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The Dynamics of Cambial Stem Cell Activity

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Abstract

Stem cell populations in meristematic tissues at distinct locations in the plant body provide the potency of continuous plant growth. Primary meristems, at the apices of the plant body, contribute mainly to the elongation of the main plant axes, whereas secondary meristems in lateral positions are responsible for the thickening of these axes. The stem cells of the vascular cambium—a secondary lateral meristem—produce the secondary phloem (bast) and secondary xylem (wood). The sites of primary and secondary growth are spatially separated, and mobile signals are expected to coordinate growth rates between apical and lateral stem cell populations. Although the underlying mechanisms have not yet been uncovered, it seems likely that hormones, peptides, and mechanical cues orchestrate primary and secondary growth. In this review, we highlight the current knowledge and recent discoveries of how cambial stem cell activity is regulated, with a focus on mobile signals and the response of cambial activity to environmental and stress factors.

Contents

1. INTRODUCTION

With evolution of life on land, fierce competition for light began, and growing taller and reaching the canopy earlier than did competitors became selective advantages for plants. However, increased plant height and growth along the plant's primary axis also resulted in the demand for new designs to support aerial organs and to deliver water and nutrients to heights beyond the limits of capillary forces. Some seed plants overcame this limitation by evolving an additional stem cell population, the vascular cambium, which forms a hollow cylinder within stems and permits radial growth of primary axes. This evolutionary innovation, referred to as secondary growth, occurred several times in seed plants, which suggests that factors regulating primary growth have been co-opted in the coordination of secondary growth. Mechanical strength is provided not only by thickening of the stems through the activity of the vascular cambium, but also by specialized cell types with thickened cell walls belonging to the two tissues—the phloem and xylem—that the vascular cambium forms. These thick secondary cell walls also permit water and nutrient transport to greater heights by preventing xylem cells from collapsing.

Design of independent, spatially separated stem cell populations contributing to primary and secondary growth requires a high degree of communication between apical and lateral sites of growth. Primary and secondary growth need to be aligned for the stem to provide enough mechanical support to carry the crown. By contrast, secondary growth is a costly process owing to the deposition of secondary walls, and plants exceeding the needs demanded by primary growth may suffer a penalty in fitness. Although our understanding of long-range communication between apical and lateral stem cell populations is rudimentary, hormone, peptide, and mechanical signaling are likely factors to coordinate and align primary and secondary growth.

The differentiation of cambial derivatives into xylem and phloem cells and deposition of thick secondary cell walls have been reviewed profoundly and recently. We focus this review on the regulation of secondary growth, i.e., cambial activity, through long-range signals that may be at the core of aligning primary and secondary growth.

Cambial activity: cell division and growth of the stem cells and transient amplifying cells in the cambium

SAM: shoot apical meristem

RAM: root apical meristem

Periclinal cell division: division of cells parallel to the surface plane of an organ

2. DEVELOPMENT OF THE VASCULAR CAMBIUM

2.1. Embryonic Vascular Stem Cells

Unlike animals, plants can grow indefinitely and form new tissues and organs in response to seasonal and environmental changes and cues owing to the mitotic activity of stem cell populations in the meristems. Apical meristems comprising the shoot apical meristem (SAM) and root apical meristem (RAM) are located at the tips of the main and lateral shoots and roots and contain stem cell populations that support shoot and root elongation or primary growth. Apical meristems are formed during embryogenesis (76, 110), along with the first vascular stem cells (110).

In the model plant *Arabidopsis thaliana*, vascular development is initiated during transition from the 16-cell (dermatogen) stage to the globular stage of embryogenesis, when periclinal cell divisions of inner protoderm cells within the lower tier of the embryo distinguish the first vascular stem cells from the ground tissue (110). Subsequent oriented and coordinated divisions of these vascular stem cells form a structure comprising a central xylem layer surrounded by phloem poles with procambial cells packed between them, resembling the vascular pattern observed in postembryonic roots. Meanwhile, vasculature develops in the cotyledons and connects to existing vascular strands in the embryonic axis. Numerous marker gene expression studies indicate that, although the establishment of the vascular stem cells and the specification of the xylem and phloem tissues occur during embryo formation, vascular differentiation (i.e., secondary cell wall formation) does not start before seed germination (130).

2.2. The Procambium

The primary vasculature of plants arises from the activity of the SAM and RAM (40). In dicot and gymnosperm stems, the primary vasculature is arranged in discrete bundles (also referred to as fascicles) surrounding a central pith. Each bundle contains a procambium, which comprises the vascular stem cells giving rise to the primary xylem on the side facing the pith and the primary phloem on the side facing the outside of the stem (**Figure 1*a***). Phloem tissue is responsible for shoot-to-root transport of photoassimilates, solutes, signaling molecules, and plant hormones. Conversely, xylem is responsible for water transport from roots to shoots and structural support. Meanwhile, cells of the interfascicular region, between the vascular bundles, differentiate into parenchymatic cells. In contrast to shoots, the primary vasculature in roots consists of a central xylem axis that is enclosed by the phloem, with the procambium lying between the two transport tissues (40).

2.3. Interfascicular Cambium Formation and Establishment of the Vascular Cambium

With increasing apical growth, shoots and roots require more structural support, water, and nutrients (40, 72). Many plant species, i.e., gymnosperms and dicots, encounter these limitations by induction of secondary (also referred to as lateral) growth. This growth is largely driven by the activity of the vascular cambium.

