A fundamental developmental transition in *Physcomitrium patens* is regulated by evolutionarily conserved mechanisms

Richard Jaeger | Laura A. Moody

Department of Plant Sciences, University of Oxford, Oxford, UK

Correspondence
Laura A. Moody, Department of Plant Sciences, University of Oxford, South Parks Rd, Oxford OX1 3RB, UK.
Email: laura.moody@plants.ox.ac.uk

Funding information
Office of the Royal Society, Grant/Award Number: URF/R1/191310

Abstract

One of the most defining moments in history was the colonization of land by plants approximately 470 million years ago. The transition from water to land was accompanied by significant changes in the plant body plan, from those than resembled filamentous representatives of the charophytes, the sister group to land plants, to those that were morphologically complex and capable of colonizing harsher habitats. The moss *Physcomitrium patens* (also known as *Physcomitrella patens*) is an extant representative of the bryophytes, the earliest land plant lineage. The protonema of *P. patens* emerges from spores from a chloronemal initial cell, which can divide to self-renew to produce filaments of chloronemal cells. A chloronemal initial cell can differentiate into a caulonemal initial cell, which can divide and self-renew to produce filaments of caulonemal cells, which branch extensively and give rise to three-dimensional shoots. The process by which a chloronemal initial cell differentiates into a caulonemal initial cell is tightly regulated by auxin-induced remodeling of the actin cytoskeleton. Studies have revealed that the genetic mechanisms underpinning this transition also regulate tip growth and differentiation in diverse plant taxa. This review summarizes the known cellular and molecular mechanisms underpinning the chloronema to caulonema transition in *P. patens*.

KEYWORDS

actin, auxin, chloronema to caulonema transition, development, morphological evolution, *Physcomitrium patens*

1 | INTRODUCTION

Gametophyte body plans of charophyte algae are represented by diverse forms, from those that comprise simple unicells, to those that are morphologically complex and multicellular. A number of key evolutionary innovations that developed in water were inherited by plants following the colonization of land approximately 470 million years ago (Delwiche & Cooper, 2015). This included the emergence of apical initial cells that could direct growth, and self-renew and differentiate to provide, and then replenish, cells that are required by a plant during its lifetime. The morphological diversity observed across the terrestrial biosphere can thus be attributed to changes in apical initial cell behavior.
The moss *Physcomitrium patens* (also known as *Physcomitrella patens*) is an extant representative of the bryophytes, the earliest land plant lineage (Rensing et al., 2020). *P. patens* thus represents an ideal model system in which to answer fundamental questions about land plant evolution at the molecular level. The predominantly haploid life cycle of *P. patens* begins with spore germination and the formation of a chloronemal initial, which divides to produce chloronemal filaments. A chloronemal initial cell can then continue to divide to self-renew or can differentiate into a caulonemal initial cell, which can divide to produce caulonemal filaments. Caulonemal cells can divide in two planes to produce side branch initials that give rise to either chloronemata, caulonemata, or three dimensional, reproductively competent gametophores (Figure 1a–c). Gametes combine during fertilization to form the diploid zygote, and subsequently the multicellular sporophyte, which undergoes meiosis to release haploid spores (D. Cove, 2005).

Chloronema and caulonema, collectively known as the protonema, are morphologically distinguishable cell types and represent two phases of filamentous growth in *P. patens*. Chloronemal cells facilitate efficient photosynthesis, and as such, are typically chloroplast rich. Conversely, caulonemal cells are rapidly growing “colonizing” cells that contain fewer chloroplasts (B. B. Menand, Calder, et al., 2007). Chloronemal cells are additionally characterized by the formation of transverse cell plates (Figure 1d) whereas caulonemal cells form cell plates that are obliquely oriented (Figure 1e). The reorientation of cross walls is dependent on the highly dynamic microtubule cytoskeleton (Hiwatashi et al., 2008).

The protonema bears a close resemblance to multicellular and filamentous representatives of the charophytes and may therefore represent the most ancestral morphology of the land plants. The subsequent acquisition of additional layers of multicellular complexity enabled plants to survive on land more effectively, and to colonize progressively drier habitats across the globe (Delwiche & Cooper, 2015). Although invariably confined to a single cell, the ancestral toolkit underpinning tip growth was comprehensively integrated into flowering plant body plans; the formation of pollen tubes provided a more efficient means of reproduction, and the formation of root hairs (epidermal

---

**FIGURE 1** The *Physcomitrella patens* chloronema to caulonema developmental transition. (a) *P. patens* (Gransden strain) chloronemata (ch) and caulonemata (ca), collectively known as protonemata. Scale bar, 100 µm. (b)–(e) Confocal micrographs of propidium iodide stained caulonemal filament with secondary protonema (b), caulonemal filament with developing bud (c), chloronemal filament (d), and caulonemal filament (e). Scale bar, 20 µm. Note that chloronemal cells are typically shorter and form transverse cell plates (arrows in (d)) whereas caulonemal cells are longer and narrower and form oblique cell plates (* in (e)) [Color figure can be viewed at wileyonlinelibrary.com]
outgrowths) improved water and nutrient absorption efficiency. Thus, chloronemata, caulonemata, and indeed rhizoids have tip growth mechanisms that are similar to those found in the root hairs and pollen tubes of flowering plants (reviewed in Rounds & Bezanilla, 2013). Unlike their flowering plant counterparts, tip growing cells in the mosses possess the unique capacity to divide. The mechanisms underpinning the emergence of those tip growing cells in *P. patens* is remarkably well conserved throughout plants, as well as in fungi (Boyce et al., 2005): the emergence of root hairs from the root epidermis, the development of pollen tubes, and the formation of side branches in *P. patens* all require coordinated control of cell polarization, local cell wall loosening and cell expansion. These processes have been shown to be regulated by ROP GTPases, which localize to future bulge sites and mediate actin-induced bulge formation (Chen et al., 2003; Jones et al., 2002; Molendijk et al., 2001; P. Yi & Goshima, 2020).

A common toolkit comprising a subset of actin-associated proteins mediates tip growth in both chloronemata and caulonemata (Vidali et al., 2007, 2009, 2010). However, the differentiation of a chloronemal initial cell into a caulonemal initial cell is governed by an additional layer of regulation, which involves, but is not limited to, components of the Arp2/3 and SCAR/WAVE complexes (Augustine et al., 2008, 2011; Finka et al., 2008; Perroud & Quatrano, 2006, 2008). It has also been shown that the transition from a chloronemal cell into a caulonemal cell occurs in an auxin-dependent manner, and that a gradient of cell identity is detectable in developing filaments (Jang & Dolan, 2011). The chloronema to caulonema transition can therefore easily be reversed by antiauxins such as p-chlorophenoxyisobutyric acid (PCIB), or by polar auxin transport inhibitors such as N-1-naphthylphthalamic acid (NPA) (D. J. Cove & Ashton, 1984). The emergence of root hairs as outgrowths from the root epidermis, and the development of pollen tubes, are processes also regulated by auxin (Z. Ding et al., 2012; Grebe et al., 2002; Kleine-Vehn et al., 2006; Löfke et al., 2015). Cytokinin acts as a negative regulator of the chloronema to caulonema transition (Thelander et al., 2005). This is perhaps unsurprising since cytokinin is known to operate antagonistically to auxin throughout a range of important developmental transitions. The chloronema to caulonema transition is also triggered by a range of environmental conditions such as light quality and nutrient availability. Low light and high nutrient conditions tend to favor the maintenance of chloronema development. Conversely, high light and low nutrient conditions promote the chloronema to caulonema transition, and also provide the stimulus for extensive branching (Jenkins & Cove, 1983). Enhanced formation of caulonemata represents a convenient strategy to improve the acquisition of nutrients from the surrounding environment. This is a response that is mimicked in flowering plants whereby an upregulation of local auxin responses effects increased root hair formation in response to low nutrient conditions (Bhosale et al., 2018; C. Zhang et al., 2018).

