Tansley review

Mosaic modularity: an updated perspective and research agenda for the evolution of vascular cambial growth

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Summary

Secondary growth from a vascular cambium, present today only in seed plants and isoetalean lycophytes, has a 400-million-yr evolutionary history that involves considerably broader taxonomic diversity, most of it hidden in the fossil record. Approaching vascular cambial growth as a complex developmental process, we review data from living plants and fossils that reveal diverse modes of secondary growth. These are consistent with a modular nature of secondary growth, when considered as a tracheophyte-wide structural feature. This modular perspective identifies putative constituent developmental modules of cambial growth, for which we review developmental anatomy and regulation. Based on these data, we propose a hypothesis that explains the sources of diversity of secondary growth, considered across the entire tracheophyte clade, and opens up new avenues for exploring the origin of secondary growth. In this hypothesis, various modes of secondary growth reflect a mosaic pattern of expression of different developmental–regulatory modules among different lineages. We outline an approach that queries three information systems (living seed plants, living seed-free plants, and fossils) and integrates data on developmental regulation, anatomy, gene evolution and phylogeny to test the mosaic modularity hypothesis and its implications, and to inform efforts aimed at understanding the evolution of secondary growth.

I. Introduction

Secondary growth refers to the addition of tissues laterally, by the activity of secondary meristems (cambia). Secondary growth from a vascular cambium (hereafter, secondary growth) produces vascular tissues: secondary xylem (wood) and secondary phloem (Fig. 1). While wood formation is most conspicuously associated with the arborescent growth habit, many so-called herbaceous plants undergo secondary growth. Stressing the importance of secondary growth to plant biology, Barghoorn (1964) included cambial growth on his list of fundamental advances in plant evolution. While mechanical support provided by secondary xylem enabled

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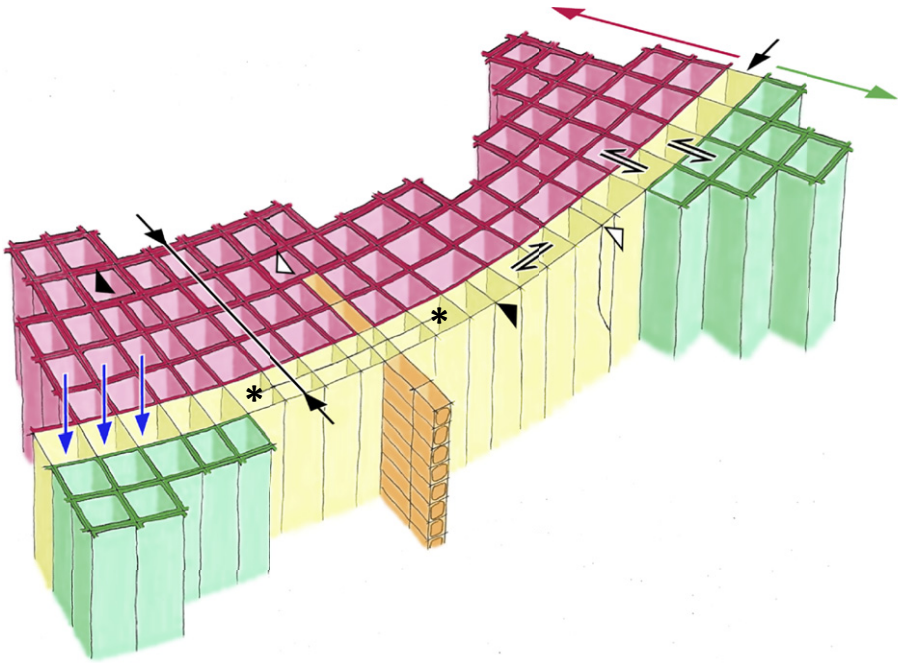

Fig. 1 Idealized diagram of vascular cambial region summarizing some of the features, processes and interactions in secondary growth; the vascular cambium is represented as a single layer of fusiform initials (yellow, black arrow) with exaggerated thickness. Periclinal divisions of fusiform initials (between asterisks) add new layers of secondary tissues that differentiate into secondary xylem (red) and secondary phloem (green), producing radial files of cells (e.g. between the two collinear black arrows). Secondary xylem differentiates centripetally (red arrow) and secondary phloem (green arrow) centrifugally. Symmetric anticlinal divisions of fusiform initials generate additional radial files of cells of the axial system (black arrowheads). Asymmetric anticlinal divisions of cambial initials (white arrowheads) generate the radial system (vascular rays; orange); the asymmetric division at right separates a ray initial (right) from a fusiform initial (left). Tangential signaling between fusiform initials (⇋) synchronizes periclinal divisions around the cambial layer. Radial signaling between the cambium and differentiating secondary tissues (⇋) regulates radial patterning. Polar transport of hormones (auxin, GA) through the cambial layer and region (blue arrows) maintains meristematic identity, promotes periclinal divisions, and regulates differentiation in the secondary xylem and secondary phloem.

the evolution of larger sporophytes, growing evidence indicates that secondary growth evolved initially as an innovation for improved water conduction in small plants with simple organization (Gerrienne *et al.*, 2011; Hoffman & Tomescu, 2013; Strullu-Derrien *et al.*, 2014).

Secondary growth occurs in only two clades among living plants: seed plants, which have explored this developmental feature to a tremendous extent, and *Isoetes*, the sole living relative of the arborescent lepidodendrid lycophytes (Gifford & Foster, 1989) (Supporting Information Notes S1). The fossil record reveals that secondary growth is much more widely spread phylogenetically (Cichan & Taylor, 1990; Rothwell *et al.*, 2008), having evolved in multiple extinct euphyllophyte lineages – progymnosperms (Arnold, 1940; Beck, 1976), Stenokoleales (Beck & Stein, 1993; Momont *et al.*, 2016), sphenopsids (Cichan & Taylor, 1983; Cichan, 1985), rhacophytalean and zygopterid ferns (Andrews & Phillips, 1968; Dennis, 1974), and cladoxylopsids (Arnold, 1940; Meyer-Berthaud *et al.*, 2004). Secondary growth also extends down the phylogenetic tree close to the base of the euphyllophyte clade, *c.* 409 Myr ago (Ma) (Gerrienne *et al.*, 2011; Hoffman & Tomescu, 2013; Gensel, 2018).

The deep origins and broad taxonomic presence of secondary growth by Devonian–Carboniferous times inform about evolutionary tempo, but the mode of evolution of secondary growth is less well understood. Nevertheless, the fossil record reveals several patterns: secondary growth is present in both lycophytes and euphyllophytes, the two major tracheophyte clades that diverged in the Silurian (> 425 Ma); within euphyllophytes, secondary growth is present in multiple lineages and at deep nodes; secondary growth is also known deep in the lycophyte fossil record (377 Ma or earlier; Andrews *et al.*, 1971); and some regulatory mechanisms associated with secondary growth are shared across all tracheophytes (Rothwell *et al.*, 2008). These observations generate a series of questions regarding the evolution of secondary growth: Is secondary growth in lycophytes and euphyllophytes homologous or homoplastic? If homologous, had the common ancestor of lycophytes and euphyllophytes already evolved secondary growth, or only the regulatory potential for secondary growth (deep homology)? If homoplastic, is secondary growth in lycophytes and euphyllophytes an instance of parallelism or convergence (*sensu* Scotland, 2011)? Similar questions apply within the euphyllophyte clade to instances of secondary growth in diverse lineages.

To provide an integrative framework and spark renewed impetus toward addressing such questions, here we approach secondary growth as a tracheophyte-wide attribute of development and structure. In this context, we introduce a new perspective on secondary growth as a modular assemblage of developmental processes and we review evidence that supports the modular nature of secondary growth. Together, these suggest a working hypothesis, which explains the various types of secondary growth seen across the tracheophyte clade, as the result of mosaic expression of developmental-regulatory modules among different lineages. We further

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formulate a three-pronged approach for testing this hypothesis and addressing the origin and evolution of secondary growth.

II. Secondary growth as an assemblage of developmental modules

1. Is the search for modularity in secondary growth justifiable?

Complex developmental processes are not always cleanly reduced to constituent parts, and their regulatory mechanisms usually form highly integrated systems. Nevertheless, currently there is agreement that modularity represents a real property of these systems. For morphological traits, modularity occurs in developmental, genetic, functional and evolutionary contexts (Klingenberg, 2008). Developmental modularity can be present at different levels of organization, including that of underlying regulatory mechanisms, as demonstrated empirically in several cases (Bissell & Diggle, 2010; Etchells *et al.*, 2013; Minelli, 2017). In a prime example, which emphasizes the integration of data from developmental biology and paleontology, Wu *et al.* (2018) identified a series of morpho-regulatory modules responsible for the developmentally of the avian feather. They demonstrated that deployment of these modules in different combinations produces different types of dermal appendages that are seen in extant birds and extinct feathered dinosaurs, all of which are thought to have evolved from archosaur scales.