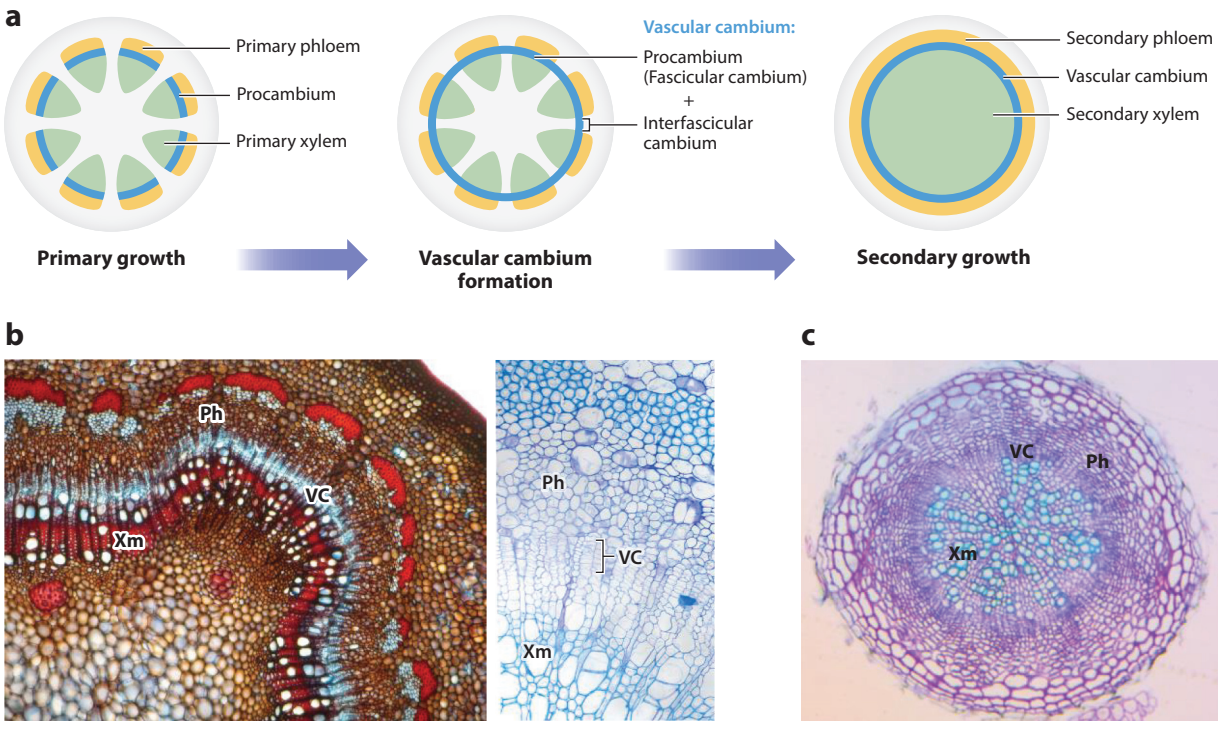


Figure 1

Vascular cambium anatomy. (*a*) Schematic illustration of secondary growth progression in a dicot stem from primary growth to vascular cambium formation and secondary growth, showing the (*yellow*) primary/secondary phloem, (*blue*) procambium/interfascicular cambium/cambium, and (*green*) primary/secondary xylem. (*b*) Stem cross sections from 2-month-old hybrid aspen tree (fifth internode) showing early secondary growth. A thin cross section is shown on the right. (*c*) Root cross section from 26-day-old *Arabidopsis* showing secondary growth. Abbreviations: Ph, secondary phloem; VC, vascular cambium; Xm, secondary xylem.

In stems, the onset of secondary growth is accompanied by reactivation of the procambium within the vascular bundles. Simultaneously, parenchyma cells located in the interfascicular region between the bundles transdifferentiate into interfascicular cambial cells and acquire meristematic activity. This results in the formation of a complete ring of vascular cambium (**Figure 1**). The ontogeny of the vascular cambium in roots was recently characterized at cellular resolution (121); vascular cambium originates only from those procambial and pericycle cells that are physically connected with primary xylem at the onset of secondary growth (121).

2.4. Maintenance of the Vascular Cambium and Differentiation of Cambial Daughter Cells

The vascular cambium contains files of meristematic cells consisting of vascular stem cells (also referred to as initials) and their immediate derivatives known as transient amplifying cells (also referred to as mother cells) (25, 40, 63, 72). Two types of stem cells exist in the vascular cambium: fusiform initials and ray initials. Fusiform initials are larger than ray initials and are longitudinally oriented with a highly vacuolated cytoplasm. By dividing periclinally (parallel to the surface plane of stems/roots), they increase the number of cells radially, generating the secondary phloem (bast) in the centrifugal direction and the secondary xylem (wood) in the centripetal direction (**Figure 1**). Small ray initials, by contrast, are the precursors of ray parenchyma cells, which form

radial arrays linking the secondary phloem and secondary xylem. These cells have roles in storage and transport of substances such as photoassimilates and signaling molecules.

Although transient amplifying cells are anatomically indistinguishable from cambial stem cells, only stem cells retain the ability to generate both secondary phloem and secondary xylem elements. However, transient amplifying cells have stably acquired the cell fate of one of these tissues (16, 40, 72). Moreover, transient amplifying cells may differentiate directly into different tissues or may divide several times before terminal differentiation. Interestingly, recent genetic lineage tracing studies of *Arabidopsis* roots and hypocotyls provided cell fate maps during secondary growth and demonstrated that vascular cambium is uniseriate, such that in each radial file a single, bifacial stem cell produces both xylem and phloem cell lineages (117, 121). Likewise, clonal analysis of genetically labeled sectors in *Populus* stems during secondary growth revealed the existence of a single layer of stem cells in the regenerating vascular cambium (16).

The secondary xylem contains conducting tracheary elements (i.e., tracheids in gymnosperms and vessel members in dicots), supporting fibers (only in dicots), and axial parenchyma cells (40). Secondary phloem contains conducting sieve elements (i.e., sieve cells in gymnosperms and sieve tube members in angiosperms), associated cells (i.e., companion cells in angiosperms), axial parenchyma cells, and fibers. In woody plants, after cells acquire a xylem or phloem cell fate, they elongate or expand via intrusive or symplastic growth (40, 119). Tracheary elements and fibers then deposit secondary cell walls, lignify, and undergo programmed cell death.

To support increases in girth, the vascular cambial ring must also expand laterally (40, 72). This is accomplished by anticlinal divisions of the vascular stem cells, whereby the cells of the cambium divide perpendicularly to the surface plane of the stems/roots, increasing the number of radial cell files.

3. SIGNALS IN SECONDARY GROWTH

Although dissected cambial zones remain alive for several months, their microsurgical removal from the internode halts cell division (6). When larger pieces of stem tissue containing the cambium are isolated, as is commonly done during in vitro propagation of commercially important species, cell division often continues. However, under such conditions, the orientation of the division planes is seemingly random, and cells in such calli are isodiametric rather than elongated. Therefore, although cambial tissues may retain some capacity for autonomous cell division over extended periods, the absence of primary meristems causes a loss of tissue organization and prevents cellular differentiation. Thus, cambial activity and the differentiation of derivatives from vascular stem cells are regulated via long-range signals derived from apical meristems rather than solely by local factors.

3.1. Auxin

Several lines of evidence indicate that auxin is instrumental in regulating cambial stem cells' activity and the differentiation of their derivatives. For example, secondary growth is halted by removing the main auxin source, i.e., the shoot apex (125), but aspects of secondary growth can be restored by administration of exogenous auxins via the cut surface (1, 14, 64). This strongly suggests that shoot apex–derived auxin plays a key role in regulating cambial activity and radial growth. In trees, shoot apices and leaves are the main sites of auxin biosynthesis (125). From its site of biosynthesis, auxin is transported rootward along the plant's main growth axis. Rootward auxin transport is sensitive to the auxin transport inhibitor *N*-1-naphthylphthalamic acid and requires