The transition from a chloronemal initial cell into a caulonemal initial cell represents a fundamental biological transition in both evolutionary and developmental contexts. This review summarizes the known cellular and molecular mechanisms underpinning the chloronema to caulonema transition in *P. patens*.

## 2. THE CYTOSKELETON

Tip growth and differentiation are processes highly regulated by the actin cytoskeleton in a range of different cell types including pollen tubes, root hairs, and fungal hyphae. It is therefore unsurprising that the chloronema to caulonema transition is also highly dependent on organizational changes to the cytoskeleton, and that a common genetic toolkit regulates tip growth in these cell types. In chloronemal initial cells, cortical microtubules extend from the nucleus toward the tip, and actin filaments are oriented longitudinally and are enriched at the apical dome. In caulonemal initial cells, cortical microtubes extend from the nucleus and converge on a concentrated apical actin spot (Figure 3). Treatment with latrunculin B interferes with the process of actin polymerization and causes tip growth inhibition in both of these cell types (Harries et al., 2005). The tubulin cytoskeleton is not essential for tip growth in *P. patens* but acts in concert with the actin cytoskeleton to direct growth (Doonan et al., 1988).

### 2.1. ACTIN DEPOLYMERIZING FACTOR (ADF) AND ACTIN INTERACTING PROTEIN 1 (AIP1)

ADF and AIP1 mediate actin turnover by severing and depolymerizing actin filaments. ADF and AIP1 are required to regulate the organization of actin filaments in root hairs (Dong et al., 2001; Ketelaar et al., 2004), and in pollen tubes (Chen et al., 2003; Diao et al., 2019). In *P. patens*, ADF and AIP1 are each represented by a single gene copy (Augustine et al., 2008, 2011; Carlier et al., 1997). The complete loss of ADF leads to lethality, but RNAi-mediated silencing of ADF leads to the formation of stunted plants with unpolarized cells. Furthermore, the actin filaments in these lines are highly disorganized, and not uniformly oriented like those in wild type protonemal cells. ADF is therefore essential for the organization of the actin cytoskeleton (Augustine et al., 2008). *AIP1* knockout lines (Δ*aip1*) produce shorter
chloronemal cells than wild type and are unable to make the chloronema to caulonema transition. Partial caulonema induction can occur following the ectopic expression of ADF, or when Δαιp1 mutants are grown in the dark. In Δαιp1 mutants, F-actin bundles overaccumulate demonstrating that actin turnover in these lines is defective. AIP1 is thus required to promote actin turnover and to regulate F-actin dynamics during the differentiation of caulonemata (Augustine et al., 2011).

2.2 | Actin-related protein 2/3 (Arp2/3) complex

The Arp2/3 complex is required for the nucleation of actin polymerization, and its disruption leads to striking perturbations in root hair development (Mathur et al., 2003). The Arp2/3 complex is made up of seven subunits, all of which have been identified in P. patens. Arp2, ArpC4, and ArpC5 subunits are represented by single genes whereas Arp3, ArpC1, ArpC2, ArpC3 subunits are each represented by duplicated genes (PpARP3α and PpARP3β, PpARP1α and PpARP1β, PpARP2α and PpARP2β, PpARP3α and PpARP3β) (Finka et al., 2008).

RNAi-mediated disruption of both ARPC1α and ARPC1β, or a knockout of ARP3α alone, generates mutants that are unable to make the chloronema to caulonema transition. The arpc1 RNAi mutants are completely unable to initiate gametophore formation, whereas in arp3α mutants, gametophores develop from chloronemal filaments, are severely stunted and lack rhizoids (Finka et al., 2008; Harries et al., 2005). Disruption of the ARPC4 gene produces mutants with tip growth defects that are much smaller than wild type. The chloronema to caulonema occurs in these mutants, but caulonemal cells exhibit severe tip growth defects even though actin organization is not significantly disrupted. Gametophores also develop but these are stunted compared with wild type and give rise to rhizoids with similar tip growth defects. Thus, ARPC4 is therefore not required for caulonemal initial cell formation but is essential for tip growth in both caulonemal cells and rhizoids (Perroud & Quatrano, 2006). On the other hand, ARPC1α/1β and ARP3α are essential for tip growth and enable the chloronema to caulonema transition by positively regulating actin polymerization (Finka et al., 2008; Harries et al., 2005).

2.3 | The WAVE/SCAR complex

The BRICK1 subunit of the WAVE/SCAR complex is required for the accumulation of ARPC4 and actin at the tips of apical cells. The phenotype of (Δbrk1) deletion mutants resembles that of arpc4 mutants but is more severe. Cross walls distinguishing chloronema from caulonema are ambiguous, and these cells divide less rapidly than wild type. Like arpc4 mutants, gametophore development initiates, but rhizoids fail to undergo tip growth. It seems plausible that BRICK1 mediates additional interactions with other regulators of tip growth and development (Perroud & Quatrano, 2008).

3 | AUXIN

Auxin regulates essential developmental processes in plants including cell division, expansion and differentiation (reviewed in Vanneste & Friml, 2009). Auxin signaling is
largely dependent on a transcriptional pathway that comprises transport inhibitor response 1/auxin signaling F-box (TIR1/AFB) proteins, auxin/indole-3-acetic acid (AUX/IAA) transcriptional repressors, and auxin response factor (ARF) transcription factors. When bound to TIR1/AFB, auxin promotes the degradation of AUX/IAA proteins via the 26S proteasome, enabling the transcriptional activation of auxin-inducible genes (Dharmasiri et al., 2005; Gray et al., 2001; Kepinski & Leyser, 2005; X. Tan et al., 2007) - (Figure 2). Within plant tissues, spatial distribution patterns of auxin are defined by local auxin biosynthesis and also directional transport mechanisms that are facilitated by PIN-FORMED (PIN) proteins (Barbez et al., 2012; Brumos et al., 2018; Mravec et al., 2009). It is well accepted that auxin can operate antagonistically to cytokinin, and that developmental progression is often reliant on changes in the ratio of auxin to cytokinin within specific cell types (Bishopp et al., 2011; Cheng et al., 2013). Orthologues of the core auxin signaling machinery can be found in all land plant lineages, including _P. patens_ (De Smet et al., 2011).

### 3.1 Auxin biosynthesis

The SHI/STY genes encode transcription factors that regulate auxin biosynthesis (Eklund, Ståldal, et al., 2010). The SHI/STY gene family is represented by two gene members in _P. patens_, PpSHI1 and PpSHI2. PpSHI1 and PpSHI2 are not expressed in chloronemal cells but exhibit strong expression in both caulonemal and rhizoids. Mutants in which either PpSHI1 or PpSHI2 have been disrupted are small and produce fewer caulonemal cells than wild type, which correlates with reduced IAA levels. Conversely, the chloronema to caulonema transition is greatly accelerated when PpSHI1 is ectopically overexpressed and this correlates with enhanced IAA levels. PpSHI1 and PpSHI2 therefore drive local auxin biosynthesis to generate maxima necessary for the differentiation of both caulonemal cells and rhizoids (Eklund, Thelander, et al., 2010). Recent studies have shown that, although chloronemal and caulonemal initial cells can produce auxin, these cells are unable to perceive (or sense) auxin. Thus, low auxin sensing activity appears to act as a pre-requisite for maintaining initial cell identity in these cell types (Thelander et al., 2019). Interestingly, in cells derived from chloronemal or caulonemal initial cells, auxin sensing is restored thus enabling the fundamental processes of cell division, expansion and differentiation. It is not clear how the acquisition of auxin sensing is regulated, but it is clear that auxin is a key driver for cell fate transitions throughout the development of land plants.

