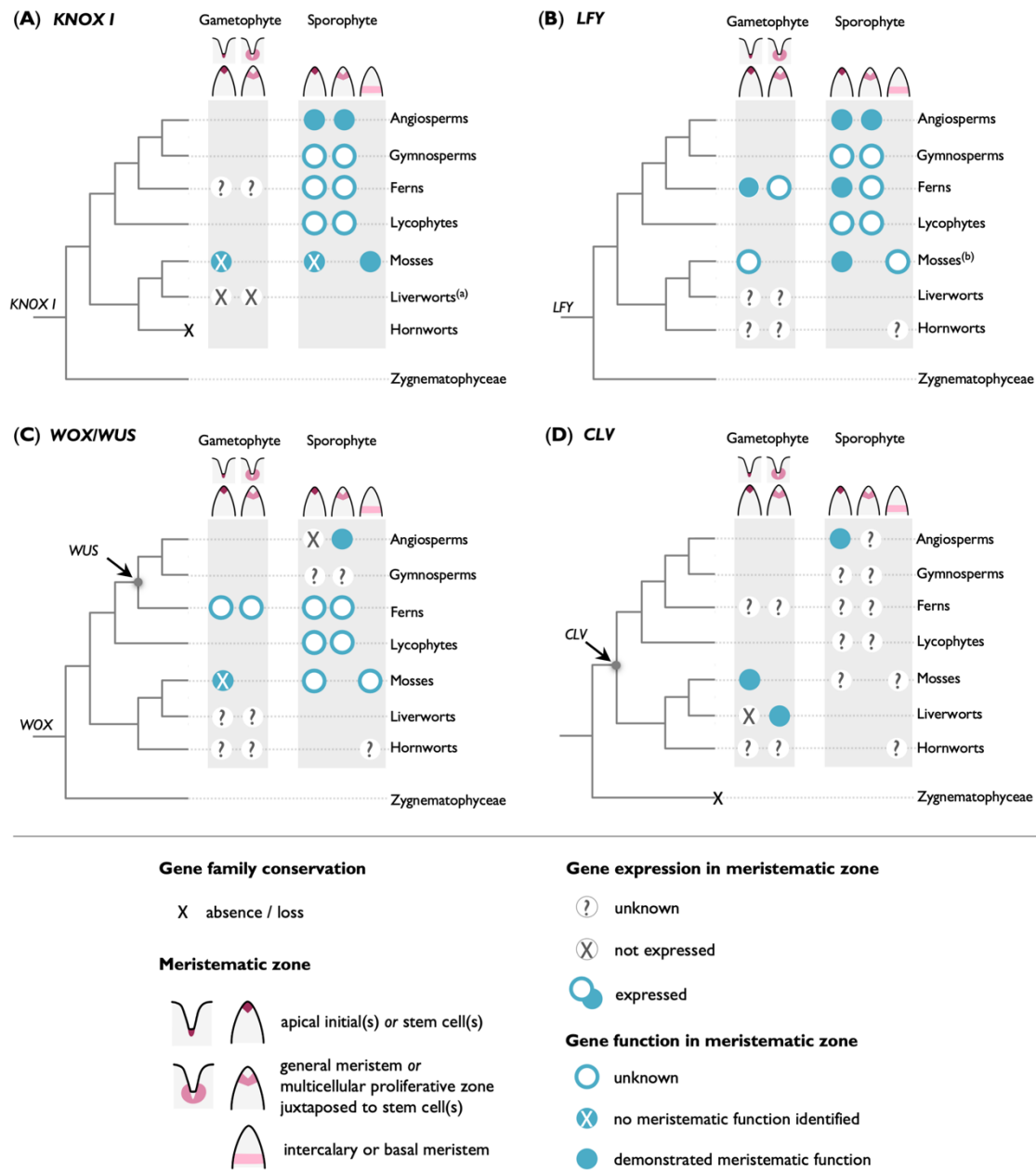
517 **Figure 1**



518
519

520 **Fundamental types of vascular plant shoot apices defined by Newman [10].** These three types represent an
521 abstraction of the initial cell niche's structure, position, and division patterns within vascular plant shoot
522 apical meristems. (A) In the monoplex apex, a single layer of cell initiation is situated at the surface of the
523 apex. Typically, it comprises a single apical cell undergoing division parallel to the oblique, outer walls. In
524 certain cases, multiple juxtaposed initial cells may be present. The monoplex type is characteristic of ferns
525 and lycophytes. (B) The simplex apex also features a single layer of cell initiation at the surface of the apex.
526 Initial cells may divide both periclinally and anticlinally, *ie.* parallel and perpendicular to the surface,
527 respectively. This apex type predominates among the gymnosperms, and is found in some ferns and
528 lycophytes, such as lycopodiales and isoetales. (C) In the duplex apex, two discrete yet contiguous regions of
529 cell initiation exist. The outer region may consist of one to several cell layers (only one is depicted on the
530 diagram), and cells within each layer divide solely anticlinally. In the inner region, cells undergo division both
531 periclinally and anticlinally. This distinction in cell division behaviour between the outer and inner regions
532 also defines the tunica-corpora zonation. The duplex apex is characteristic of angiosperms and is present in
533 some gymnosperms like *Gnetum*, *Ephedra* and *Welwitschia*. In the diagrams, initial cells are represented in
534 purple, and the general meristem, arising from the coordinated proliferation of these initials, is shown in
535 grey. Only initial cells and their immediate derivatives are shown. Arrows indicate the general direction of
536 cell displacement in the distinct layers. Diagrams were adapted from [10,16].

537
538



540

541 **Evolution of key gene families and regulatory pathways associated with meristematic function in land**

542 **plants and their algal sister lineage.** (A-D) Gene conservation, expression and function data are inferred from

543 [42,57,60–66,127–132] for class I *KNOTTED1-LIKE HOMEODOMAIN* (*KNOX I*), [51,70–74,117,122,133,134] for