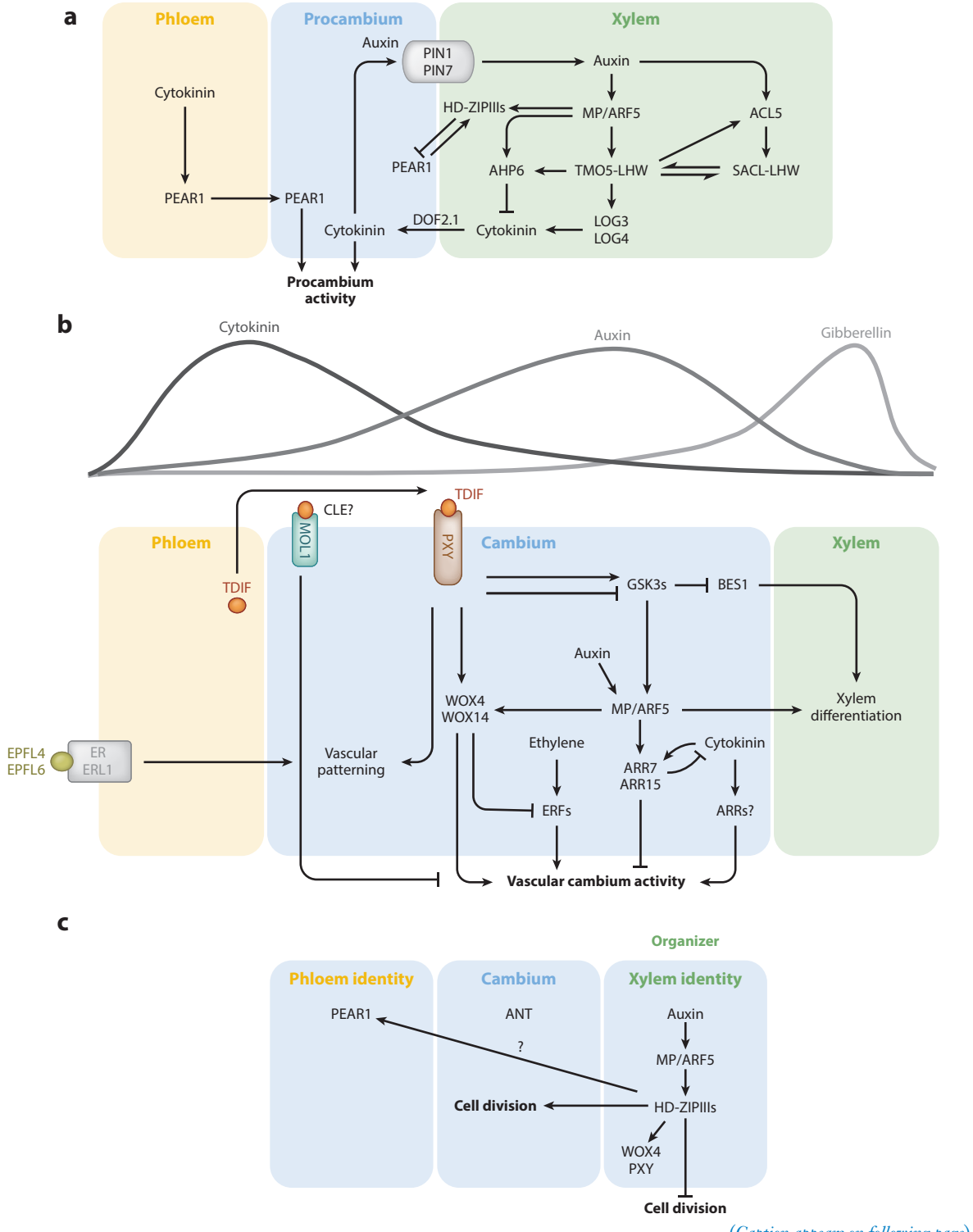
PIN-FORMED (PIN): auxin efflux carrier protein family

the activity of auxin efflux carriers belonging to the PIN-FORMED (PIN) family (96). In *Arabidopsis*, *PIN1* appears to be expressed in the procambial and cambial zones of the inflorescences with a rootward-oriented localization (7–9, 45, 107). Conversely, in *Arabidopsis* roots, PIN1 seems to be absent from mature procambial stem cells (97). It thus remains to be determined whether *PIN1* is actually expressed in the cambial stem cells. Three-dimensional reconstructions based on highly resolved confocal images or imaging using a yet-to-be identified cambial marker will be required to resolve this question.

PIN1 expression is induced when decapitated shoots are fed with auxin and is repressed in the absence of auxin (8). Moreover, *PIN* expression and rootward auxin transport are strongly repressed in an *Arabidopsis revoluta* (*rev*) mutant (152), which is deficient in the expression of a gene encoding a class III homeodomain leucine zipper transcription factor (HD-ZIPIII) that positively regulates auxin biosynthesis (19). Although there is ample evidence that shoot-derived auxin is required for secondary growth, it remains to be determined whether PIN auxin efflux carriers are required to steer cambial activity. *rev* mutants have a striking phenotype in terms of the differentiation of the interfascicular fibers. However, their phenotype with respect to cambial activity has not been reported (152), and overexpression of a microRNA-resistant *REV* leads to severe defects in vascular patterning and tissue polarity rather than obvious defects in cambial activity (105). However, there have been contradictory reports about the effects of PIN on the anatomy of inflorescences in *Arabidopsis*. Specifically, a *pin1* loss-of-function mutation reportedly had no effect on the area occupied by secondary xylem within the hypocotyl (101), but *PIN1* and *PIN3* are required for growth driven by the activity of the interfascicular cambium in inflorescence stems (1). It is important to note that both these reports were based on proxies of cambial activity rather than directly measured cell division rates.

Descriptive work has partially closed the knowledge gap due to the absence of robust functional data on auxin's influence over secondary growth. Studies of cryotome sections across wood-forming tissues from poplar and spruce indicated that auxin concentrations are highest in the cambial zone (57, 131, 137, 138) and decrease gradually upon moving away from the cambium toward the secondary phloem or the xylem (**Figure 2*b***). As suggested for the SAM (27), the highest auxin response in the youngest cambial derivatives with xylem identity (121) does not colocalize with the auxin concentration maximum. This decrease is more pronounced during latewood formation than in earlywood (136). On the basis of these observations, an auxin gradient may provide spatial information to cambial stem cells and their derivatives (12, 126): Cells exposed to high auxin concentrations are located in the cell division zone, those experiencing intermediate auxin concentrations expand, and those experiencing low concentrations undergo secondary wall deposition. Owing to the general robustness of auxin gradients (132), this hypothesis has not yet been tested experimentally.

The localization and expression patterns of *PIN1* may explain the high auxin concentrations in the cambium, but it is not clear how auxin is redistributed from the cambium in the centrifugal and centripetal directions. Potential contributors to active redistribution of cambial auxin include PIN4 and PIN7, which are localized nonpolarly to the plasma membrane (8), and PIN1, which is localized to the rootward end of the vascular cells. Redistribution by cell division and diffusion across plasmodesmata should also be considered (12). Factors determining the gradient are believed to include influx from the source, cell expansion, and tip growth as well as auxin decay. WALLS ARE THIN1, a tonoplast-localized auxin transporter expressed in the cambial zone, may be responsible for auxin retention in cambial cells (103). For an auxin gradient to influence cell differentiation, cells require a graded perception system, and very different molecular processes must be activated or repressed in each of the wood formation zones. Therefore, either the auxin receptors must have a large dynamic output range, or there must be multiple subsets of