Thus, approaching secondary growth as a modular assemblage of developmental processes is justified biologically. Furthermore, in addressing the evolution of developmental modules it is conceptually advantageous to individuate them according to the specific roles they perform in the production of organismal morphology (Austin & Nuño de la Rosa, 2018). Therefore, treating secondary growth as a modular assemblage of developmental processes is also desirable epistemically. However, is there evidence that secondary growth is a modular assemblage and, if so, how might constituent modules be identified? According to Klingenberg (2008), modules are sets of traits that are internally integrated by mutual interactions, but are relatively independent from other modules. Thus, to answer these questions we need to identify aspects of secondary growth that exhibit developmental and regulatory independence. Experiments and observations on living and fossil plants provide a wealth of relevant data, summarized below, that highlight processes of secondary growth that are uncoupled developmentally, suggesting corresponding putative regulatory modules.

2. Evidence for developmental-regulatory modules

In seed plant stems, assembly of the vascular cambium as a continuous meristematic layer involves sectors of residual procambium, sandwiched between primary xylem and phloem of the cauline vascular bundles (fascicular cambium), as well as sectors recruited from mature pith ray tissue (interfascicular cambium) (Fig. 2a). Zhu *et al.* (2018) demonstrated that cambial growth is initiated and proceeds even if interfascicular cambium sectors are not specified (Fig. 2b). This suggests that *specification of interfascicular cambium* from pith ray cells *may be independent from other cambial growth processes* in terms of regulation. When interfascicular cambium specification is repressed, the geometry of secondary tissues is reminiscent of cambial variants with dissected xylem seen in lianas (e.g. *Alicia*; Angyalossy *et al.*, 2015) (Fig. 3a) and of the vascular segments of the Carboniferous seed fern *Medullosa* (Dunn *et al.*, 2003) (Fig. 3b). The lobed secondary xylem in the roots of Permian glossopterid seed ferns (*Vertebraria*; Decombeix *et al.*, 2009) (Fig. 3c) and the Devonian cladoxylopsid *Xinicaulis* (Xu *et al.*, 2017) reflects similar repression of interfascicular cambium specification. This indicates that *specification of interfascicular cambium is independent from other cambial growth processes in shoots as well as roots*, and suggests that *similar regulatory independence is present in distinct plant lineages*.

Periclinal divisions in the cambium are the hallmark developmental process, driving force and ultimate cause of secondary growth. These divisions, in a plane parallel with the surface of the host organ (Fig. 1), are also known as additive or tangential divisions and add new cell layers parallel to the cambium, leading to an increase in girth. In most plants the meristematic activity of the vascular cambium is indeterminate, indicating the presence of regulatory mechanisms with homeostatic role. However, evidence that vascular cambial growth can be determinate (Fig. 2c) comes from fossil plants with limited secondary growth – such as the Carboniferous sphenopsid *Sphenophyllum* (Eggert & Gaunt, 1973) (Fig. 3d) – and from occurrences of successive cambia and polyxylic development across the spermatophyte clade, including living cycads (Fig. 3e), gnetales and angiosperms, and extinct corystosperms (Chamberlain, 1935; Bodnar & Coturel, 2012; Angyalossy *et al.*, 2015; Pace *et al.*, 2018). Additionally, evidence from *Arabidopsis* suggests that cell division in the cambium and vascular organization are genetically separable (Etchells *et al.*, 2013). All this evidence indicates that *a regulatory module responsible for homeostasis of periclinal divisions can be switched on and off independently of other developmental processes*.

The vascular cambium of seed plants is bifacial, producing secondary tissues that differentiate both centripetally (secondary xylem) and centrifugally (secondary phloem) (Fig. 1). The identity of these tissues is determined by a regulatory program that controls radial polarity across the cambial zone (Du & Groover, 2010; Ursache *et al.*, 2013; Bhalerao & Fischer, 2016). The fossil record has revealed vascular plant lineages – lepidodendrid lycophytes, zygopterid ferns (Fig. 3f), and possibly also cladoxylopsids, rhacophytaleans and stenokolealeans – with unifacial vascular cambium (Fig. 2d) producing exclusively secondary xylem and no secondary phloem (Cichan & Taylor, 1990). These occurrences suggest that *regulation of radial polarity in secondary tissues is independent of other regulatory aspects of secondary growth*. Consistent with this interpretation, Bossinger & Spokevicius (2018) demonstrated that secondary xylem and phloem differentiation is controlled independently in the cambial zone.

Typical secondary growth produces even thicknesses of secondary tissues. Contrasting this regular pattern, cambial variants with furrowed xylem (phloem arcs or wedges) documented in several angiosperm families (Angyalossy *et al.*, 2015; Fig. 3g,h) show differential expansion of the cambium and different

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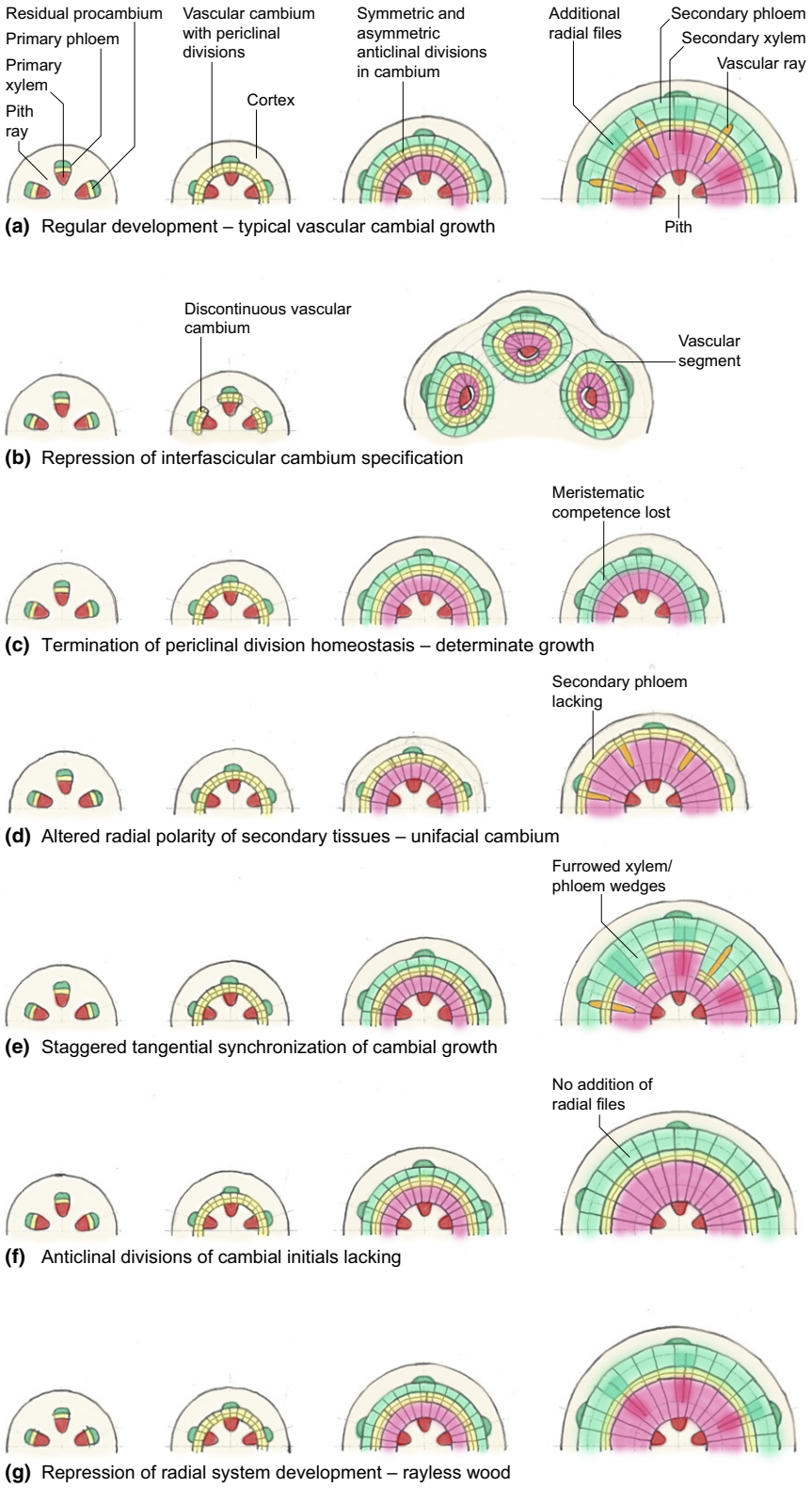

Fig. 2 Modes of secondary growth identified in extant or extinct tracheophytes that support the modular nature of secondary growth; refer also to Figs 3 and 4 – for (b): Fig. 3(a, b); for (c): Fig. 3(d, e); for (d): Fig. 3(f); for (e): Fig. 3(g, h); for (f): Fig. 3(d, f, i); for (g): Fig. 4(a,b). Color key: beige, primary tissues (pith, cortex, pith rays); red, primary xylem; pink, secondary xylem (with darker areas laid down following symmetric anticlinal division); yellow, vascular cambium; light green, secondary phloem (with darker areas laid down following symmetric anticlinal division); dark green, primary phloem; orange, vascular rays; presence/absence of symmetric anticlinal divisions not emphasized in (b–d); ray presence/absence not emphasized in (b, c).