### 3.2 Auxin transport

PIN proteins mediate directional auxin transport that is determined by their asymmetric localization within a cell. PIN proteins facilitate both intracellular and intercellular auxin transport and are required to generate auxin concentration gradients that underpin important developmental transitions in plants (Barbez et al., 2012; Mravec et al., 2009). Disruption of auxin transport has been shown to affect root hair formation (Fischer et al., 2006; Ikeeda et al., 2009; Kleine-Vehn et al., 2006; Löfke et al., 2015; Schmidt & Schikora, 2001) and pollen tube growth and development (Z. Ding et al., 2012). Before root hair initiation, PIN proteins are differentially expressed in root hair cells (trichoblasts) and non-hair cells (atrichoblasts) (Löfke et al., 2015). Relatively low PIN expression increases intracellular auxin levels within trichoblasts, and promotes actin-mediated root hair initiation, or bulge formation (Ganguly et al., 2010; Jones et al., 2002; Löfke et al., 2015; Molendijk et al., 2001). By maintaining cellular auxin homeostasis, root hairs can be appropriately spaced for their optimal function. The localization of PIN proteins is reliant on actin-dependent trafficking processes (Grebe...
et al., 2002; Kleine-Vehn et al., 2006). In turn, the organization of actin is under the control of auxin, a phenomenon that is also observed in the brown algae (Nick et al., 2009; Sun et al., 2004). Thus, there is a fundamental link between auxin homeostasis and the cytoskeleton.

An invariable feature of all PIN proteins is the presence of a hydrophilic loop that is flanked by several conserved N- and C-terminal transmembrane domains (Sauer & Kleine-Vehn, 2019). Canonical “long” PINs have longer hydrophilic loops than those of noncanonical “short” PINs. “Long” PINs localize to the plasma membrane, exhibit polar localization patterns and facilitate intercellular auxin transport. On the other hand, non-canonical PINs localize to the endoplasmic reticulum and are believed to be essential for maintaining auxin homeostasis within cells (Adamowski & Friml, 2015).

Four PIN proteins are encoded by the P. patens genome; 3 canonical “long” PINs (PINA-C) and 1 noncanonical “short” PIN (PIND) (Bennett et al., 2014; Viaene et al., 2014). Disruption of P. patens PIN function perturbs auxin export. Changes in PIN localization thus alter the dynamics and transport of auxin within the tissues, leading to a variety of developmental abnormalities. PINA and PINB overexpression enhances auxin export, reduces cellular auxin levels, and delays caulonemata differentiation (Viaene et al., 2014). Conversely, loss of PIN function in long PIN double mutants (pinApinB) reduces auxin export, increases cellular auxin levels, and accelerates the chloronema to caulonema transition. Gametophores also appear earlier in pinApinB mutants than in wild type (Bennett et al., 2014; Viaene et al., 2014). The PIN proteins exhibit a striking polar localization in both chloronemal initial cells and in caulonemal initial cells. However, a reduced level of PIN activity in caulonemal initial cells indicates that relatively high auxin levels must be sustained in these cells to maintain their identity (Figure 3). Thus, the establishment of a PIN-mediated auxin concentration gradient is likely a pivotal event in the chloronema to caulonema transition.

There are three genes encoding Aux/IAA proteins in P. patens; IAA1A, IAA1B, and IAA2 (Paponov et al., 2009). IAA1A and IAA1B protein sequences are nearly identical, and both share 69% identity with IAA2 (Prigge et al., 2010). All three proteins share a conserved EAR motif (LxLxPP) which has been shown to interact with TPL proteins (Causier et al., 2012). Mutants lacking both IAA1B and IAA2 (Δiaa1biaa2) are phenotypically indistinguishable from wild type. However, mutants lacking all three genes (Δaux/iaa) exhibit an accelerated chloronema to caulonema transition, and thus resemble wild type plants treated with auxin (Lavy et al., 2016). The ectopic expression of an EAR motif-deleted version of PpIAA1A in the Δiaa1biaa2 mutant lead to an accelerated chloronema to caulonema transition. Additionally, in the absence of exogenous auxin, the accumulation of auxin-inducible transcripts was higher in these mutants than in wild type. These studies therefore demonstrated that the IAA1A EAR motif contributes to, but is not essential for, Aux/IAA-mediated repression (Tao & Estelle, 2018).

Mutations in the auxin-responsive degron motifs of any of the three Aux/IAA genes results in the generation of noa-resistant (nar) mutants that fail to make caulonemal cells (Prigge et al., 2010). In the absence of auxin, IAA proteins are required for transcriptional repression and are thus negative regulators of the chloronema to caulonema transition.

### 3.4 TIR1/AFB proteins

TIR1/AFB proteins are required for the perception of auxin and operate in conjunction with Aux/IAA transcriptional repressors in angiosperms (Parry et al., 2009). There are four TIR1/AFB homologues in P. patens (PpAFB1-4), and two additional genes (PpXFB1-2) that are more distantly related to their angiosperm counterparts. RNAi-induced silencing of all four PpAFB genes leads to suppression of the chloronema to caulonema transition, which cannot be restored following auxin treatment. RNAi-induced silencing of all six genes has no additive effect, and mutants in which both PpXFB1 and PpXFB2 have been knocked down, are indistinguishable from wild type. PpAFB2 and PpAFB4 have been shown to interact with P. patens Aux/IAA proteins in the presence of auxin (Prigge et al., 2010). Furthermore, ectopic overexpression of PpAFB2 leads to auxin hypersensitivity, and accelerates the formation of caulonemal cells that are longer than those found in wild type (Lavy et al., 2012). Thus, the auxin-mediated chloronema to caulonema transition is mediated by PpAFBs and the Aux/IAA co-receptors (Prigge et al., 2010).
3.5 | ARFs

The ARF proteins are organized into three phylogenetic clades. Clade A ARFs are regulated by miR167, clade B ARFs are regulated by miR390-dependent tasiARFs and miR1219, and clade C ARFs are regulated by miR160 (Plavskin et al., 2016; Plavskin & Timmermans, 2012). Clade A comprises 8 transcription factors (PpARFa1-8) that constitute activating ARFs. Conversely, representatives from Clades B (PpARFb1-4) and C (PpARFc1-2) appear to contribute to the repression of auxin responses (Plavskin & Timmermans, 2012).

It has been shown that repressor and activating ARFs can target the same set of auxin-inducible genes. Reduced levels of repressing ARFs cause levels of activating ARFs to increase. Interestingly, this does not lead to an enhanced auxin response. In fact, the response appears to trigger the recruitment of Aux/IAA proteins to auxin-responsive gene promoters to repress transcription. For that reason, the ectopic overexpression of the activating ARF PpARFa8 generates auxin-resistant mutants with a significant reduction in levels of auxin-inducible gene activation. Ectopic overexpression of the repressor ARF PpARFb4 can reverse the constitutive auxin phenotype of auxin-inducible gene expression line reduces hypersensitivity to auxin. It has been proposed that DGT suppresses PpAFB2 and is essential for the transcriptional response to auxin (Lavy et al., 2012).