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Regulation of cell divisions in the early procambium and vascular cambium. (*a*) Auxin maximum in protoxylem cells induces production of cytokinin through the TMO5-LHW pathway. Cytokinin diffuses to the procambium, where it promotes periclinal cell divisions and stimulates the auxin efflux carrier proteins PIN1 and PIN7, which direct auxin flow toward the protoxylem. Auxin also induces *AHP6* expression and suppresses CK signaling in the protoxylem. ACL5 antagonizes TMO5-LHW activity by stimulating SACLs, which can also heterodimerize with LHW. Mobile PEAR1 transcription factor promotes periclinal cell divisions. Auxin-induced HD-ZIPIII proteins antagonize the function of *PEAR1*. (*b*) Hormone concentration gradients (cytokinin, auxin, and gibberellin) and signaling networks across the cambium. CLE family member TDIF peptide is produced in the phloem and presumably moves to the cambium, where it binds to its receptor PXY. Downstream of TDIF-PXY signaling, WOX4 and WOX14 control stem cell proliferation, and GSK3s control xylem differentiation and cambium activity through phosphorylation of MP/ARF5. Together with TDIF-PXY signaling, EPFL-ER signaling controls vascular patterning. The TDIF-PXY pathway also participates in crosstalk with hormonal pathways including auxin, ethylene, and cytokinin. MOL1 is another receptor that opposes PXY activity in cambium regulation. (*c*) Spatial signaling network defines the stem cell organizer of the vascular cambium. Auxin-induced *HD-ZIPIII* genes maintain the organizer cells in a nondividing state and promote stem cell identity and cell proliferation in the adjacent cells. Stem cells are marked by *ANT* expression. *HD-ZIPIII*s promote *PEAR1* expression non-cell autonomously on the phloem side of the cambium. Abbreviations: ACL5, ACAULIS5; AHP6, ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6; ANT, AINTEGUMENTA; ARF, AUXIN RESPONSE FACTOR; ARR, ARABIDOPSIS RESPONSE REGULATOR; BES1, BRI1-EMS SUPPRESSOR 1; CLE, CLAVATA3/EMBRYO SURROUNDING REGION-RELATED; EPFL, EPIDERMAL PATTERNING FACTOR-LIKE; ER, ERECTA; ERL1, ERECTA-LIKE1; GSK3, GLYCOGEN SYNTHASE KINASE 3; HD-ZIPIII, CLASS III HOMEODOMAIN LEUCINE ZIPPER TRANSCRIPTION FACTOR; LHW, LONESOME HIGHWAY; LOG, LONELY GUY; MOL1, MORE LATERAL GROWTH1; MP, MONOPTEROS; PEAR1, PHLOEM EARLY DOF 1; PIN, PIN-FORMED; PXY, PHLOEM INTERCALATED WITH XYLEM; SACL, SAC51-LIKE; TDIF, TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR; TMO5, TARGET OF MONOPTEROS5; WOX, WUSCHEL-RELATED HOMEOBOX.

receptors, each of which has a different and narrow binding affinity range that is expressed in a specific differentiation domain along the gradient and induces a specific downstream signaling cascade. The binary nature of the auxin receptor means that many such subsets could arise from the dimerization of different receptor protein pairs. The hypothesis that different auxin receptor pairs are expressed in differentiation-domain-specific patterns is supported by coexpression of subsets of *Aux/IAA* (*Auxin/Indole-3-Acetic Acid*) genes with markers of specific wood formation processes (64). Differentiation-domain-specific expression of auxin coreceptors may also explain reported discrepancies between the expression patterns of auxin response reporters driven by DR5 and direct auxin concentration measurements (1, 24, 129). Testing this hypothesis by manipulating auxin gradients in wood-forming tissues would probably be practically difficult (131), but doing so either by analyzing receptor loss-of-function alleles or by ectopically expressing coreceptor pairs to adjust auxin sensitivity at specific locations along the gradient should be feasible. For this purpose, stabilized AUX/IAA proteins, which have been successfully used to manipulate cambial activity (92), could be expressed under the control of differentiation-zone-specific promoters.

3.2. Gibberellin

Gibberellic acid (GA): a class of plant hormones; also referred to as gibberellin

Gibberellic acid (GA), a category of plant hormones associated with cell elongation, also plays an important role in secondary growth. For example, overexpression of the biosynthetic gene *Gibberellin 20-oxidase* (*GA20ox*) and exogenous application of GA result in increased secondary growth, longer xylem fibers, and augmented numbers of xylem fibers in *Populus* (34, 64, 83). More significantly, grafting experiments revealed that shoot-derived GA is required for secondary growth in *Arabidopsis* hypocotyls (101). In line with a function in xylem fiber production, GA concentrations across wood-forming tissues are highest in the developing xylem (57, 58) (**Figure 2*b***). GA, a weak acid like IAA, requires efflux carriers to be transported between cells. The GA_{12} transporter NITRATE TRANSPORTER1/PEPTIDE TRANSPORTER 3 (NPF3) was the first GA transporter to be identified, and it localizes to the endodermis in the primary root

of *Arabidopsis* (128), a tissue layer homologous to the starch sheath in secondary tissues. Whether members of the NPF3 family are involved in the establishment of a GA peak in the developing xylem and whether the GA gradient is of functional importance both remain to be determined. *WUSCHEL-RELATED HOMEOBOX* (*WOX*) *14* may act upstream of GA biosynthesis. Overexpression of *WOX14* activates GA, and defects in a *wox14* mutant can be partly rescued by exogenous GA application (31).

The GA concentration peak probably overlaps with the expression domain of the class I KNOX transcription factors *SHOOT MERISTEMLESS* (*STM*) and *KNOTTED-LIKE FROM ARABIDOPSIS THALIANA 1* (*KNAT1*) in the cambium (63, 74). Class I KNOX transcription factors may directly and negatively regulate GA levels (15, 23, 61, 106). In contradiction of this idea, cambial phenotypes of GA biosynthesis as well as *stm* and *knat1* mutants are similar; both mutant classes have reduced fiber formation and cambial activity (56, 74, 101). Furthermore, fiber formation in a *knat1* mutant is insensitive to exogenous GA (56). Hence, GA is most likely acting through KNAT1 on xylem formation rather than KNAT1 on GA homeostasis. Alternatively, GA concentration and class I KNOX expression peaks may not overlap spatially, and class I KNOX action may deplete the cambium of GA and prevent premature differentiation of cambial stem cells and derivatives. The *Arabidopsis* hypocotyl provides a simple platform to address these open leads. Spatial coexpression patterns of GA reporters and class I KNOX transcription factors as well as GA measurements in class I KNOX mutants are approaches that will help clarify interactions between this class of homeodomain transcription factors and GA signaling.

WOX: WUSCHEL-RELATED HOMEOBOX

STM: SHOOT MERISTEMLESS

KNAT: KNOTTED-LIKE FROM ARABIDOPSIS THALIANA

MP: MONOPTEROS

3.3. Cytokinin

Cytokinin plays a central role in procambium/cambium initiation and development (28, 82, 91, 94). During procambium development in *Arabidopsis* roots, if cytokinin perception by histidine kinase receptors is disrupted or procambium cells are depleted of cytokinin by the expression of *CYTOKININ OXIDASE2* (*CKX2*), the rate of periclinal cell divisions in procambium is reduced, leading to the formation of small vascular bundles in which all vascular cells differentiate into protoxylem cells (79–81, 109). This suggests that cytokinin is critical for determining the identity of procambial cells and controlling their division activity. A mutually inhibitory feedback loop between cytokinin and auxin specifies the boundaries between the procambium and xylem in root vasculature (13) (**Figure 2*a***). In this loop, cytokinin controls the expression and bisymmetric localization of the auxin efflux carrier proteins PIN1 and PIN7, which direct auxin flow toward the central xylem axis. Conversely, auxin induces expression of *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*), an inhibitor of cytokinin signaling, in the xylem.