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proportions of secondary xylem vs phloem, between adjacent radial sectors (Fig. 2e). These result from differences in the amount of secondary tissue that the cambium produces centrifugally and centripetally. In turn, these suggest discontinuity in the circumferential synchronization of cambial divisions and radial patterning, between such sectors with differential development. Whether these discontinuities reflect local differences within the cambial layer or more broadly reaching differentiation of sectorial developmental domains, they suggest that *tangential synchronization of developmental processes within the vascular cambium is controlled by an independent regulatory module*.

The circumference of the vascular cambium increases by anticlinal divisions oriented perpendicular to the surface of the host organ (also known as multiplicative of radial divisions; Fig. 1), to accommodate the thickness of secondary xylem added centripetally. These divisions, which add new radial cell files in the secondary tissues, are absent in a few extinct lineages (Fig. 2f) – sphenopsids (*Sphenophyllum*, *Rotafolia*) (Fig. 3d,i), rhacophytalean and zygopterid ferns (Fig. 3f) – which suggests that *regulation of anticlinal cambial divisions may represent an independent module* among processes that control secondary growth.

Specialized asymmetric anticlinal divisions of cambial cells produce ray initials, generating the system of vascular rays (Fig. 1). This radial tissue system, which along with the axial system (consisting primarily of longitudinal conducting cells) defines the secondary tissues of most plants, is repressed in several angiosperms (Carlquist, 2015) (Fig. 4a,b). This suggests that the radial system is not indispensable for development and functioning of secondary tissues and is under independent regulation. Importantly, Lev-Yadun (1994; Fig. 4c,d) provided direct experimental evidence that *differentiation of the radial and axial systems of secondary tissues is uncoupled developmentally and controlled by independent regulatory programs*.

Another potential example of modularity is given by monocots. Monocots lack a typical vascular cambium, but some species have evolved a lateral meristem. This monocot cambium does not produce wood and, instead, makes secondary vascular bundles embedded within ground tissue (Fig. 3j). RNA sequencing was recently used to compare gene expression in the monocot cambium and its recent derivatives with the cambium and differentiating xylem of *Populus* and *Eucalyptus* (Zinkgraf *et al.*, 2017). The results showed that the monocot cambium expresses some of the same key regulators as the vascular cambium, suggesting that lateral meristems can evolve or can be reactivated as developmental pathways through recruitment or reactivation of expression of key meristematic genes. The monocot cambium also illustrates a certain degree of modularity of functions, in that the lateral meristem functions of these cambia are uncoupled from the type of tissues they produce (e.g. vascular bundles vs wood).

The evidence summarized here indicates that multiple processes within secondary growth are partly independent from each other, supporting the view that *secondary growth is a modular assemblage of developmental processes*. Combining what we know about meristematic growth with this evidence for processes uncoupled developmentally, we can deconstruct secondary growth into putative developmental-regulatory modules: specification of cambial identity leading to assembly of the cambial layer and initiation of cambial activity; control of the orientation of periclinal divisions of cambial initials (fusiform initials); homeostasis of meristematic activity in the cambium (growth in-/determinacy); radial patterning of secondary tissue identity (unifacial/bifacial cambia); circumferential synchronization of periclinal divisions; control of the orientation of anticlinal divisions of cambial initials; and asymmetric division of cambial initials generating the radial system (vascular rays). Below, we review these developmental processes of vascular cambial growth, examining their associated anatomical features and molecular–genetic regulation. This approach allows an appreciation of both their *regulatory integration* – these processes interact and may have overlapping regulatory mechanisms – and the potential for *modular deployment*. Because knowledge of secondary growth regulation comes exclusively from stems of living seed plants (overwhelmingly angiosperms), our discussions focus primarily on the typical seed plant stem, and much of the information is based on a few model species (notably *Arabidopsis* and poplar).

3. Assembly of the cambial layer and maintenance of meristematic identity

Developmental anatomy Secondary growth starts with assembly of the vascular cambium as a continuous meristematic layer (Box 1). This layer includes meristematic sectors of residual procambium (fascicular cambium; Fig. 2a) located between the xylem and phloem of primary vascular bundles. Initiation of cambial activity in these sectors is marked by periclinal divisions, which begin just before cessation of elongation, as the last tracheary elements in the primary xylem mature (Eames & MacDaniels, 1947). Fascicular cambium sectors are separated by fully differentiated parenchyma cells of pith rays and assembly of a continuous cambial layer requires recruitment into this layer of pith ray cells that form cell files tangentially continuous with the fascicular cambium sectors. Formation of these cambial sectors (interfascicular cambium) between fascicular cambium sectors involves *de novo* specification of meristematic identity in mature pith ray cells. This process starts in cells adjacent to the fascicular cambium and progresses toward the center of the pith ray.

Regulation The fascicular cambium sectors are continuous, physically and ontogenetically, with the procambium strands specified during the patterning of primary meristems at the shoot apex. Regulated movement of auxin is a fundamental process underlying vascular strand development and polarity of procambial and cambial cells (Dengler, 2001). Procambial strands are specified, at the apical meristem, as pathways of highest concentration of basipetal auxin transport. The vascular cambium, too, is characterized by high auxin concentrations (Tuominen *et al.*, 1997; Björklund *et al.*, 2007) and basipetal auxin transport (Snow, 1935; Lachaud & Bonnemain, 1984; Agusti *et al.*, 2011). Together, these observations suggest that continuity of basipetal auxin transport is required for maintenance of meristematic identity in the residual procambium and for assembly of the vascular cambium. However, only a few cambium-specific genes appear to depend directly on

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Box 1 Structure of the vascular cambium.

Two opposing perspectives on the nature of the cambium have been considered, historically. One views the cambium as a uniseriate meristematic layer flanked on either side by layers of derivatives (xylem and phloem mother cells) that divide periclinally a few times before maturing (e.g. Bailey, 1943; Eames & MacDaniels, 1947; Bannan, 1955, 1968). Alternatively, the cambium has been viewed as a multiseriate zone of initials (cambial zone), the layers of which maintain meristematic identity for some time (e.g. Evert, 1963; Catesson, 1964; Philipson *et al.*, 1971). However, across the taxonomic breadth of woody plants, the cambium encompasses a range of structural configurations. Philipson *et al.* (1971) pointed to differences between conifers, among which most species seem to support the uniseriate cambium view, and angiosperms, some of which seem to fit the multiseriate cambium view. Studies by Catesson (1964) and Gahan (1989) on rates of cell division across the cambial region support Klekowski's (1988) view of this region as consisting of layers with different levels of meristematic activity – a layer characterized by slow cell divisions flanked by regions within which cell division is rapid but limited. Thus, the two opposing views may represent end terms of a continuum. The identification of cell–cell signaling mechanisms coordinating the cambium (e.g. TDIF: Ito *et al.*, 2006) would suggest that signaling is more fundamental than these anatomical conceptual frameworks. Nevertheless, the question still lingers, with a recent study (Bossinger & Spokevicius, 2018) demonstrating a single layer of true initials in the poplar cambium, by *in vivo* single-cell transformation. This study also showed that initials can be lost and replaced, suggesting that initial identity is based on position and not lineage.

auxin signaling (Nilsson *et al.*, 2008; Agusti *et al.*, 2011). The direction of auxin transport also determines the orientation of fusiform initials in the cambium, as demonstrated by experiments where the auxin flow direction was constrained by girdling of helical strips of cambium (MacDaniels & Curtis, 1930; Zagorska-Marek & Little, 1986). Transporting auxin, fusiform initials develop a longitudinal polarization of PIN auxin transporters that they maintain even upon excision, as shown in cambium patches grafted into the stock cambium with rotated orientation (Thair & Steeves, 1976).

Given the continuity of procambium and vascular cambium, it should not be a surprise that genes involved in regulation of the latter are related to those active in the former, and that similarities in regulation exist between shoot apical meristem and vascular cambium (Schrader *et al.*, 2004; Groover *et al.*, 2006; Du & Groover, 2010; Agusti *et al.*, 2011; Gursanscky *et al.*, 2016). A feed-forward loop that involves HD-ZIP III genes, auxin, PIN and the auxin response factor MP/ARF5 is essential for the formation of procambium strands at the shoot apical meristem in *Arabidopsis* (Jouannet *et al.*, 2015; Müller *et al.*, 2016). These same regulatory components, carrying similar roles in the functioning or establishment of the vascular cambium in *Populus*, suggest that a conserved HD-ZIP III–auxin–PIN–MP/ARF5 signaling pathway is shared between procambium formation and vascular cambium establishment (Zhu *et al.*, 2018).