5 | ROOT HAIR DEFECTIVE SIX-LIKE (RSL)

Genes encoding RSL transcription factors are characterized by the presence of both a conserved C-terminal basic helix-loop-helix (bHLH) domain and an RSL domain. RSL genes are found extensively in all land plant genomes and also within the charophyte algae (N. D. Pires et al., 2013). Phylogenetic studies have revealed that two classes of RSL genes (Classes I and II) exist in land plants, and that these likely arose in the charophyte lineage, or in the earliest colonizers of land (Bonnot et al., 2019; N. D. Pires et al., 2013). RSL genes regulate the development of epidermal structures, most notably root hairs and rhizoids, but also asexual propagules or secretory hairs in extant land plants (Jang & Dolan, 2011; Jang et al., 2011; Kim & Dolan, 2016; Masucci & Schiefelbein, 1994; B. Menand, Yi, et al., 2007; Proust et al., 2016; K. Yi et al., 2010). In P. patens, RSL genes positively regulate the development of rhizoids, auxillary hairs, and caulonema (Jang & Dolan, 2011; B. Menand, Yi, et al., 2007; Proust et al., 2016). There are seven RSL genes in P. patens; two Class I (PpRSL1 and PpRSL2) and five Class II (PpRSL3-7) genes. Mutants lacking Class I genes, Pprsl1 Pprsl2 double mutants, exhibit an absence of caulonemal cell formation, even in the presence of auxin. Consequently, mutants comprise only chloronemal cells, and gametophores develop from chloronemal cells instead of from caulonemal cells (Jang & Dolan, 2011; Menand, Calder, et al., 2007). Auxin positively regulates the expression of PpRSL1 and PpRSL2, which, when co-expressed ectopically, are sufficient to drive a premature chloronema to caulonema transition. Somewhat strikingly, the expression of PpRSL1 diminishes as the chloronema to caulonema transition proceeds. Exogenous auxin treatment can extend the expression of PpRSL1 throughout the filament, which in turn accelerates the development of caulonemal cells (Jang & Dolan, 2011; Prigge et al., 2010). Thus, the differential regulation of these genes within this type. This phenotype is recapitulated in dgt△ null mutants, which can produce gametophores even in the presence of high auxin concentrations. Additional investigation showed that the expression of a range of auxin-inducible genes was significantly reduced in dgt△ mutants compared with wild type. Furthermore, the generation of a dgt△ null mutation in the PpAFB2 overexpression line reduces hypersensitivity to auxin. It has been proposed that DGT suppresses PpAFB2 and is essential for the transcriptional response to auxin (Lavy et al., 2012).

4 | DIADE OTROPICA (DGT)

Genes encoding the cyclophilin DGT were originally discovered in tomato. Mutants defective in DGT function exhibit a range of developmental perturbations that are associated with altered responses to auxin. It has been shown that DGT modulates changes in PIN localization and consequently polar auxin transport, which are required to generate auxin maxima during aspects of growth and development (Ivanchenko et al., 2015). Using a candidate gene approach, it has been shown that the causative mutations of several nar mutants (described earlier) reside in P. patens DGT. These mutants exhibit reduced sensitivity to auxin and produce fewer caulonomal cells that are shorter than those found in wild
cell-identity-gradient is essential for the chloronema to caulonema transition (Jang & Dolan, 2011).

Class II RSL genes are also required for the chloronema to caulonema transition in *P. patens*. Single knockout mutants of Class II RSL genes did not differ significantly from wild type. However, *Pprsl3* *Pprsl4* double mutants produce fewer caulonemal cells than wild type, resulting in a protonemata composed almost exclusively of chloronemal cells. Interestingly, the expression of Class II RSL genes is affected in *Pprsl1* *Pprsl2* mutants, but only in the presence of auxin; transcript levels of *PpRSL5* and *PpRSL6* increase, whereas those of *PpRSL3* decrease (N. D. Pires et al., 2013). Thus, auxin regulates the expression of both Classes I and II RSL genes, which collectively positively regulate the chloronemal-to-caulonemal transition in *P. patens*.

6 | LOTUS JAPONICUS ROOTHAIRLESS1-LIKE (LRL)

The *LRL* genes encode group XI bHLH transcription factors that are characterized by a conserved LRL domain (N. Pires & Dolan, 2010). *LRL*-related genes positively regulate the development of tip-growing cells with a rooting function, such as rhizoids and root hairs, in a number of evolutionary divergent land plants (Breuninger et al., 2016; Bruex et al., 2012; W. Ding et al., 2009; Karas et al., 2009; Tam et al., 2015).

There are two *LRL*-related genes in *P. patens*; *PpLRL1* and *PpLRL2*. Both genes positively regulate the chloronema to caulonema transition, and are required for the developmental induction of caulonemal cells in response to phosphate starvation (Tam et al., 2015; Wang et al., 2008). *Pplrl1* and *PpLRL2* single mutants produce fewer caulonemal cells than wild type, and *Pplrl1* *PpLRL2* double mutants exhibit a complete absence of caulonemal cells even when subjected to phosphate starvation. Auxin positively regulates the expression of *PpLRL1* and *PpLRL2*, which are predominantly expressed in caulonemal cells that are located in the center of growing protonema. These genes are not expressed in distal regions of the protonema that are almost exclusively occupied by caulonemal cells. Thus, an expression gradient of *PpLRL1* and *PpLRL2* expression appears to be an intrinsic requirement for the chloronema to caulonema transition (Tam et al., 2015). The *PpLRL1/PpLRL2*-mediated cell-identity-gradient is reminiscent of that exhibited by the *P. patens* RSL genes (Jang & Dolan, 2011). In *Arabidopsis*, *AtRHD6* and *AtRSL1* (Karas et al., 2009). However, in *P. patens* no such observation has been made. Thus, *PpLRL1* and *PpLRL2* likely promote the chloronema to caulonema transition independently of the RSL genes (Tam et al., 2015).

7 | LIGHT

Cryptochromes are required for the perception of blue light, which regulates aspects of development in angiosperms, including de-etiolation and flowering (Yu et al., 2010). In *P. patens*, blue light negatively regulates the chloronema to caulonema transition but positively regulates the formation of side branch apical initials that give rise to caulonema. These responses are mediated by the cryptochrome-related genes *PpCRY1a* and *PpCRY1b*. In the presence of blue light, mutants lacking *PpCRY1a* and/or *PpCRY1b* exhibit reduced branching compared with wild type. Furthermore, in the presence of white or blue light, the chloronema to caulonema transition is accelerated in these mutants compared with wild type. The phenotype of *cry1b* single mutants is more severe than that of *cry1a* single mutants, and *cry1a cry1b* mutants are most severely affected (Imaizumi et al., 2002).

Exogenous auxin treatment increases the ratio of caulonemal to chloronemal cells in mutants relative to wild type plants in the presence of white and blue light, but not in red light. At high doses of auxin, *cry1a*, and *cry1b* single mutants phenocopy the low dose response of the *cry1a cry1b* double mutant. Interestingly, in the absence of exogenous auxin, the accumulation of the auxin-inducible transcripts *PpGH3L1* and *PpIAA1* was significantly higher in *cry1a cry1b* mutants compared with wild type. In response to auxin treatment, the accumulation of the *PpIAA1* transcript occurred more rapidly in *cry1a cry1b* mutants compared with wild type. However, the accumulation of the *PpGH3L1* transcript was suppressed in *cry1a cry1b* mutants compared with wild type (Imaizumi et al., 2002). Cryptochrome-mediated inhibition of the chloronema to caulonema transition is thus likely due to a repression of auxin signaling, a similar phenomenon to that observed in *Arabidopsis* (Imaizumi et al., 2002; Xu et al., 2018).

8 | SUGAR

Trehalose-6-phosphate synthases (TPS) catalyze the synthesis of trehalose 6-phosphate (Tre-6P) from glucose 6-phosphate and UDP-glucose and are essential for the regulation of trehalose metabolism in plants. Fluctuations in Tre-6P levels are associated with significant changes in plant growth and development, and perturbations in trehalose metabolism have been linked to the development of root hairs, pollen tubes, and embryos (Eastmond et al., 2002; Gussin et al., 1969; Paul et al., 2018; Van Houtte et al., 2013).

There are six *TPS* genes in *P. patens*; two Class I (*PpTPS1* and *PpTPS2*) and four class II (*PpTPS3-6*) genes (Avonce et al., 2010). Mutants lacking either
(tps1Δ or tps2Δ) or both (tps1Δ tps2Δ) of the Class I genes produce fewer caulonemal cells than wild type, with double mutants most severely affected. In tps1Δ tps2Δ mutants, the chloronemal to caulonemal transition cannot be restored even in the presence of auxin. Furthermore, enhanced repression of caulonema formation occurs following cytokinin treatment, demonstrating that mutants are hypersensitive to cytokinin. Comparative analyses of Tre-6P levels between these mutants suggest that PpTPS1 is likely to be more active than PpTPS2, since the concentration of Tre-6P is significantly lower in tps1Δ mutants than in tps2Δ mutants. Interestingly, low levels of Tre-6P are detectable in tps1Δtps2Δ mutants, but this is likely due to a basal level of TPS activity exhibited by the Class II TPS proteins (Phan et al., 2020).