544 *LEAFY* (*LFY*), [48,76–78,80,81,84,85,135,136] for *WUSCHEL homeobox* (*WOX*)/ *WUSCHEL* (*WUS*) and

545 [84,89,90,94,95,137,138] for *CLAVATA* (*CLV*). *MpKNOX1* (a), and *PpLFY1* and *PpLFY2* (b) are expressed

546 throughout sporophyte development in *M. polymorpha* and *P. patens*, respectively. “Notch” diagrams

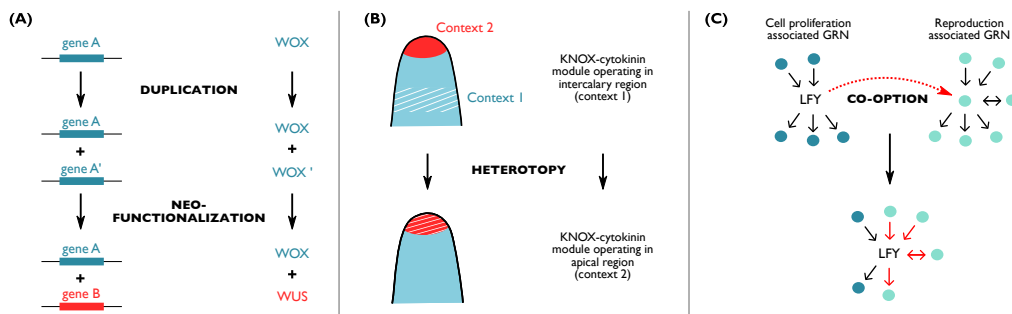
547 represent gametophyte meristems in hornworts, liverworts and ferns, and “tip” diagrams represent other

548 types of gametophyte and sporophyte meristems. Liverwort sporophytes are not represented as they grow

549 by diffuse cell division and expansion.

550

551 **Figure 3**



552
 553 **Evolutionary mechanisms underpinning meristem diversification in land plants.** (A) *WOX* gene family
 554 underwent duplication and neo-functionalization leading to the emergence of the *WUS* clade in ferns and
 555 seed plants. Similar mechanisms have been proposed to explain the diversification of *CLE* gene function. (B)
 556 A KNOX-cytokinin regulatory module is active in the intercalary region of a moss sporophyte and sustains
 557 indeterminate meristem function in the sporophyte of angiosperms, suggesting that heterotopy might have
 558 allowed the redeployment of this module from non-apical to apical regions during evolution. (C) *LFY* activity
 559 is associated with vegetative and reproductive tissues in vascular plants, and more particularly flowers in
 560 angiosperms. Data from bryophytes point towards a more general role in cell proliferation, suggesting that
 561 *LFY* could have been secondarily co-opted into reproductive and floral development. GRN means gene
 562 regulatory network.

563

564

565 **Declaration of interests**

566 The authors have no competing interests to declare.

567

568

569 **Declaration of generative AI and AI-assisted technologies in the writing process**

570 After drafting this manuscript, the authors who are non-native English speakers [139] used ChatGPT
 571 (version 3.5) in order to enhance the readability of the English text. After using this tool, the authors
 572 reviewed and edited the content as needed and take full responsibility for the content of the
 573 publication.

574

575

576

577 **References**

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