The mechanism for recruitment of pith ray cells into the interfascicular cambium is incompletely understood. Periclinal divisions of pith ray cells start at locations adjacent to the fascicular cambium and lateral signaling from the latter is probably involved (Little *et al.*, 2002; Sehr *et al.*, 2010; Mazur *et al.*, 2014). In the early stages of cambium assembly, while pith ray cells are being recruited into the cambial pathway, periclinal divisions are already underway in the residual procambium (Sehr *et al.*, 2010). Auxin diffusing tangentially from the basipetal flux that transits the fascicular cambium has been suggested as a factor responsible for interfascicular cambium formation (Little *et al.*, 2002; Mazur *et al.*, 2014). Signals other than auxin but associated with auxin-dependent activity in the fascicular cambium may also be responsible for recruitment of pith ray cells into the interfascicular cambium (Agusti *et al.*, 2011; Kucukoglu *et al.*, 2017). Sehr *et al.* (2010) proposed that the fascicular cambium-derived signal that induces pith ray cells into cambial identity is in part physical in nature: mechanical tension induced in pith ray cells by radial expansion of the adjacent fascicular cambium. Nevertheless, multiple studies suggest that specific gene products are also required for interfascicular cambium initiation.

Defects in interfascicular cambium initiation have been documented in loss-of-function mutants of *pxy* (*tdr*) (Etchells & Turner, 2010; Agusti *et al.*, 2011), a receptor-like kinase involved in cell polarity and the orientation of cambial cell divisions, and of *HB4*, a HD-ZIP III gene potentially associated with auxin signaling pathways (Zhu *et al.*, 2018). Thus, *PXY* and *HB4* seem to be required for interfascicular cambium development. It is unclear whether *PXY* regulates this process directly or indirectly, by promoting the activity of the fascicular cambium (Agusti *et al.*, 2011). Additional genes potentially involved in interfascicular cambium initiation include *COV*, *HCA* and *HCA2*/*Dof5.6* (which is not allelic with *HCA*), each of which seems to act in a different pathway (Guo *et al.*, 2009). Whereas HCA2 promotes interfascicular cambium formation (Guo *et al.*, 2009), which is also seen in *hca* mutants, probably via effects on the cytokinin transduction pathway (Pineau *et al.*, 2005), *COV1* is considered a repressor of interfascicular cambium specification or activity (Parker *et al.*, 2003).

Meristem homeostasis is maintained at the shoot apex by antagonistic interaction between the KNOX I transcription factors *STM* and *BP*, and the myb transcription factor *AS1* (Byrne *et al.*, 2000). Whereas STM/BP promote cell division and exclude AS1 expression and cell differentiation in the meristem central zone, thus maintaining meristematic identity, AS1 is expressed on the flanks of the meristem, where it down-regulates STM/BP and promotes cell division and differentiation to form leaf primordia. In *Populus*, *STM* and *BP* orthologs (*ARBORKNOX1* (*ARK1*) and *ARK2*, respectively) are expressed in both the shoot apical meristem and the cambial zone (Groover *et al.*, 2006; Du *et al.*, 2009), where they promote meristematic activity and repress cell differentiation. This suggests that a developmental module regulating the balance of cell division and cell differentiation was co-opted from the shoot apical meristem by the cambium, although no AS1-like genes have been characterized to date that are associated with secondary growth. Additionally, in *Arabidopsis* hypocotyls and roots, *BP*

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promotes differentiation of secondary xylem, contrary to its role in the stem (Liebsch *et al.*, 2014; Woerlen *et al.*, 2017), which suggests that the role of these genes in regulating the balance of cell division and cell differentiation is more complex.

4. Periclinal division of cambial initials – rate and plane of division, circumferential synchronization

Developmental anatomy Coordination of rates and planes of cell division is fundamental for cambial growth. Rates of division, in coordination with those of cell differentiation, determine meristem homeostasis and the rate of secondary growth. The orientation of the plane of division determines the geometry and structure of secondary tissues. Periclinal divisions occur in both types of cambial initials – fusiform initials and ray initials (Fig. 1) – and generate the alignment of secondary tissues in radial cell files (as seen in cross sections), an anatomical fingerprint of secondary growth (Fig. 1). Periclinal divisions of fusiform initials add to the axial system of secondary tissues and those of ray initials add to the radial system, elongating the vascular rays.

Ray initials are generally isodiametric, whereas fusiform initials are elongated. Fusiform initials up to 1.6 mm long have been reported in angiosperms and up to 4–5 mm long in conifers, with aspect ratios between 5 : 1 and 25 : 1 or more in angiosperms, and 50 : 1 to > 100 : 1 in conifers (Bailey, 1920; Eames & MacDaniels, 1947; Philipson *et al.*, 1971). Rates of cell division in fusiform initials are relatively low (one every 4–6 d, in conifers; Bannan, 1962), possibly due to their length (Fahn, 1990): in *Pinus* the cell plate initiated in the middle of the cell requires about 19 h to reach its distant ends and complete cytokinesis (Wilson, 1964).

Cambial growth progresses at roughly the same rate all around, consistent with a certain level of synchronization of cell divisions around the cambial layer. At the scale of the whole stem, cambial initials divide at similar rates and the derivatives produced belong to the same tissue (Eames & MacDaniels, 1947). However, at smaller scales synchronization between adjacent cambial sectors is not always tight (Steeves & Sussex, 1972): in adjacent cell files at a given moment one might be forming xylem and the other phloem (Newman, 1956).

Regulation Polar auxin transport is a major determinant of periclinal divisions. This follows from the requirement for auxin flow through meristematic cells to maintain cambial identity and initiate cambial activity, and is confirmed by studies showing that auxin flow, above a threshold level, is required for cambial reactivation following dormancy (Snow, 1935; Avery *et al.*, 1937; Savidge & Wareing, 1981; De Groote & Larson, 1984). GA_3 is also needed for reactivation of the cambium (Wareing *et al.*, 1964; Lachaud, 1983). Along a transect through the cambial region, auxin levels peak in the cambium, whereas GA_3 levels are highest in differentiating secondary xylem (Björklund *et al.*, 2007). While periclinal divisions depend on both auxin and GA_3, such responses to hormonal cues are components of broader regulatory programs, wherein they interact with gene networks.

Peptide signaling across secondary tissues has been identified as a key mode of regulation of the cambium (Ito *et al.*, 2006), including the orientation of cell division planes (Etchells & Turner, 2010). The TDIF peptide is encoded by the CLE41 and CLE44 genes in *Arabidopsis*. CLE41/44 are expressed in the phloem, but TDIF is secreted and perceived by the receptor PXY/TDR localized in the plasma membrane of cambial zone cells. This signaling results in upregulation of WOX4, a transcription factor that promotes cell division within the cambial zone. This same mechanism appears to play a fundamental role in orienting planes of cell division in the cambial zone. Aberrant division planes are found in *pxy/tdr* loss-of-function mutants, and when the CLE41 ligand is spatially misexpressed. Although the specific mechanism affected is uncertain, it appears that proper spatial signaling of TDIF ligand is important for division planes. This same mechanism has been shown to regulate cambium functioning in poplar (Etchells *et al.*, 2015). More generally, the role of TDIF peptide signaling in vascular cell differentiation is conserved among euphyllophytes (Hirakawa & Bowman, 2015).

Additional peptide-based signaling mechanisms are involved in regulating cambium divisions and other fundamental features of secondary growth. The receptors encoded by *MOL* (Gursanscky *et al.*, 2016) and *RUL* act as repressor and enhancer of cambial activity, respectively (Agusti *et al.*, 2011). Given that numerous uncharacterized receptor and CLE-peptide encoding genes are expressed during secondary growth, it seems likely that additional signaling mechanisms await discovery. For example, the TMO5-LHW transcription factor dimer was shown to control periclinal cell divisions in the *Arabidopsis* root, embryonically and post-embryonically, promoting indeterminate growth (De Rybel *et al.*, 2013), but it is still unclear what role this plays in secondary growth.

Biophysical forces appear to also play important roles, poorly defined currently, in regulating division planes in the cambium. Radial pressure, resulting from the interaction between an expanding inner core of secondary xylem and the constraint imposed by extracambial tissues, is required for correct orientation of periclinal divisions. This may be because cell division planes are determined by microtubule orientation, in turn influenced by mechanical stresses on the cell (Louveaux & Hamant, 2013; Sampathkumar *et al.*, 2014). Excised cambial region tissues form disorganized callus, unless subjected to oriented pressure, which generates cambium-like patterns of cell division (Brown & Sax, 1962; Lintilhac & Vesecky, 1984). Steeves & Sussex (1972) have suggested that radial pressure is important not only for correct orientation of periclinal divisions, but also for maintenance of normal differentiation in the secondary tissues.