Tre-6P is required for carbohydrate utilization, and glucose and sucrose induce caulonema formation in wild type P. patens (Phan et al., 2020; Schluepmann et al., 2003). Sugar sensing is not completely abolished in tps1Δ tps2Δ mutants, but these mutants are less responsive to glucose and sucrose treatment than wild type and indeed tps1Δ mutants. Collectively, these findings demonstrate that PpTPS1 and PpTPS2 are required for the perception of both sugars and hormones, but likely act with other factors such as Class II TPS enzymes or SNF1-related kinases (SnRKs) (Phan et al., 2020). This is in line with reports of a link between Tre-6P concentration and sugar availability, as well as Tre-6P mediated inhibition of SnRK1 in Arabidopsis (Lunn et al., 2006; Y. Zhang et al., 2009).

P. patens SnRK1-related genes (PpSNF1a and PpSNF1b) are negative regulators of the chloronema to caulonema growth transition since snf1a snf1b double mutants produce excessive numbers of caulonemal cells. Interestingly, snf1a snf1b double mutants exhibit increased sensitivity to auxin and reduced sensitivity to cytokinin (Thelander et al., 2005). The opposite is true for both tps1Δ mutants and tps1Δtps2Δ mutants, which exhibit reduced sensitivity to auxin treatment and increased sensitivity to cytokinin (Phan et al., 2020). Thus, the developmental phenotypes observed in these mutants are likely due to perturbations in the underlying auxin-cytokinin crosstalk.

9 | GIBBERELLINS

The gibberellin signaling pathway in early divergent land plants has not been defined as such. However, ent-kaurene represents a common precursor for gibberellin biosynthesis that is found in both P. patens and throughout the land plants. The bifunctional diterpene cyclase ent-kaurene synthase (CPS/KS) synthesizes ent-kaurene from geranylgeranyl diphosphate (GGDP) via ent-copalyl diphosphate. Ent-kaurene oxidase (KO) then catalyzes three subsequent steps culminating in the production of the diterpenoid ent-Kaurenoic acid, and further steps lead to the production of bioactive gibberellins (Hayashi et al., 2006; Yamaguchi, 2008).

In P. patens, the absence of either PpCPS/KS or PpKO leads to suppression of the chloronema to caulonema transition. The Ppcps/ks phenotype is complemented by the application of ent-kaurene or ent-kaurenoic acid, and differentiation in wild type is enhanced with the addition of red light. Furthermore, treatment of protonemata with uniconazole, an inhibitor of kaurene oxidase, recapitulates the mutant phenotype. Interestingly, GA3 and GA4 do not appear to be bioactive in P. patens. However, the GA9 methyl ester can promote the chloronema to caulonema transition and can also restore differentiation to the Ppcps/ks mutant. Interestingly, Ppcps/ks mutants are less responsive to auxin than wild type. Thus, it appears that the chloronema to caulonema transition is underpinned by additional and highly complex crosstalk between gibberellin and auxin signaling pathways (Hayashi et al., 2010).

10 | ABOCISIC ACID (ABA)

ABA is a hormone that is required for the regulation of seed dormancy and germination, and to confer tolerance to stresses such as desiccation and freezing (reviewed in Vishwakarma et al., 2017). Abscisic acid insensitive 3 (ABI3) plays a pivotal role in the ABA response throughout all land plants, including P. patens (Khandelwal et al., 2010; Sakata et al., 2010; T. Tan et al., 2017).

There are three ABI3-related genes in P. patens; ABI3A, ABI3B and ABI3C. Triple deletion mutants (Δabi3) exhibit an accelerated chloronema to caulonema transition, and gametophores appear much earlier in these mutants than in wild type. Furthermore, chloronemal branching is inhibited, which is indicative of an enhanced auxin response. Exogenous application of ABA blocks the chloronema to caulonema transition and promotes chloronemal filament branching in both wild type and Δabi3. Under standard growth conditions, endogenous levels of auxin within Δabi3 mutants are significantly higher than those found in wild type. Thus, ABA negatively regulates the chloronema to caulonema transition by antagonizing the auxin response (Zhao et al., 2018).

11 | SUMMARY

The chloronema to caulonema transition represents a fundamental biological transition in both evolutionary and developmental contexts. Caulonemata differentiation is highly
FIGURE 4 Model for the chloronema to caulonema transition in Physcomitrium patens. Auxin positively regulates the chloronema to caulonema transition, and this response is facilitated by root hair defective six-like (RSL) and lotus japonicus roothairless1-like (LRL) transcription factors. Other positive regulators of the chloronema to caulonema transition include Actin Interacting Protein 1 (AIP1), the actin-related protein 2/3 (Arp2/3) complex, glucose and the GA<sub>3</sub> methyl ester (GA<sub>3</sub>‐ME). The chloronema to caulonema transition is negatively regulated by abscisic acid (ABA), cytokinin, blue light, and the SnRK1-related kinases PpSNF1a and PpSNF1b [Color figure can be viewed at wileyonlinelibrary.com]

dependent on auxin-induced remodeling of the actin cytoskeleton but is also regulated by complex hormone crosstalk that overlaps with light and sugar signaling pathways (Figure 4). Interestingly, many studies have revealed that the development of tip growing cells in P. patens, and those of flowering plants, are regulated by common genetic mechanisms. P. patens is highly genetically tractable, largely due to the recent development of innovative techniques designed to identify mutations in sterile mutants generated by forward mutagenesis (X. Ding et al., 2018; Moody et al., 2018). Thus, future studies of the chloronema to caulonema transition will enable fundamental questions to be answered about the processes of stem cell formation, cell expansion, and differentiation across diverse taxa.

ACKNOWLEDGMENT
The work was funded by a Royal Society University Research Fellowship to Laura A. Moody (URF\R1\191310).

CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

AUTHOR CONTRIBUTIONS
Richard Jaeger and Laura A. Moody contributed to the writing of the original manuscript. Laura A. Moody edited the manuscript following suggestions from two anonymous reviewers.