Another interaction between cytokinin and auxin that affects procambium initiation and regulation was recently discovered (28, 94): The basic helix-loop-helix (bHLH) transcription factor *TARGET OF MONOPTEROS5* (*TMO5*) was identified as a direct target of the auxin-dependent transcription factor MONOPTEROS (MP)/AUXIN RESPONSE FACTOR 5 (ARF5) (29, 111) (**Figure 2*a***). In the form of a heterodimer with another bHLH transcription factor, LONESOME HIGHWAY (LHW), TMO5 is crucial for vascular tissue establishment in *Arabidopsis* embryos and periclinal cell divisions in the primary root procambium (29, 94); ectopic coexpression of *TMO5* and *LHW* is sufficient to induce periclinal cell divisions. The closest homologs of *TMO5* and *LHW* in *Arabidopsis* also act redundantly in the regulation of cell division activity. Transcriptional profiling studies revealed that the cytokinin biosynthesis genes *LONELY GUY3* (*LOG3*) and *LOG4* act downstream of the TMO5-LHW complex (28, 94). However, the TMO5-LHW dimer accumulates in the xylem, which does not divide periclinally, and *LOG* gene expression overlaps with this domain. Moreover, higher-order *log* mutants can suppress the increased periclinal cell division

phenotypes associated with *TMO5-LHW* overexpression. These suggest that cytokinin, which is produced by LOG activity, acts downstream of this complex as a diffusible non-cell-autonomous factor and is required for TMO5-LHW-dependent periclinal cell division activity in procambial cells (28, 94).

A negative feedback loop restricts the levels of TMO5-LHW heterodimers (65, 140) (**Figure 2*a***). In this loop, TMO5-LHW and auxin upregulate *ACAULIS5* (*ACL5*) expression, which encodes for an enzyme that synthesizes thermospermine—a polyamine signaling molecule. Thermospermine promotes the accumulation of SAC51-LIKE (SACL) proteins, which are also members of the bHLH family. SAC51-LIKE proteins can dimerize with LHW protein, thereby competing with TMO5 protein for dimerization. By contrast, in the absence of thermospermine, translation of SAC51-LIKE open reading frames is blocked owing to the presence of a regulatory sequence in their promoters.

A recent study showed the importance of cytokinin-inducible PHLOEM EARLY DOF 1 (PEAR1) and PEAR2 transcription factors in the initiation of radial growth in *Arabidopsis* primary roots (89) (**Figure 2*a***). While *PEAR* genes are expressed in the phloem sieve element precursor cells, which generates the procambium through periclinal cell divisions as the development continues in the roots, protein products of *PEAR*s move across the neighboring cells. Mutant studies indicated that overexpression of these factors increases the number of cell files in the roots, and higher-order combinatorial mutants of *pear1*, *pear2*, and their closest homologs (*dof6*, *tmo6*, *hca2*, and *obp2*) display reduction in radial growth, suggesting that these mobile transcription factors redundantly control cell proliferation around the protophloem sieve elements. Interestingly, PEARs seem to induce expression of auxin-regulated *HD-ZIPIII*s. In contrast, HD-ZIPIII proteins antagonize the function of *PEAR*s on the xylem side of the procambium to inhibit periclinal cell divisions. Very recently, it was also revealed that downstream of TMO5-LHW-dependent cytokinin biosynthesis, *DOF2.1* and its closest homologs control vascular proliferation (120). Putative orthologs of *TMO5* and *LHW* are expressed in the wood-forming zones of aspen trees in a tissue-specific manner (127). It would therefore be interesting to determine whether cambial activity and wood formation can be induced by manipulating the expression of these genes in trees.

The regulatory effects of cytokinin on cambial activity during secondary growth have been probed by generating transgenic hybrid aspen trees that overexpress the cytokinin-degrading enzyme *CKX2* and therefore have reduced cytokinin levels (91). The resulting mutant trees exhibit fewer cambial cell divisions and have thinner trunks compared with wild-type trees. Cytokinin's effects on cambial development in *Arabidopsis* roots were studied via mutating members of the cytokinin biosynthesis gene family *ISOPENTENYLTRANSFERASE* (*IPT*) (82). Quadruple *ipt1 ipt3 ipt5 ipt7* mutants cannot form a cambium, leading to complete suppression of secondary growth in roots. This phenotype can be rescued by treatment with exogenous cytokinin. More recently, the effects of elevated cytokinin signaling on the vascular development of *Populus* trees were characterized (57), revealing that cambial cell division and biomass production are strongly stimulated upon increasing cytokinin levels in the stem by overexpressing *IPT7* under the wood-specific *LMX5* promoter.

Measurements of cytokinin and auxin levels in the wood-forming zone of *Populus* revealed that auxin concentrations are highest in the cambial zone, whereas cytokinin concentrations peak in the developing phloem (57, 131) (**Figure 2*b***). Interestingly, *pLMX5::IPT7* trees display elevated auxin levels in addition to increased cambial cytokinin levels and augmented signaling responses of both hormones in their respective expression domains (57). Studies of *Arabidopsis* root secondary growth demonstrated that the genes encoding the transcription factors *AINTEGUMENTA* (*ANT*) and *D-type cyclin CYCD3;1* are cytokinin-responsive positive regulators of cambial activity, suggesting that they may be some of the downstream members of cytokinin signaling pathways active during

vascular development (102). However, further studies will be needed to clarify the role of cytokinin and downstream partners in promoting cambial activity and wood formation.

3.4. Other Hormones

In addition to its role in tension wood formation (discussed below), the phytohormone ethylene promotes cambial cell divisions (38, 78). Treatment with either gaseous ethylene or aminocyclopropane-1-carboxylate (ACC; the precursor of ethylene) stimulates vascular cambial activity and wood formation in hybrid aspen trees (78). *ACC oxidase* (*ACO*), which encodes the enzyme that catalyzes the conversion of ACC into ethylene, is expressed strongly in developing secondary xylem of hybrid aspen. Moreover, *ACO* overexpression in hybrid aspen stems produces phenotypes similar to those observed in vitro, with transgenic trees displaying increased cambial cell proliferation and secondary xylem production. Genetic evidence suggests that ethylene also promotes radial growth in *Arabidopsis* (38): Whereas *ethylene overproducer1* (*eto1*) mutants display increased numbers of vascular cells in hypocotyls and inflorescence stems, mutation of *ETHYLENE RESPONSE FACTOR* (*ERF*) genes reduces the numbers of cells in the vascular tissues during primary and secondary growth.

A recent study also connected the branching hormone known as strigolactone (SL) to secondary growth regulation in *Arabidopsis* (1): Localized treatment with the synthetic SL analog GR24 stimulates cambial activity and secondary growth in inflorescence stems, whereas SL biosynthesis mutants *more axillary branches 1* (*max1*), *max3*, and *max4* and the SL-signaling mutant *max2* exhibit reduced cambium-initiated secondary growth in the stem. These results suggest that SL has a positive effect on meristematic activity in the vascular cambium. Interestingly, this regulatory activity is likely to be downstream of auxin signaling because auxin induces the expression of SL biosynthesis genes (52), and combining the *max1* or *max2* mutations with the auxin signaling mutation *auxin resistant 1* (*axr1*) does not increase the severity of interfascicular cambium defects (1).