5. Radial patterning of tissue identity – xylem vs phloem

Developmental anatomy Periclinal divisions in the cambium insert layers of secondary tissues between the central primary tissues (primary xylem ± pith) and the outer layers of primary tissues (phloem, ground tissues, epidermis). In the process, radial patterning is established, including layers of secondary xylem left inwards by the centrifugally expanding cambium, and secondary phloem layers, pushed outwards in front of the cambium. Such a

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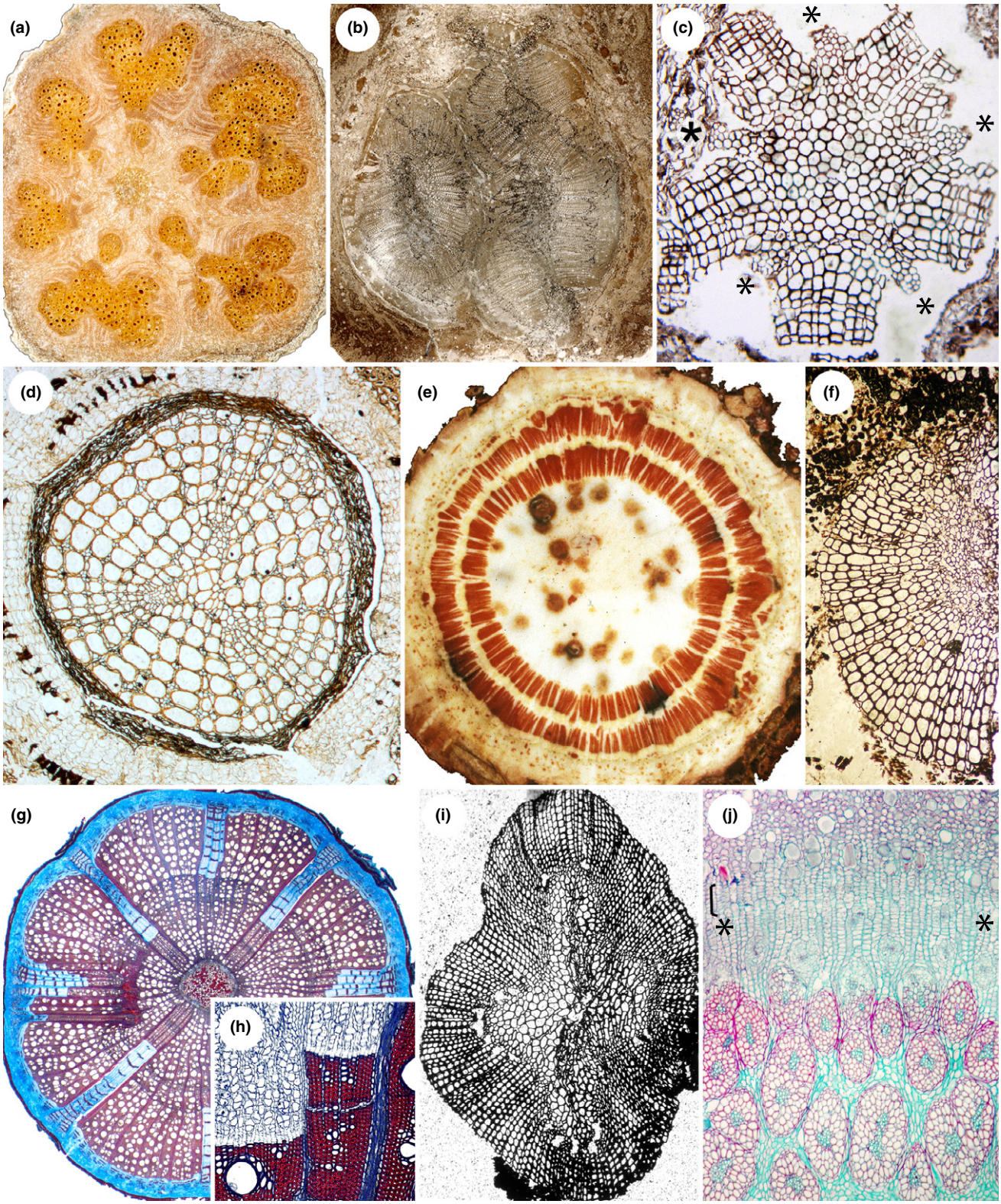

bifacial cambium is a synapomorphy of lignophytes (Rothwell & Serbet, 1994), the clade including seed plants and the extinct seed-free progymnosperms. In seed plants, xylem increments laid down by the cambium are wider than the phloem increments produced during the same time period (Esau, 1965), possibly because derivatives on the xylem side divide more times before maturing than those on the phloem side (Wilson, 1964). Nevertheless, ratios of secondary xylem to secondary phloem vary widely among species – 1 : 1 to 10 : 1 in conifers (Wilson, 1964); 4 : 1 in *Eucalyptus* (Waisel *et al.*, 1966).

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Fig. 3 Anatomy of different modes of secondary growth that support the modular nature of secondary growth (refer also to Fig. 2). (a) Dissected xylem in *Alicia anisopetala* stem (image: Marcelo R. Pace) and (b) multiple vascular segments in stem of the Carboniferous pteridosperm *Medullosa steinii* (image: Michael T. Dunn), both reminiscent of secondary tissue geometry produced under repression of interfascicular cambium specification. (c) Permian glossopterid pteridosperm root (*Vertebraria*) with lobed secondary xylem reflecting repressed specification of (interfascicular) cambium around the tips of primary xylem lobes (asterisks) (image: Anne-Laure Decombeix). (d) Stems of the Carboniferous sphenopsid *Sphenophyllum* (image: Edith L. Taylor & Rudolph Serbet) exhibit determinate secondary growth and absence of anticlinal divisions. (e) Polyxylic wood (two successive cambia) in *Cycas* trunk reflecting iterative determinate secondary growth; secondary xylem is stained red (image: Dennis Wm. Stevenson). (f) Stem of Carboniferous pteridophyte *Zygopteris illinoiensis* with unifacial cambium (note absence of secondary phloem, especially at top, where tissue preservation is complete) and lacking anticlinal divisions. (g) *Pyrostegia venusta* and (h) *Dolichandra unguiculata* stems with furrowed xylem suggesting discontinuity in circumferential synchronization of cambial activity; xylem is red, phloem is blue (images: Marcelo R. Pace). (i) Stem of Devonian sphenopsid *Rotafolia songziensis* with secondary xylem lacking anticlinal divisions (image: Wang Deming). (j) Stem of *Cordyline* showing a monocot cambium (roughly between the asterisks) that produces vascular bundles (bottom) and secondary cortex (bracket).

In a radial transect, the cambium is flanked by derivatives that divide periclinally several times, with the resulting cells maturing into secondary xylem and secondary phloem. A cambial initial and its derivatives form a continuous radial file of cells (Fig. 1), wherein cell age increases away from the cambium – centripetally in the xylem and centrifugally in the phloem – documenting successive stages of cell differentiation (Fig. 1). These developmental series provide an anatomical framework for studying interactions between hormones and gene networks that regulate cell division and differentiation.

Regulation Information on the factors regulating the radial patterning of secondary tissues comes from multiple sources. Experiments with bud removal followed by hormone application demonstrate that auxin by itself leads to formation of xylem only, without phloem production, whereas GA_3 by itself induces production of both xylem and phloem derivatives, and differentiation in the phloem, but without xylem maturation (Wareing *et al.*, 1964; DeMaggio, 1966; Digby & Wareing, 1966; Lachaud, 1983). Furthermore, the two hormones regulate the differentiation of distinct cell types in secondary xylem by controlling expression of fiber- vs vessel-specific NAC transcription factors (Johnsson *et al.*, 2018).

In callus cultures, phloem differentiates from cells adjacent to mature phloem explants, and xylem adjacent to mature xylem explants (Kühn, 1971), consistent with noncell autonomous identity specification in cambial derivatives, by signaling from adjacent maturing tissues. Interestingly, grafting experiments suggest that radial polarity is present in the parenchymatous tissue of interfascicular areas before initiation of cambial activity (Siebers, 1971), possibly reflecting polarity established in the primary meristem ring/residual meristem (Kaussmann, 1963; Esau, 1965) at the shoot apex.