ORCID
Laura A. Moody https://orcid.org/0000-0002-1306-6020

REFERENCES
Adamowski, M., & Friml, J. (2015). PIN-dependent auxin transport: Action, regulation, and evolution. The Plant Cell, 27, 20–32.
Augustine, R. C., Pattavina, K. A., Tüzel, E., Vidali, L., & Bezanilla, M. (2011). Actin interacting protein1 and actin depolymerizing factor drive rapid actin dynamics in Physcomitrella patens. The Plant Cell, 23, 3696–3710.
Augustine, R. C., Vidali, L., Kleinman, K. P., & Bezanilla, M. (2008). Actin depolymerizing factor is essential for viability in plants, and its phosphoregulation is important for tip growth. The Plant Journal, 54, 863–875.
Avonce, N., Wuysts, J., Verschooten, K., Vandesteene, L., & Dijck, P. V. (2010). The Cytophaga hutchinsonii ChTTPS: First characterized bifunctional TPS-TPP protein as putative ancestor of all eukaryotic trehalose biosynthesis proteins. Molecular Biology and Evolution, 27, 359–369.
Barbez, E., Kubéš, M., Rolčík, J., Béziat, C., Pěnčík, A., Wang, B., Rosquete, M. R., Zhu, J., Dobrev, P. I., Lee, Y., Zažímalová, E., Petrášek, J., Geisler, M., Friml, J., & Kleine-Vehn, J. (2012). A novel putative auxin carrier family regulates intracellular auxin homeostasis in plants. Nature, 485, 119–122.
Bennett, T. A., Liu, M. M., Aoyama, T., Bierfreund, N. M., Braun, M., Coudert, Y., Dennis, R. J., O’Connor, D., Wang, X. Y., White, C. D., Decker, E. L., Reski, R., & Harrison, C. J. (2014). Plasma membrane-targeted PIN proteins drive shoot development in a moss. Current Biology, 24, 2776–2785.
Bhosale, R., Giri, J., Pandey, B. K., Giehl, R. F. H., Hartmann, A., Traini, R., Truskina, J., Lee, M. M., Anam, S., Vatén, A., Help, H., El-Showk, S., Scheres, B., Helariutta, K., Mähönen, A. P., Sakakibara, H., & Helariutta, Y. (2011). Phloem-associated proteins drive rapid actin dynamics in Physcomitrella patens. The Plant Cell, 23, 3696–3710.
Bison, A., Lehesranta, S., Vatén, A., Help, H., El-Showk, S., Scheres, B., Helariutta, K., Mähönen, A. P., Sakakibara, H., & Helariutta, Y. (2011). Plasma membrane-targeted cytokinin regulates polar auxin transport and maintains vascular pattern in the root meristem. Current Biology, 21, 927–932.
Bonnot, C., Hetherington, A. J., Champion, C., Breuninger, H., Kelly, S., & Dolan, L. (2019). Neofunctionalisation of basic helix–loop–helix proteins occurred when embryophytes colonised the land. New Phytologist, 223, 993–1008.
Boyce, K. J., Hynes, M. J., Andrianopoulos, A. (2005). The Ras and Rho GTPases genetically interact to co-ordinately regulate cell polarity during development in Penicillium marneffei. Molecular Microbiology, 55, 1487–1501.
Breuninger, H., Thamm, A., Streubel, S., Sakayama, H., Nishiyama, T., & Dolan, L. (2016). Diversification of a transcription factor family led to the evolution of antagonistically acting genetic regulators of root hair growth. Current Biology, 26, 1622–1628.
Brueck, A., Kainkaryam, R. M., Wieckowski, Y., Kang, Y. H., Bernhardt, C., Xia, Y., Zheng, X., Wang, J. Y., Lee, M. M., Benfey, P., Woolf, P. J., & Schiefelbein, J. (2012). A gene regulatory network for root epidermis cell differentiation in Arabidopsis. PLOS Genetics, 8, e1002446.
Brumos, J., Robles, L. M., Yun, J., Vu, T. C., Jackson, S., Alonso, J. M., Stepanova, A. N. (2018). Local auxin
biosynthesis is a key regulator of plant development. Developmental Cell, 47, 306–318.

Carlier, M. F., Laurent, V., Santolini, J., Melki, R., Didry, D., Xia, G. X., Honig, Y., Chua, N. H., & Pantaloni, D. (1997). Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: Implication in actin-based motility. Journal of Cell Biology, 136, 1307–1322.

Causier, B., Lloyd, J., Stevens, L., & Davies, B. (2012). TOPLESS co-repressor interactions and their evolutionary conservation in plants. Plant Signaling & Behavior, 7, 325–328.

Chen, C. Y.-H., Cheung, A. Y., & Wu, H.-M. (2003). Actin-depolymerizing factor mediates Rac/Rop GTPase-regulated pollen tube growth. The Plant Cell, 15, 237–249.

Cheng, Z. J., Wang, L., Sun, W., Zhang, Y., Zhou, C., Su, Y. H., Li, W., Sun, T. T., Zhao, X. Y., Li, X. G., Cheng, Y., Zhao, Y., Xie, Q., & Zhang, X. (2013). Pattern of auxin and cytokinin responses for shoot meristem induction results from the regulation of cytokinin biosynthesis by AUXIN RESPONSE FACTOR3. Plant Physiology, 161, 240–251.

Cove, D. (2005). The moss Physcomitrella patens. Annual Review of Genetics, 39, 339–358.

Cove, D. J., & Ashton, N. W. (1984). The hormonal regulation of gametophytic development in Bryophytes. In A. F. Dryer, & J. G. Duckett (Eds.), Experimental biology of bryophytes (pp. 177–201). Academic Press.

Delwiche, C. F., & Cooper, E. D. (2015). The evolutionary origin of a terrestrial flora. Current Biology, 25, R899–R910.

De Smet, I., Voûü, U., Lau, S., Wilson, M., Shao, N., Timme, R. E., Swarup, R., Kerr, I., Hodgman, C., Bock, R., Bennett, M., Jürgens, G., & Beeckman, T. (2011). Unraveling the evolution of auxin signaling. Physiology, 155, 209–221.

Dharmasiri, N., Dharmasiri, S., & Estelle, M. (2005). The F-box protein TIR1 is an auxin receptor. Nature, 435, 441–445.

Ding, W., Yu, Z., Tong, Y., Huang, W., Chen, H., & Wu, P. (2009). A transcription factor with a bHLH domain regulates root hair development in rice. Cell Research, 19, 1309–1311.

Ding, X., Pervere, L. M., Bascom, C., Bibeau, J. P., Khurana, S., Xia, G. X., Hong, Y., Chua, N. H., & Pantaloni, D. (1997). Actin depolymerizing factor (ADF/cofilin) enhances the rate of filament turnover: Implication in actin-based motility. Journal of Cell Biology, 136, 1307–1322.

Eastmond, P. J., Van Dijken, A. J. H., Spielberg, M., Kerr, A., Tissier, A. F., Dickinson, H. G., Jones, J. D. G., Smeekens, S. C., & Graham, I. A. (2002). Trehalose-6-phosphate synthase 1, which catalyses the first step in trehalose synthesis, is essential for Arabidopsis embryo maturation. Plant Physiology, 29, 225–235.

Eklund, D. M., Ståldal, V., Valsecchi, I., Cierlik, I., Eriksson, C., Hiratsu, K., Ohme-Takagi, M., Sundström, J. F., Thelander, M., Ezcurra, I., & Sundberg, E. (2010). The Arabidopsis thaliana STYLISH3 protein acts as a transcriptional activator regulating auxin biosynthesis. The Plant Cell, 22, 349–363.

Eklund, D. M., Thelander, M., Landberg, K., Ståldal, V., Nilsson, A., Johansson, M., Valsecchi, I., Pederson, E. R. A., Kowalczyk, M., Ljung, K., Ronne, H., & Sundberg, E. (2010). Homologues of the Arabidopsis thaliana SHI/STV/LRP1 genes control auxin biosynthesis and affect growth and development in the moss Physcomitrella patens. Development, 137, 1275–1284.

Finka, A., Saidi, Y., Goloubinoff, P., Neuhaus, J.-M., Zryž, J.-P., & Schaefer, D. G. (2008). The knock-out of ARP3a gene affects F-actin cytoskeleton organization altering cellular tip growth, morphology and development in moss Physcomitrella patens. Cell Motility and the Cytoskeleton, 65, 769–784.

Fischer, U., Ikeda, Y., Ljung, K., Serralbo, O., Singh, M., Heidstra, R., Palme, K., Scheres, B., & Grebe, M. (2006). Vectorial information for Arabidopsis planar polarity is mediated by combined AUX1, EIN2, and GNOM activity. Current Biology, 16, 2143–2149.

Ganguly, A., Lee, S. H., Cho, M., Lee, O. R., Yoo, H., & Cho, H.-T. (2010). Differential auxin-transporting activities of PIN-FORMED proteins in Arabidopsis root hair cells. Plant Physiology, 153, 1046–1061.

Gray, W. M., Kępinski, S., Rouse, D., Leyser, O., & Estelle, M. (2001). Auxin regulates SCFTIR1-dependent degradation of AUX/IAA proteins. Nature, 414, 271–276.

Grebe, M., Friml, J., Swarup, R., Ljung, K., Sandberg, G., Teroulu, M., Palme, K., Bennett, M. J., & Scheres, B. (2002). Cell polarity signaling in Arabidopsis involves a BFA-sensitive auxin influx pathway. Current Biology, 12, 329–334.