Jasmonic acid signaling also promotes secondary growth (115). In *Arabidopsis*, jasmonic acid treatment stimulates lateral expansion of the interfascicular cambium. Furthermore, mutations to the *JASMONATE ZIM-DOMAIN10* (*JAZ10*) and *JAZ7* genes, which repress jasmonic acid signaling, stimulate interfascicular cambial activity, and increase basal stem diameter in the case of *jaz10*.

3.5. Peptide Signaling

Peptide-receptor signaling modules have diverse regulatory functions in plants (70). At least one of these signaling modules regulates procambium/cambium development in *Arabidopsis* and involves a proteolytically processed and posttranslationally modified 12-amino-acid-long peptide ligand called TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF) (55, 59, 95). TDIF is encoded by the *CLAVATA3* (*CLV3*)*/EMBRYO SURROUNDING REGION-RELATED 41* (*CLE41*) and *CLE44* genes in the *Arabidopsis* genome, which are expressed mainly in the phloem tissue and neighboring cells (39, 55, 59). Upon cleavage and modification, TDIF is presumably released into the apoplastic space and diffuses toward the cambial cells, where it is bound by the plasma membrane–associated leucine-rich repeat receptor-like kinase (LRR-RLK) protein PHLOEM INTERCALATED WITH XYLEM (PXY)/TDIF RECEPTOR (TDR) (39, 42, 55) (**Figure 2*b***). Interestingly, a recent study presented that SOMATIC EMBRYOGENESIS RECEPTOR KINASEs (SERKs) act as coreceptors of TDIF/CLE41 with PXY/TDR to control cell proliferation in the procambium (150). Interactions between the peptide ligand TDIF/CLE41 and the PXY/TDR receptor have three independent effects on vascular development-related

ERF: ETHYLENE RESPONSE FACTOR

TDIF: TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR

CLAVATA3 (CLV3)/EMBRYO SURROUNDING REGION-RELATED (CLE): family of signaling peptides

LRR-RLK: leucine-rich repeat receptor-like kinase

PXY: PHLOEM INTERCALATED WITH XYLEM

processes: They promote vascular cell divisions in the procambium/cambium, inhibit xylem cell differentiation, and control vascular patterning (39, 54, 55, 143) (**Figure 2*b***). Most information on the roles of *CLE41* and *CLE44* genes comes from studies involving their overexpression in plants or peptide treatment assays. In keeping with their postulated roles, *35S::CLE41* plants display stunted growth with vascular organization defects such as a striking xylem mixed with phloem phenotype in both inflorescence stems and hypocotyls. These mutant plants also exhibit increased vascular cell proliferation in hypocotyls and interrupted xylem vessel formation in leaves.

WOX4 and *WOX14* homeodomain transcription factors are downstream targets of the TDIF/CLE41-PXY/TDR signaling pathway regulating vascular cell divisions in *Arabidopsis* (37, 54) (**Figure 2*b***). Both genes display cambium-associated expression patterns in inflorescence stems and hypocotyls. Single *wox4* or *wox14* mutants exhibit reduced cell division activity in the cambial tissue, and double mutants exhibit more severe cell division defects, suggesting that *WOX4* and *WOX14* act redundantly and positively to regulate cambial activity in *Arabidopsis*. Moreover, expression of both genes is induced by overexpression of *CLE41* and in response to synthetic TDIF/CLE41 peptide, suggesting that they are positively regulated by TDIF/CLE41-PXY/TDR signaling. Earlier reports showed that expression of the *WOX* homeodomain transcription factors *WUSCHEL* (*WUS*) and *WOX5* is regulated by the CLE peptide family members CLV3 and CLE40, respectively, to control stem cell differentiation in the SAM and the RAM (18, 113, 122). Therefore, despite anatomical differences between apical and lateral meristems, and differences in the nature (positive or negative) of the regulatory effects on stem cell division activity, all three major meristems—SAM, RAM, and procambium/cambium—may be regulated by *WOX* transcription factors downstream of CLE peptide signaling. It remains to be determined whether a similar mechanism regulates the activity of cork cambium in trees (also see the sidebar titled The Cork Cambium). Expression of several *ERF*s is induced in *pxy/tdr* or *wox4* mutant backgrounds (38) (**Figure 2*b***). As mentioned above, ERFs are also required for normal vascular cell division activity in the procambium/cambium, and the combination of *pxy/tdr* mutations with ethylene signaling mutations increases the severity of these cell division defects. These results suggest that ethylene signaling and TDIF/CLE41-PXY/TDR signaling also interact at the genetic level during vascular development.

Very recently, orthologs of *CLE41*, *PXY/TDR*, and *WOX4* genes have also been characterized in hybrid aspen (36, 69). Co-overexpression of *CLE41* and *PXY/TDR* genes in hybrid aspen under tissue-specific promoters corresponding to the original expression domains of both genes

THE CORK CAMBIUM

Although the vascular cambium is primarily responsible for secondary growth, the cork cambium (also referred to as phellogen) also contributes (40). By dividing periclinally, the cork cambium produces the cork (also referred to as phellem) toward the outside of the stem/root and the phelloderm toward the inside. These tissues are collectively known as the periderm, and their role is to protect the vasculature against biotic and abiotic stresses. Early anatomical studies revealed that the cork cambium in roots originates from the pericycle, whereas in stems it can originate from several tissues including the epidermal layers, subepidermal layers, or phloem. Like the vascular cambium, the cork cambium's cell division activity is sensitive to seasonal and climatic changes. Although development and regulation of the cork cambium have been studied less extensively than those of the vascular cambium, a recent publication showed that periderm development in roots and hypocotyls of the model plant *Arabidopsis* proceeds in a fashion similar to that seen in woody species (146). This work also included anatomical observations and marker analyses that were used to determine the ontogeny of periderm development.

increases wood formation (36). Other experiments showed that *WOX4* plays a major role in controlling cell identity and division activity in the vascular cambium of hybrid aspen (69). Downregulation of *WOX4* homologs by RNA interference in hybrid aspen causes more dramatic phenotypic changes than is observed in annual species; in the most extreme cases, the resulting reductions in cambial activity and wood formation are severe enough to prevent the trees from remaining upright.

BIL1: BRASSINOSTEROID-INSENSITIVE 2-LIKE 1

WOX4 expression can be enhanced by auxin treatment in *Arabidopsis* stems (**Figure 2*b***), and both *PXY* and *WOX4* are vital for auxin-dependent cambium stimulation (124). Remarkably, a recent study showed that the auxin-dependent transcription factor MP/ARF5 promotes xylem production from cambial cells (**Figure 2*b***) and regulates *WOX4* expression possibly by binding to the *WOX4* promoter (17).