A now classic mechanism regulating vascular polarity is defined by the antagonistic action of HD-ZIP III and KANADI transcription factors. In *Arabidopsis* cauline bundles, phloem-expressed KANADIs and xylem-expressed HD ZIP III promote abaxial (phloem) vs adaxial (xylem) identities, respectively (Emery *et al.*, 2003; Ilegems *et al.*, 2010). The same mechanism is probably involved in polarity regulation during secondary growth (Schrader *et al.*, 2004). In poplar, the HD-ZIP III genes *popREVOLUTA* (Robischon *et al.*, 2011) and *HB4* (Zhu *et al.*, 2018) are expressed

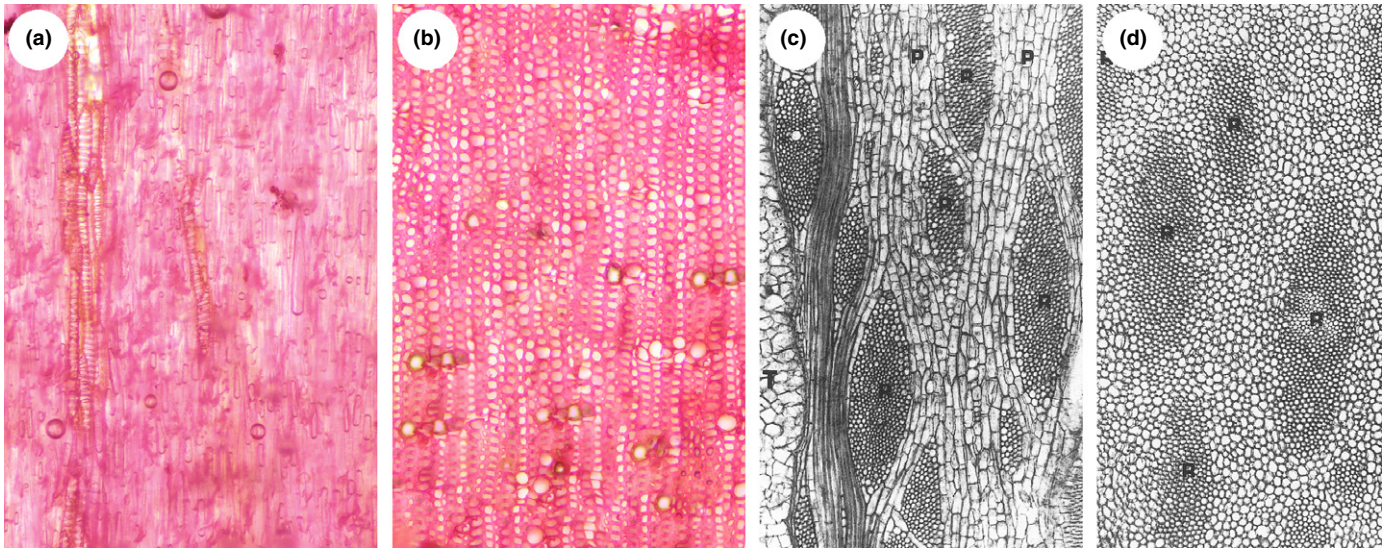

Fig. 4 Rayless wood seen in longitudinal tangential section in *Kalanchoe tomentosa* (a) and in cross-section in *K. beharensis* (b); the large cells in both images are vessel elements. (c, d) Longitudinal tangential sections of *Ficus sycomorus* wood; (c) regular wood showing radial system (rays; R) and differentiated axial system (vessel with tyloses, T; fibers, dark vertical band; axial parenchyma, P); (d) wood produced post-girdling, wherein only the radial system (R) is differentiated, whereas the axial system consists of undifferentiated isodiametric parenchyma; (c) and (d) from Lev-Yadun (1994), with permission.

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in developing xylem. Overexpression of either of these genes results in formation of ectopic cambium within cortex layers, which can produce secondary vascular tissues of reversed polarity (i.e. xylem towards the stem periphery).

First described in the *Arabidopsis* root, a signaling mechanism influencing tissue patterning and cell division involves the transcription factor SHR, which regulates noncell autonomously the asymmetric periclinal divisions that form the endodermis (Helariutta *et al.*, 2000; Nakajima *et al.*, 2001). The radial movement of SHR is negatively regulated by the SCR transcription factor (Sabatini *et al.*, 2003; Koizumi *et al.*, 2012). Interestingly, studies in poplar described the expression of an SHR ortholog in the secondary phloem and rays (Schrader *et al.*, 2004; Miguel *et al.*, 2016). Additional research is needed to determine if this gene is involved, in poplar secondary tissues, in a similar mechanism as in roots. This could be a fruitful area for research, given the central role of SHR signaling in regulating vascular patterning and cell division, through regulation of cytokinin levels (Cui *et al.*, 2011).

Also in the sphere of noncell autonomous regulation of tissue identity and radial patterning, Wang *et al.* (2018) report interactions between ligands and receptors from multiple tissue layers in the *Arabidopsis* stem: xylem, phloem, procambium and endodermis. These interactions coordinate the organization, proliferation and sizes of cells across both vascular and external, nonvascular layers, and suggest that similar regulatory relationships, where tissue growth is controlled via signals moving across different layers, may coordinate tissue expansion throughout the plant body.

6. Anticlinal division of cambial initials – plane of division and asymmetry

Developmental anatomy To accommodate the addition of secondary xylem layers internal to the cambium, the latter expands radially. Two processes contribute to the associated increase in circumference: growth of cambial initials in tangential dimension, and anticlinal divisions of initials. As the tangential growth of cambial initials is limited, anticlinal divisions are the main process driving the expansion of cambial circumference. Symmetric anticlinal divisions produce two fusiform initials, whereas asymmetric divisions cut off ray initials (Esau, 1965) (Figs 1, 2a). Symmetric divisions are obliquely oriented in most gymnosperms and angiosperms (Bailey, 1923). Longitudinal symmetric divisions are encountered in angiosperms with storied cambia, wherein fusiform initials form regular horizontal rows and anticlinal divisions are synchronized in vertical files of initials (Derr & Evert, 1967). Oblique divisions depart from the long axis of the fusiform initial at low to very high angles. Following oblique division, the two fusiform daughter cells grow apically between adjacent cells elongating past one another. Such intrusive growth of fusiform initials may extend over several years (Esau, 1965) and is probably guided by polar auxin transport trajectories. Symmetric divisions occur once every 3.7 yr, on average, in the same radial file of tracheids in *Thuja*, and in some species they are more frequent toward the end of the growth season (Bannan, 1956, 1957; Esau, 1965).

Regulation The factors regulating the position and frequency of symmetric and asymmetric anticlinal divisions are largely unknown. Tangential tension building up in fusiform initials due to stretching of the cambium is probably involved in rearrangement of microtubules, which orients the plane of division; it is possible that a threshold tension level triggers division. Ray initiation has been suggested to be regulated by the differentiating vascular tissues, with ray locations determined by channels of a stimulus that moves between phloem and differentiating xylem (Carmi *et al.*, 1972). Since rays tend to be regularly spaced around the circumference, a possible explanation is that existing rays determine the position of new rays. Could a mechanism analogous to that controlling the positioning of leaf primordia on the apical meristem be at work? Is it possible that existing rays inhibit formation of new rays until radial expansion distances neighboring rays far enough to drop the levels of an unknown inhibitor signal between them below a threshold value? This would make sense if signaling from ray initials circulates tangentially through the cambium.

For symmetric anticlinal divisions, Nilsson *et al.* (2008) showed that auxin signaling restricts them spatially to a narrow zone of cambial derivatives on the xylem side. The mechanism involved in the spatial regulation of anticlinal divisions is unclear, but appears to be more sensitive to changes in auxin responsiveness than the mechanism responsible for periclinal divisions. Additional evidence may come from studies such as the one by Tarelkina & Novitskaya (2018), which showed that high exogenous sucrose causes increased frequencies of anticlinal divisions in the cambial zone and distribution over a wider zone on both phloem and xylem sides of the cambium. Noting higher frequencies of anticlinal divisions in cambial initials adjacent to rays, these authors also suggested an inducing signal transmitted from the phloem via vascular rays.

III. Is vascular cambial growth less than the sum of its parts? A mosaic modularity hypothesis

The evidence reviewed here demonstrates that secondary growth is a multi-faceted developmental feature, and significant integration and coordination is required across multiple regulatory mechanisms. Furthermore, secondary growth is responsive to environmental cues (water stress, daylength, wounding and myriad other factors), and thus underlying developmental mechanisms integrate information from the environment and modify development to produce anatomies suitable for given conditions.

What we have learned from angiosperms points toward deep intertwining of regulatory programs for component processes, suggesting that secondary growth is more than the sum of its parts. If the same type of information was available for other plant lineages, now extinct, that had evolved secondary growth, we would probably find similarly deep integration of regulatory programs, in each of those lineages. However, in a broader perspective, vascular cambial growth can also be considered as a developmental and structural syndrome that takes different expressions in different plant lineages or developmental contexts. In this perspective, secondary growth emerges as a modular assembly, wherein different combinations of regulatory

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programs for individual developmental modules lead to different *modes of secondary growth*.