Gussin, A. E. S., McCormack, J. H., Waung, L. Y.-L., & Gluckin, D. S. (1969). Trehalose: A new pollen enzyme. Plant Physiology, 44, 1163–1168.

Han, M., Park, Y., Kim, I., Kim, E. H., Yu, T. K., Rhee, S., & Suh, J. Y. (2014). Structural basis for the auxin-induced transcriptional regulation by Aux/IAA17. Proceedings of the National Academy of Sciences of the United States of America, 111, 18613–18618.

Harries, P. A., Pan, A., & Quatrano, R. S. (2005). Actin-related protein2/3 complex component ARPC1 is required for proper cell morphogenesis and polarized cell growth in Physcomitrella patens. The Plant Cell, 17, 2327–2339.

Hayashi, K., Horie, K., Hiwatashi, Y., Kawaide, H., Yamaguchi, S., Hanada, A., Nakashima, T., Nakajima, M., Mander, L. N., Yamane, H., Hasebe, M., & Nozaki, H. (2010). Endogenous diterpenes derived from ent-kaurene, a common gibberellin precursor, regulate protonema differentiation of the moss Physcomitrella patens. Plant Physiology, 153, 1085–1097.
Hayashi, K., Kawaide, H., Notomi, M., Sakigi, Y., Matsuo, A., & Nozaki, H. (2006). Identification and functional analysis of bifunctional ent-kaurene synthase from the moss Physcomitrella patens. FEBS Letters, 580, 6175–6181.

Hiwatashi, Y., Obara, M., Sato, Y., Fujita, T., Murata, T., & Hasebe, M. (2008). Kinesins are indispensable for interdigitation of phragmoplast microtubules in the moss Physcomitrella patens. The Plant Cell, 20, 3094–3106.

Ikeda, Y., Men, S., Fischer, U., Stepanova, A. N., Alonso, J. M., Ljung, K., & Grebe, M. (2009). Local auxin biosynthesis modulates gradient-directed planar polarity in Arabidopsis. Nature Cell Biology, 11, 731–738.

Imai, T., Kadota, A., Hasebe, M., & Wada, M. (2002). Cryptochrome light signals control development to suppress auxin sensitivity in the moss Physcomitrella patens. The Plant Cell, 14, 373–386.

Ivanchenko, M. G., Zhu, J., Wang, B., Medvecka, E., Du, Y., Azzarello, E., Mancuso, S., Megraw, M., Filichkin, S., Dubrovsky, J. G., Friml, J., & Geisler, M. (2015). The cyclophilin DIAGEOTROPICA gene affects auxin transport in both root and shoot to control lateral root formation. Development, 142, 712–721.

Jang, G., & Dolan, L. (2011). Auxin promotes the transition from chloronema to caulonema in moss protonema by positively regulating PpRSL1and PpRSL2 in Physcomitrella patens. New Phytologist, 192, 319–327.

Jang, G., Yi, K., Pires, N. D., Menand, B., & Dolan, L. (2011). RSL genes are sufficient for rhizoid system development in early diverging land plants. Development, 138, 2273–2281.

Jenkins, G. I., & Cove, D. J. (1983). Light requirements for regeneration of protoplasts of the moss Physcomitrella patens. Planta, 157, 39–45.

Jones, M. A., Shen, J.-J., Fu, Y., Li, H., Yang, Z., & Grierson, C. S. (2002). The Arabidopsis Rop2 GTPase is a positive regulator of root hair initiation and tip growth. The Plant Cell, 14, 763–776.

Karas, B., Amyot, L., Johansen, C., Sato, S., Tabata, S., Kawaguchi, M., & Szczyniakowski, K. (2009). Conservation of Lotus and Arabidopsis basic helix-loop-helix proteins reveals new players in root hair development. Plant Physiology, 151, 1175–1185.

Kepinski, S., & Leyser, O. (2005). The Arabidopsis F-box protein TIR1 is an auxin receptor. Nature, 435, 446–451.

Ketelaar, T., Anthony, R. G., & Hussey, P. J. (2004). Green fluorescent protein-mTalin causes defects in actin organization and cell expansion in Arabidopsis and inhibits actin depolymerizing factor’s actin depolymerizing activity in vitro. Plant Physiology, 136, 3990–3998.

Khandelwal, A., Cho, S. H., Marella, H., Sakata, Y., Perroud, P. F., Pan, A., & Quatrano, R. S. (2010). Role of ABA and ABI3 in desiccation tolerance. Science, 327, 546.

Kim, C. M., & Dolan, L. (2016). ROOT HAIR DEFECTIVE SIX-LIKE Class I genes promote root hair development in the grass Brachypodium distachyon. PLoS Genetics, 12, e1006211.

Kleine-Vehn, J., Dhonoukhe, P., Swarup, R., Bennett, M., & Friml, J. (2006). Subcellular trafficking of the Arabidopsis auxin influx carrier AUX1 uses a novel pathway distinct from PIN1. The Plant Cell, 18, 3171–3181.

Korasick, D. A., Westfall, C. S., Lee, S. G., Nanao, M. H., Dumas, R., Hagen, G., Guilfoyle, T. J., Jez, J. M., & Strader, L. C. (2014). Molecular basis for AUXIN RESPONSE FACTOR protein interaction and the control of auxin response repression. Proceedings of the National Academy of Sciences of the United States of America, 111, 5427–5432.

Lavy, M., Prigge, M. J., Tao, S., Shain, S., Kuo, A., Kirchsteiger, K., & Estelle, M. (2016). Constitutive auxin response in Physcomitrella reveals complex interactions between Aux/IAA and ARF proteins. eLife, 5, e13325.

Lavy, M., Prigge, M. J., Tigi, K., & Estelle, M. (2012). The cyclophilin DIAGEOTROPICA has a conserved role in auxin signaling. Development, 139, 1115–1124.

Löfke, C., Scheuring, D., Dünser, K., Schöller, M., Luschnig, C., & Kleine-Vehn, J. (2015). Tricho- and atrichoblast cell files show distinct PIN2 auxin efflux carrier exploitations and are jointly required for defined auxin-dependent root organ growth. Journal of Experimental Botany, 66, 5103–5112.

Lunn, J. E., Feil, R., Hendriks, J. H. M., Gibon, Y., Morcuende, R., Osuna, D., Scheible, W. R., Carillo, P., Hajirezaei, M. R., & Stitt, M. (2006). Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADP glucose pyrophosphorylase and higher rates of starch synthesis in Arabidopsis thaliana. Biochemical Journal, 397, 139–148.

Masucci, J. D., & Schiefelbein, J. W. (1994). The rhd6 mutation of Arabidopsis thaliana alters root-hair initiation through an auxin- and ethylene-associated process. Plant Physiology, 106, 1335–1346.

Mathur, J., Mathur, N., Kernebeck, B., Hülskamp, M. (2003). Mutations in actin-related proteins 2 and 3 affect cell shape development in Arabidopsis. The Plant Cell, 15, 1632–1645.

Menand, B. B., Calder, G., & Dolan, L. (2007). Both chloronemal and caulonemal cells expand by tip growth in the moss Physcomitrella patens. Journal of Experimental Botany, 58, 1843–1849.

Menand, B., Yi, K., Jouannic, S., Hoffmann, L., Ryan, E., Linstedt, P., Schaefer, D. G., & Dolan, L. (2007). An ancient mechanism controls the development of cells with a rooting function in land plants. Science, 316, 1477–1480.

Molendijk, A. J., Bischoff, F., Rajendrakumar, C. S. V., Friml, J., Braun, M., Gilroy, S., & Palme, K. (2001). Arabidopsis thaliana Rop GTPases are localized to tips of root hairs and control polar growth. EMBO Journal, 20, 2779–2788.

Moody, L. A., Kelly, S., Coudert, Y., Nimchuk, Z. L., Harrison, C. J., & Langdale, J. A. (2018). Somatic hybridization provides segregating populations for the identification of causative mutations in sterile mutants of the moss Physcomitrella patens. New Phytologist, 218, 1270–1277.