A recent study of young roots of *Arabidopsis* showed that locally high auxin signaling on the xylem side of the cambium defines a stem cell organizer of the vascular cambium (121) (**Figure 2*c***). In the organizer, expression of *HD-ZIPIII* genes is induced through *MP/ARF5*, *ARF7*, and *ARF19*, which maintain the organizer cells in a nondividing state and promote stem cell identity in the adjacent cells. Stem cells display high expression of *ANT*. This study also exhibited that *PXY/TDR* and *WOX4* genes are expressed highly on the xylem side of the cambium in the organizers and act downstream of *ARF*s and *HD-ZIPIII*s. However, how this new data fits with known roles/signaling pathways of PXY/TDR and WOX4 needs to be studied further. Interestingly, *MP/ARF5* was also able to induce *PEAR1* gene expression non-cell autonomously on the phloem side of the cambium; therefore, future research should investigate how organizer cells regulate stem cell identity in the adjacent cells and *PEAR1* expression on the phloem side of the cambium, as well as regulation of differential proliferation rates of the xylem and phloem cells on both sides of the vascular cambium.

Although WOX4 activity is necessary for regulating vascular cell divisions in the procambium/cambium downstream of the TDIF/CLE41-PXY/TDR signaling module, it is not involved in the repression of xylem cell fate (68). Instead, it appears that GLYCOGEN SYNTHASE KINASE 3 (GSK3) proteins, including BRASSINOSTEROID-INSENSITIVE 2 (BIN2), regulate xylem differentiation by repressing the transcription factor BRI1-EMS SUPPRESSOR 1 (BES1) (**Figure 2*b***). There is thus a link between brassinosteroid signaling and TDIF/CLE41-PXY/TDR signaling (68). Conversely, WOX14 promotes vascular cell differentiation and lignification in *Arabidopsis* inflorescence stems by increasing the concentration of bioactive GAs (31). Hence, the functions of WOX4 and WOX14 diverge at the level of vascular cell differentiation.

There is evidence that EPIDERMAL PATTERNING FACTOR-LIKE (EPFL)-ERECTA (ER) signaling interacts with the TDIF/CLE41-PXY/TDR signaling module to regulate vascular patterning (37, 134, 135) (**Figure 2*b***). Accordingly, *Arabidopsis* plants bearing the *er* or *epfl4 epfl6* mutations together with the *pxy/tdr* mutation exhibit more severe vascular organization defects than do single *pxy/tdr* mutants during both primary and secondary vascular development. However, the nature of the interaction between EPFL-ER signaling and TDIF/CLE41-TDR/PXY signaling is unclear because the active ER and PXY/TDR signaling domains are different (37, 42, 55, 135). The genes encoding EPFL4 and EPFL6 are expressed mainly in endodermal tissues (134, 135). Although *ER* is expressed more widely in the epidermis, phloem, and xylem tissues, ER activity in the phloem is necessary and sufficient for its correct function. Moreover, a recent report showed that ER and its paralog ERECTA-LIKE1 (ERL1) control vascular growth in hypocotyls of *Arabidopsis* by preventing premature GA-mediated fiber formation (56).

Very recently a connection between cytokinin signaling and the TDIF/CLE41-PXY/TDR signaling module was identified in the regulation of *Arabidopsis* cambial development (51). As mentioned above, most GSK3 family members suppress xylem differentiation, but one, BRASSINOSTEROID-INSENSITIVE 2-LIKE 1 (BIL1), seems to inhibit cambial activity

by interacting with and phosphorylating the auxin-dependent transcription factor MP/ARF5 (**Figure 2*b***). *Arabidopsis bil1* mutants display increased cambial activity and vascular tissue production in both the inflorescence stems and hypocotyls. Overexpression of a phosphorylated version of *MP/ARF5* suppresses these phenotypes observed in *bil1* mutants, suggesting that BIL1-mediated MP/ARF5 phosphorylation is required for negative regulation of the cambial activity. Interestingly, the effect of BIL1 is independent of *WOX4*; instead, it acts via the A-type *Arabidopsis response regulator* (*ARR*) genes *ARR7* and *ARR15* (**Figure 2*b***), which are negative regulators of cytokinin signaling. These *ARR*s are upregulated by MP/ARF5, and knocking out either of them increases cambial activity. In return, PXY/TDR can suppress the BIL1-MP/ARF5 interaction (**Figure 2*b***), and combining the *bil1* mutation with *pxy* can recover the effects caused by *pxy*. Overall, these results suggest that the TDIF/CLE41-PXY/TDR module acts upstream of the BIL1-MP/ARF5 module and weakens the effect of MP/ARF5 on *ARR7* and *ARR15* expression via BIL1, thereby increasing vascular cambial activity.

***Arabidopsis* response regulator (ARR):** A-type response regulator involved in cytokinin signaling

LRR-RLKs other than PXY/TDR that may regulate cambial cell proliferation were also identified by transcriptomic analysis of samples collected during interfascicular cambium initiation and secondary growth in *Arabidopsis* inflorescence stems. Notable examples include MORE LATERAL GROWTH1 (MOL1) and REDUCED IN LATERAL GROWTH1 (RUL1), which repress and promote cambial activity, respectively (2). Although the interacting peptide ligands of these LRR-RLKs remain to be identified, a recent study showed *MOL1* is expressed on the distal side of the cambium. Additionally, in double mutants of *mol1* with either *pxy/tdr* or *wox4*, the enhanced cambial cell proliferation phenotype in interfascicular regions of inflorescence stems is diminished, suggesting that *WOX4* and *PXY* may be epistatic to *MOL1* (49).

3.6. Mechanical Signals

Pressure applied to the surface of a callus induces cell division in the plane perpendicular to the stress vector (148). Similarly, the orientation of the cell plane in the epidermis of the SAM is determined by tensile forces (77), rather than by the geometric rule of the smallest division plane area through the center of a cell (10). Components of the cytoskeleton are reoriented in response to mechanical stress on the cell wall and, in turn, control the orientation of the phragmoplast (26, 145). Rearrangement of the microtubules is accompanied by a realignment of the auxin transporters in response to mechanical stress, and auxin concentration gradients may override or modify the minimal plane area rule governing the cell division plane's orientation (77, 149). With the exception of ray stem cells, cambial division planes do not follow the minimal division plane area rule, so the orientation of their division planes is presumably determined by factors other than cellular geometry, such as tensile forces and auxin concentration gradients. Evidence for such modes of action derives from decapitation experiments in *Arabidopsis*. When inflorescences, i.e., primary stems, are decapitated and additional weight is placed on the site of decapitation, secondary growth in the hypocotyl is stimulated (67, 85). Experiments involving pharmacological interference with auxin transport revealed that polar auxin transport is required for this weight-induced increase in radial growth (67). Stress patterns have not yet been predicted for the cambium, but the wavy periclinal walls of dividing cambial cells (40, 104), if not a sectioning artifact, may indicate that centripetal and centrifugal forces also have important effects in cambial stem cells. It remains to be seen whether compression induced by placing a weight at the site of decapitation imposes additional tensile forces on cambial stem cells and whether the cytoskeleton and localization of auxin transporters align with these forces.