Cast across the entire tracheophyte clade, to include all lineages that had evolved secondary growth, this modular view facilitates an upward outlook on the evolution of vascular cambial growth, with important implications. Evidence that cambial growth produces secondary vascular tissues in the absence of one or another of the different modules is consistent with *mosaic modularity*, which we propose as a working hypothesis in addressing secondary growth across the entire clade. This hypothesis implies that distinct modes of secondary growth seen in different tracheophyte lineages are the expression of developmental programs for secondary growth in which component regulatory modules are turned on or off. Therefore, secondary growth is characterized by both within-lineage regulatory integration and across-clade mosaic modularity.

The fact that different modes of cambial growth proceed in the absence of one or another of the regulatory modules argues that secondary growth is less than the sum of its parts, if considered at the level of the entire tracheophyte clade. Thus, in the spirit of a broad upward outlook, the origin and evolution of secondary growth could be explored by considering a *minimalist mode of vascular cambial growth* – for example, the 'minimum common denominator' of the different modes of secondary growth documented among tracheophytes – and looking for a *minimal set of developmental-regulatory modules* required to generate it. One could then ask whether these regulatory modules (or their main constituent genes) could have been present in the common ancestor of all vascular plants or of all euphyllophytes. Such questions could be addressed by investigating whether the minimal list of regulators are present in all living vascular plants; whether they have broadly similar functions in all these lineages; and whether minimal sets of corresponding anatomical fingerprints for the different processes they regulate are present in all extinct lineages that have secondary growth. These are as many series of questions that can be expanded to include all aspects of secondary growth regulation.

IV. A three-pronged approach to the evolution of secondary growth

Three information systems can be queried for data to test our mosaic modularity hypothesis (Fig. 5). *Living seed plants* provide information on developmental anatomy and regulation of

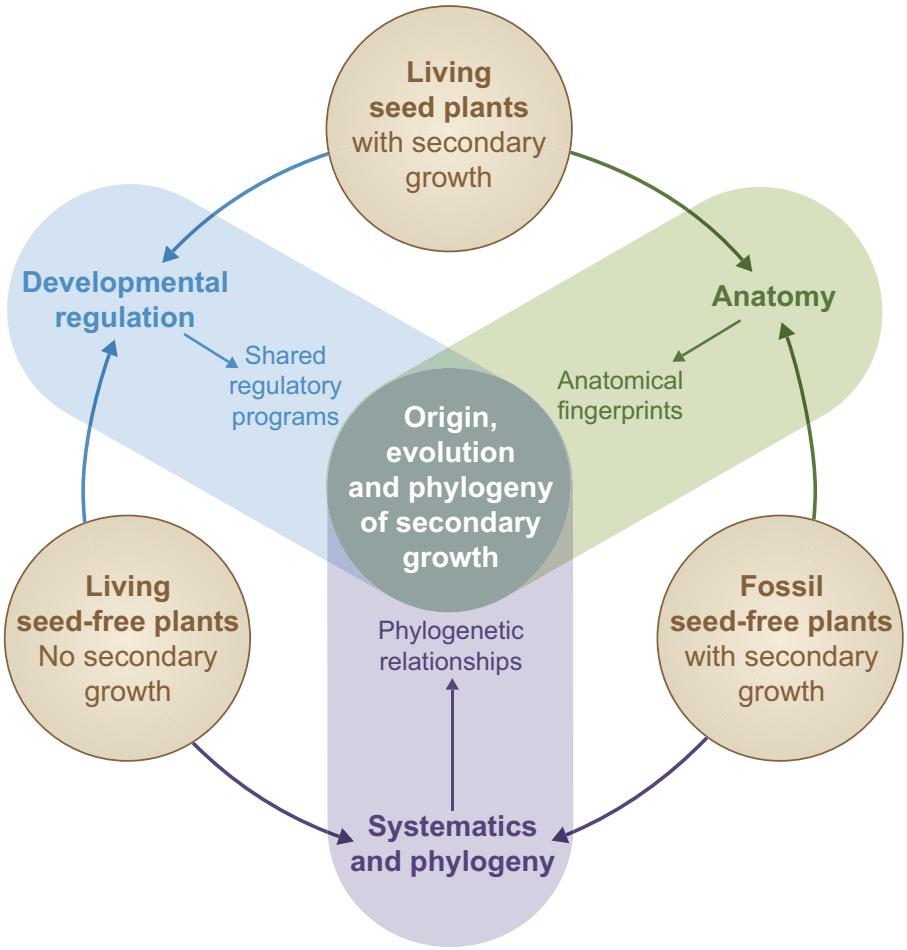

Fig. 5 An integrative approach for addressing the evolution of secondary growth. Three information systems – living seed plants (with secondary growth), fossils and living seed-free plants (devoid of secondary growth) – provide data on anatomy, developmental regulation and phylogenetic relationships, which inform each other to advance our understanding of the origin and evolution of vascular cambial growth across the entire tracheophyte clade (see also Section IV. 'A three-pronged approach to the evolution of secondary growth').

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secondary growth. *Fossils* reveal the anatomy of secondary growth, which provides insights into developmental processes, in extinct seed plants and in seed-free lineages that lack living representatives with woody growth. *Living seed-free plants* devoid of secondary growth provide information on expression patterns and functions of secondary growth regulators identified in seed plants. Additionally, fossil and living seed-free plants contribute key information for reconstructing a phylogenetic framework to reveal possible paths of evolution and inheritance of developmental processes and regulatory mechanisms.

Within the new modular paradigm we outline here, these information systems supply data for a three-pronged approach that we propose for addressing the evolution of secondary growth (Fig. 5). On this front, conceptual advances will unfold at the intersection of three lines of investigation, for which we provide an epistemic framework. First, understanding deterministic relationships between the molecular–genetic regulation of developmental processes and their anatomical expression in living seed plants allows for identification of anatomical fingerprints (e.g. Rothwell *et al.*, 2014) that provide connections between anatomical features preserved in fossils and developmental processes. Second, detailed characterization of the anatomy of secondary growth in extinct lineages can reveal such anatomical fingerprints, fostering hypotheses about the presence of developmental processes and regulatory programs in deep time. Third, understanding the distribution and functions of genes and mechanisms that underlie secondary growth in extant plants (or their homologs), in nonwoody living seed-free plants, will support comparisons that provide insight into the functions and evolution of such conserved vs lineage-specific regulators. These can represent a measure of the potential for secondary growth in each lineage. In turn, these data can provide independent tests for hypotheses based on observations of anatomical fingerprints in fossils, and can generate hypotheses about the developmental toolkit for wood production shared among lineages.

The power of integrating such approaches is exemplified by studies of polar auxin transport in secondary growth. Observations in living plants have shown that 'auxin swirls' form in the wood above branches, due to disruption of basipetal auxin transport in the stem cambium (Lev-Yadun & Aloni, 1990). Rothwell & Lev-Yadun (2005) and Rothwell *et al.* (2008) used auxin swirls as anatomical fingerprints to demonstrate polar auxin transport in the cambium of extinct archaeopterid progymnosperms, lepidodendrid lycophytes and calamitacean sphenopsids. Their results indicate that polar auxin flow through the cambium is a constant of secondary growth regulation in distant lineages, and thus potentially part of a toolkit for secondary growth shared among multiple lineages (Rothwell & Tomescu, 2018). Furthermore, differences in auxin swirl geometry predict differences between lineages in the fluxes of auxin transported through the cambium (Rothwell *et al.*, 2008).

V. Future outlook

Advancing our understanding of the evolution of developmental mechanisms that underlie vascular cambia and secondary growth will require the incorporation of new conceptual frameworks that enable integration of currently disparate data types. Encouragingly, interconnecting areas of research exist, as outlined in the three-pronged approach that we propose above, as well as new technical approaches that may soon bridge gaps between the fossil record, developmental genetic and genomic views of secondary growth.

1. Studies of extant seed plants

It is increasingly clear that co-option of genetic mechanisms regulating primary meristems played a key role in the evolution of seed plant vascular cambia (Spicer & Groover, 2010). Examples include the direct co-option of genes that are expressed in both the shoot apical meristem and the cambium (e.g. STM), as well as homologous genes that underlie functionally analogous mechanisms in both meristems (e.g. WUS in the shoot apical meristem and WOX4 in the cambium). Hormones play similarly important roles in the regulation of primary meristems and vascular cambia, including influencing rates and planes of cell division, differentiation and overall growth rates. While less well understood, it appears that biophysical forces may also be central to the function of both primary meristems and vascular cambia. These examples illustrate how new advances on fundamental aspects of plant developmental biology could be extended to provide needed insights into missing pieces of secondary growth. Major unanswered questions on development in cambial growth relate to regulation of the position and frequency of symmetric and asymmetric anticlinal divisions, specification of ray initials, coordination between development of axial and radial systems of secondary tissues, and tangential coordination of developmental processes in the vascular cambium and adjacent layers.