Mravec, J., Skůpa, P., Bailly, A., Hoyerová, K., Křeček, P., Bielach, A., Petrášek, J., Zhang, J., Gaykova, V., Sterhof, Y. D., Dobrev, P. I., Schwarzerová, K., Rolčík, J., Seifertová, D., Luschnig, C., Benková, E., Žámalová, E., Geisler, M., & Friml, J. (2009). Subcellular homeostasis of phytohormone auxin is mediated by the ER-localized PIN5 transporter. Nature, 459, 1136–1140.

Nanao, M. H., Vinos-Poyo, T., Brunoud, G., Thévenon, E., Mazzoleni, M., Mast, D., Lainé, S., Wang, S., Hagen, G., Li, H., Guilfoyle, T. J., Parcy, F., Vernoux, T., & Dumas, R. (2014). Structural basis for oligomerization of auxin transcriptional regulators. Nature Communications, 5, 3617.
Nick, P., Han, M.-J., & An, G. (2009). Auxin stimulates its own transport by shaping actin filaments. *Plant Physiology*, 151, 155–167.

Paponov, I. A., Teale, W., Lang, D., Paponov, M., Reski, R., Rensing, S. A., & Palme, K. (2009). The evolution of nuclear auxin signalling. *BMC Evolutionary Biology*, 9, 1–16.

Parry, G., Calderon-Villalobos, L. L., Prigge, M., Peret, B., Dharmasiri, S., Itoh, H., Lechner, E., Gray, W. M., Bennett, M., & Estelle, M. (2009). Complex regulation of the TIR1/AFB family of auxin receptors. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 22540–22545.

Paul, M. J., Gonzalez-Urrieta, A., Griffiths, C. A., & Hassani-Pak, K. (2018). The role of trehalose 6-phosphate in crop yield and resilience. *Plant Physiology*, 177, 12–23.

Perroud, P. F., & Quatrano, R. S. (2006). The role of ARPC4 in tip growth and alignment of the polar axis in filaments of *Physcomitrella patens*. *Cell Motility and the Cytoskeleton*, 63, 162–171.

Perroud, P. F., & Quatrano, R. S. (2008). BRICK1 is required for apical cell growth in filaments of the moss *Physcomitrella patens* but not for gametophore morphology. *The Plant Cell*, 20, 411–422.

Phan, T. L. C. H. B., Delorge, I., Avonce, N., & Van Dijck, P. (2020). Functional Characterization of Class I Trehalose Biosynthesis Genes in *Physcomitrella patens*. *Frontiers of Plant Science*, 10, 1694.

Pires, N., & Dolan, L. (2010). Early evolution of bHLH proteins in plants. *Plant Signaling & Behavior*, 5, 911–912.

Pires, N. D., Yi, K., Breuning, H., Catarino, B., Menand, B., & Dolan, L. (2013). Recruitment and remodeling of an ancient gene regulatory network during land plant evolution. *Proceedings of the National Academy of Sciences of the United States of America*, 110, 9571–9576.

Plavskin, Y., Nagashima, A., Perroud, P. F., Hasebe, M., Quatrano, R. S., Atwal, G. S., & Timmermans, M. C. P. (2016). Ancient trans-Acting siRNAs Confer Robustness and Sensitivity onto the Auxin Response. *Developmental Cell*, 36, 276–289.

Plavskin, Y., & Timmermans, M. C. P. (2012). Small RNA-regulated networks and the evolution of novel structures in plants. *Cold Spring Harbor Symposia on Quantitative Biology*, 77, 221–233.

Prigge, M. J., Lavy, M., Ashton, N. W., & Estelle, M. (2010). *Physcomitrella* patens auxin-resistant mutants affect conserved elements of an auxin-signaling pathway. *Current Biology*, 20, 1907–1912.

Proust, H., Honkanen, S., Jones, V. A. S., Morieri, G., Prescott, H., Kelly, S., Ishizaki, K., Kohchi, T., & Dolan, L. (2016). RSL Class I genes controlled the development of epidermal structures in the common ancestor of land plants. *Current Biology*, 26, 93–99.

Rensing, S. A., Goffinet, B., Meyberg, R., Wu, S. Z., & Bezanilla, M. (2020). The moss *Physcomitrium (Physcomitrella) patens*: A model organism for non-seed plants. *The Plant Cell*, 32, 1361–1376.

Rounds, C. M., & Bezanilla, M. (2013). Growth mechanisms in tip-growing plant cells. *Annual Review of Plant Biology*, 64, 243–265.

Sauer, M., & Kleine-Vehn, J. (2019). PIN-FORMED and PIN-LIKES auxin transport facilitators. *Dev. 146*, dev168088.
Vidali, L., Burkart, G. M., Augustine, R. C., Kerdaev, E., Tüzel, E., & Bezanilla, M. (2010). Myosin XI is essential for tip growth in Physcomitrella patens. The Plant Cell, 22, 1868–1882.

Vidali, L., Van Gisbergen, P. A. C., Guérin, C., Franco, P., Li, M., Burkart, G. M., Augustine, R. C., Blanchoin, L., & Bezanilla, M. (2009). Rapid formin-mediated actin-filament elongation is essential for polarized plant cell growth. Proceedings of the National Academy of Sciences of the United States of America, 106, 13341–13346.

Vishwakarma, K., Upadhyay, N., Kumar, N., Yadav, G., Singh, J., Mishra, R. K., Kumar, V., Verma, R., Upadhyay, R. G., Pandey, M., & Sharma, S. (2017). Abscisic acid signaling and abiotic stress tolerance in plants: A review on current knowledge and future prospects. Frontiers of Plant Science, 20, 161.

Wang, Y., Secco, D., & Poirier, Y. (2008). Characterization of the PHO1 gene family and the responses to phosphate deficiency of Physcomitrella patens. Plant Physiology, 146, 646–656.

Xu, F., He, S., Zhang, J., Mao, Z., Wang, W., Li, T., Hua, J., Du, S., Xu, P., Li, L., Lian, H., & Yang, H. Q. (2018). Photoactivated CRY1 and phyB Interact Directly with AUX/IAA Proteins to Inhibit Auxin Signaling in Arabidopsis. Molecular Plant, 11, 523–541.

Yamaguchi, S. (2008). Gibberellin Metabolism and its Regulation. Annual Review of Plant Biology, 59, 225–251.

Yi, K., Menand, B., Bell, E., & Dolan, L. (2010). A basic helix-loop-helix transcription factor controls cell growth and size in root hairs. Nature Genetics, 42, 264–267.

Yi, P., & Goshima, G. (2020). Rho of Plants GTPases and cytoskeletal elements control nuclear positioning and asymmetric cell division during Physcomitrella patens branching. Current Biology, 14, P2860–P2868.

Yu, X., Liu, H., Klejnot, J., & Lin, C. (2010). The cryptochrome blue light receptors. Arab. B. 8, e0135.

Zhang, C., Simpson, R. J., Kim, C. M., Warthmann, N., Delhaize, E., Dolan, L., Byrne, M. E., Wu, Y., & Ryan, P. R. (2018). Do longer root hairs improve phosphorus uptake? Testing the hypothesis with transgenic Brachypodium distachyon lines overexpressing endogenous RSL genes. New Phytologist, 217, 1654–1666.

Zhao, M., Li, Q., Chen, Z., Lv, Q., Bao, F., Wang, X., & He, Y. (2018). Regulatory mechanism of ABA and ABI3 on vegetative development in the moss Physcomitrella patens. International Journal of Molecular Sciences, 19, 2728.

How to cite this article: Jaeger, R., Moody, L. A. (2021). A fundamental developmental transition in Physcomitrium patens is regulated by evolutionarily conserved mechanisms. Evolution & Development, e12376. https://doi.org/10.1111/ede.12376