Comparative genomic methods now enable approaches that may reveal the ancestral mechanisms underlying secondary growth, and their modifications that produce the variation in anatomy seen in different lineages. Traditional systematic approaches have shown limited success in detecting or describing mechanisms underlying observed trait diversity. In contrast, comparative genomic methods can be integrated with phylogenetic frameworks to potentially identify key evolutionary steps or mechanisms underlying traits of interest. Gene co-expression networks are one example of comparative genomic approaches (Ruprecht *et al.*, 2017) that can now be applied in forest trees. In this approach, genes are clustered and assigned to gene modules based on similarity in expression across different tissues, cells or responses to experimental treatments. Such clustering, applied across a phylogenetic range of species, allows for inferences about what modules are common and, thus, potentially ancestral to all the lineages under scrutiny, and which are lineage-specific. If done at high enough resolution, such studies could reveal the most critical gene interactions required for secondary growth.

Expanding such approaches to address the evolution of secondary growth at the level of the entire tracheophyte clade is challenging because only two groups – seed plants and isoetalean lycophytes – among the many that had evolved secondary growth, have living representatives. To meet the challenge of integrating

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findings from extant and extinct plants, new computational approaches may be required, for example, to model changes in gene networks that could be responsible for observed evolutionary changes in development or anatomy. Testing such models experimentally could be facilitated by the increasing number of model systems for seed-free plants, including extant relatives of lineages with extinct members that possessed secondary growth.

Relevant answers will also come from drawing parallels with development of the periderm as a result of phellogen (cork cambium) activity. Although not discussed here due to lack of space and less thoroughly explored in terms of regulation (Wunderling *et al.*, 2018), secondary growth in the periderm shares many features with vascular cambial growth and is documented in the fossil record almost as early as the latter (Banks, 1981).

2. Studies of extant seed-free plants

In considering possible evolutionary mechanisms that explain the advent and subsequent diversification of secondary growth in seed plants, there currently is little evidence supporting significant roles of structural features of genomes or the appearance of new genes. For example, diverse angiosperm species with secondary growth have no known common structural genome features directly related to this type of growth. Indeed, angiosperms show amazing genome plasticity, including multiple whole genome duplication events and other structural variation within the many lineages harboring woody species (Amborella Genome Project, 2013). Additionally, woody gymnosperm genome and gene structures show distinct differences from angiosperms (Birol *et al.*, 2013; Nystedt *et al.*, 2013; Neale *et al.*, 2014), despite a presumably homologous nature of their secondary growth.

The fact that structural genome changes and changes in gene content do not show correlation with the woody habit suggests that the underlying gene networks are robust to perturbation. Additionally, all of the genes discussed in this review as playing significant regulatory roles in secondary growth have homologs predating seed plants (even though exploration of their expression, functions and interactions in seed-free tracheophytes is gaining impetus slowly; Floyd & Bowman, 2007; Tomescu, 2011; Ambrose & Vasco, 2016; Vasco *et al.*, 2016). These observations suggest that it is not gene content *per se*, but rather how genes interact in networks, that determines variation in secondary growth among extant seed plants, which is consistent with a modular view of secondary growth. Together with evidence for modular recruitment of some secondary growth mechanisms from primary meristems, these concepts suggest that the evolution and diversification of secondary growth were not as much driven by 'new genes' evolving, as by rewiring of existing genes.

Knowledge of the ancestral genes underlying secondary growth in seed plants and how they interact could enable interesting experiments in seed-free plants. The level of regulatory complexity separating truly herbaceous plants from those exhibiting secondary growth is unknown, but it is not implausible that differences are minimal. For example, stimulating directed auxin transport might be sufficient to promote the transition from truly herbaceous growth to cambial activity within and between vascular bundles. Genome editing could be used to test predictions from seed plant gene networks, for example by trying to revive pathways for secondary growth in extant seed-free plants from lineages that demonstrate secondary growth in the fossil record. This approach would rely on the retention of most genes and mechanisms required for formation and regulation of the cambium through their shared functions in shoot or root apical meristems. Predictions about what key genes or interactions must be reinstated to revive secondary growth would come from comparative gene network analyses between different meristems of seed- and seed-free plants. While currently speculative, such approaches illustrate how conceptual and experimental linkages could be established among currently disjunct molecular genetic, computational modeling and fossil-based studies.

Equisetum would provide a testing ground for secondary growth in the sphenopsids and maybe the more distantly related cladoxylopsids. Parallels on secondary growth in extinct fern-like plants (zygopterids, rhacophytaleans) could be drawn from experiments that employ living ferns with protostelic vascular architecture (e.g. Gleicheniaceae, Lygodiaceae, Schizaeaceae). Understanding secondary growth in the extinct lepidodendrid lycophytes would seem easily attainable, since their closest living relative, *Isoetes*, is the only extant plant that produces secondary tissues from a vascular cambium, outside the spermatophyte clade. However, this may be a hasty prognosis, considering that *Isoetes* has a specialized cambium that reflects its highly derived condition just as much as other features of its morphology and anatomy. Whereas the lepidodendrid cambium was unifacial (Cichan & Taylor, 1990), the vascular cambium of *Isoetes* produces a mixture of xylem and phloem centripetally and parenchymatous tissues centrifugally (Gifford & Foster, 1989).

3. Fossil studies

The most recent appraisal of secondary growth in a phylogenetically deep context is more than a quarter of a century old (Cichan & Taylor, 1990). The intervening period has seen the deepest record of secondary growth pushed down into the Early Devonian (Gerrienne *et al.*, 2011; Hoffman & Tomescu, 2013; Gensel, 2018), secondary growth documented in additional fossil lineages (Beck & Stein, 1993; Momont *et al.*, 2016), and discussion of secondary growth in fossils in the context of development (Gerrienne & Gensel, 2016; Decombeix & Rowe, 2018). Reassessment of the fossil record is, thus, likely to contribute new data and engender conceptual advances, and should explore a number of directions.

In-depth (re)analysis of secondary tissues in all extinct lineages should pay attention to the implications of anatomical structures for developmental processes, within the modular framework developed here. Ideally, where available, multiple representatives per lineage should be analyzed to identify differences between major groups, as well as within-lineage variability. The phylogenetic distribution of potential developmental–regulatory modules and of specific anatomical structures identified by these analyses (e.g. tracheid pitting, radial variation of tracheid size, frequency and

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distribution of anticlinal divisions) will reveal patterns of shared and derived features among and within lineages. A set of anatomical features and developmental modules representing the 'minimum common denominator' of secondary growth could be inferred by integrating shared patterns of distribution with what we learn about the mode of cambial growth of the oldest tracheophytes that exhibit secondary growth. The inferred regulators of this minimal set of modules would then predict the structure of the basic regulatory toolkit for secondary growth that could have characterized a hypothetical common ancestor, either as real process or as developmental potentiality.

How deep, phylogenetically, may such an ancestor be sought? The short interval (< 10 Myr) between the oldest euphyllophytes with secondary growth and the oldest known unequivocal euphyllophyte implies high likelihood for a common ancestor of the clade that carried the basic regulatory toolkit for secondary growth. However, the lycophyte–euphyllophyte divide, dated to no later than 425 Ma based on late Silurian lycophyte representatives (Kotyk *et al.*, 2002), remains the deepest phylogenetic divergence of clades wherein secondary growth has evolved. The presence of secondary growth in both clades, as well as the evidence for some shared mechanisms of secondary growth regulation (Rothwell *et al.*, 2008), imply that a tracheophyte ancestor of lycophytes and euphyllophytes had already evolved at least part of the basic regulatory toolkit for secondary growth before 425 Ma. Thus, a minimum set of developmental regulators for secondary growth characterizing the entire tracheophyte clade could be sought beyond the deepest roots of the euphyllophyte clade, at the intersection of the latter with the lycophytes. The need to test these predictions reiterates the importance of continued exploration of the fossil record by field studies, particularly addressing the late Silurian through Early Devonian interval.

An indirect but equally important role for fossils in illuminating the evolution of secondary growth involves resolution of the phylogenetic relationships that connect all the fossil lineages possessing secondary growth with their living relatives and representatives, across more than 400 Myr of evolution. While full resolution of these relationships is a distant desiderate, efforts in this direction are regaining impetus (Toledo *et al.*, 2018). Once phylogenetic resolution is achieved, we will be able to apply the findings about the basic regulatory toolkit for secondary growth to the closest living seed-free relatives of lineages that demonstrate secondary growth in the fossil record, to potentially revive secondary growth pathways in these plants.

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Notes S1 Glossary of terms.

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