Independently of auxin gradients, *STM* may be involved in regulating cambial activity in response to mechanical stress, and *STM* expression can be induced in the SAM by micromechanical

manipulation and may affect shoot patterning (71). In conjunction with KNAT1 activity, STM positively regulates cambial activity in the *Arabidopsis* hypocotyl; an *stm knat1* double mutant exhibits severe distortions of cambial organization (74). Simple decapitation experiments in an *stm* loss-of-function background could be performed to determine whether STM integrates mechanical signals into enhanced cambial activity and thereby helps align secondary growth with the increasing weight of the inflorescence or crown of a growing plant.

Crown weight is not the only possible source of mechanical signals interpretable by the cambium; other potentially important signals include bending stresses (see Section 4.4), compression due to strains caused by unequal growth dynamics between the cork and vascular cambium, and forces related to water transport and cell turgor. Vessel elements or tracheids differentiate in close proximity to the cambium, going from having a high internal pressure (i.e., turgor) to the negative pressure required for water conductance. It is likely that their differentiation and changing internal pressure produce tensile forces within cell walls that are transmitted to the cambial stem cells. Likewise, diurnal or seasonal patterns in water conductance that cause measurable changes in stem diameter (21) may produce mechanical cues interpreted by the cambium. It is tempting to speculate that such cues related to xylem water conductance could contribute to the determination of cell division plane patterns and regulate daily and seasonal rhythmicity and abiotic stress-induced changes in cambial activity. For example, drought stress could be rapidly sensed in cambial stem cells via the transmission of mechanical cues from water-conducting elements, and stem cells could be protected from irreversible damage by, for example, adjusting cell growth. Numerous experiments have shown that osmotic stress strongly affects wood characteristics. Interestingly, in drought-stressed poplar trees, cambial activity declines together with the activity of the cytokinin-signaling reporter gene *ARR5::GUS* (99).

4. PLASTICITY OF CAMBIAL ACTIVITY

Cambial activity is highly plastic throughout a plant's life; cell division, expansion rates, cell-type specification, and differentiation in the cambium can all be varied in response to environmental and developmental factors. These responses may protect the cambium against abiotic stresses such as increased mechanical strain as the plant grows or reduce the impact of drought stress by inducing stem cell quiescence during dry winters.

4.1. Juvenile and Mature Wood

In response to environmental factors and developmental cues, the SAM can undergo a transition from vegetative to floral status. As a result of this transition, stem cell derivatives acquire the fate of floral organs rather than leaves. Somewhat similarly, the vascular cambium has two distinct developmental stages: juvenile (also referred to as core wood) and mature (**Figure 3**). The nature of the wood that is formed depends on the age of the vascular cambia: Young cambia produce juvenile wood, and old cambia produce mature wood (155). Juvenile wood, found in gymno- and angiosperm trees, is characterized by thin walls, low density, and a greater radial increment than that of mature wood (44, 147). Breeding for increased radial growth may have increased the proportion of economically less valuable juvenile wood in *Pinus radiata* (144) and possibly other species. In gymnosperms, the transition from juvenile to mature wood occurs between the tenth and seventeenth annual rings (11, 154). Juvenile wood is formed throughout a tree's lifespan in proximity to the crown, whereas mature wood is formed further from the crown. Therefore, in the context of wood formation, juvenile and mature refer to positions within the tree and cambial age rather than tree age. The ratio of juvenile to mature wood decreases as a tree ages, so management of the cambial transition is very important for woody short-rotation crops.

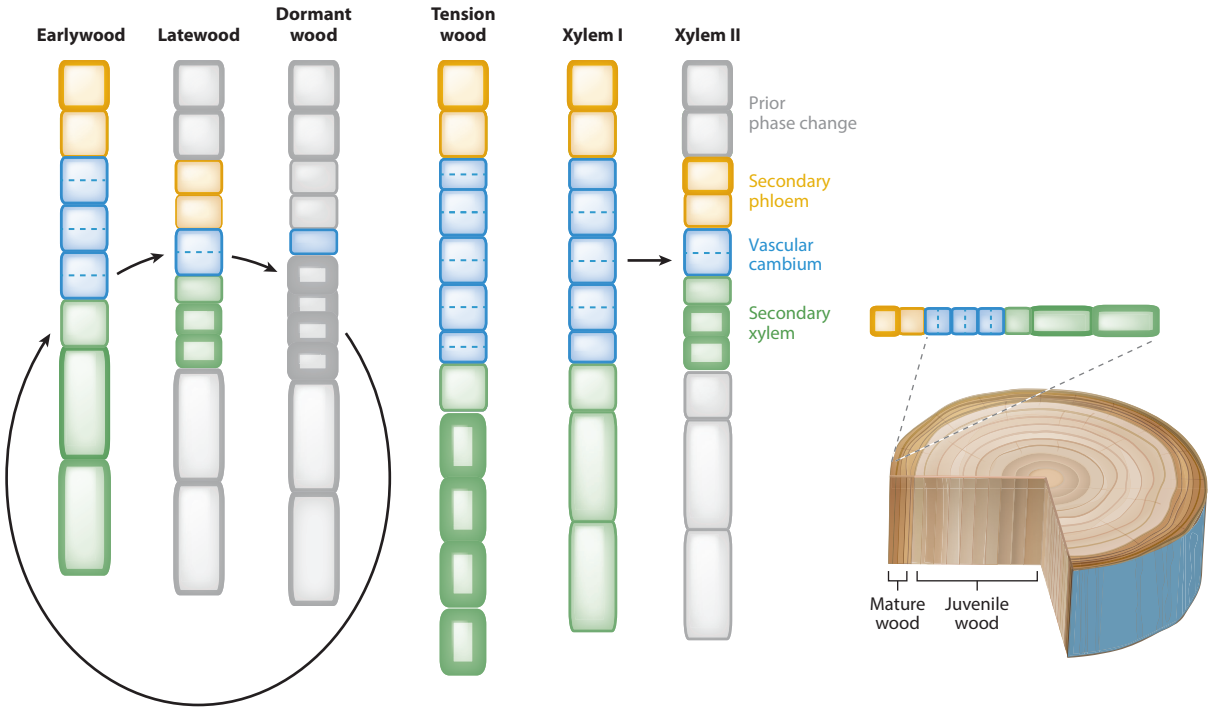


Figure 3

Phase changes in cambial development. Early-late-dormant wood transitions are annually reoccurring developmental phases in trees of temperate and boreal climates. By contrast, xylem I to xylem II and juvenile to mature wood transitions are irreversible, and tension wood formation is a transient developmental stage. The size of the cambial zone and the radial extension of stem cells and transient amplifying cells are indicative for cambial stem cell activity, with dashed lines representing the dividing cells and line strength representing cell wall thickness.

Different models have been proposed to explain the regulation of the transition from juvenile to mature wood. One such model suggests that the transition is controlled by the accumulation of auxin in the crown's vicinity and the progressive decline in auxin levels farther from the crown (142). Because auxin is predominantly synthesized in the green parts of the tree and transported rootward, this hypothesis seems plausible. Moreover, as was suggested for the regulation of the radial increment, a crown-to-root auxin gradient (12) could provide positional information to guide the transition. Relatively high auxin concentrations near the crown would correlate with the higher cambial activity characteristic of juvenile wood formation, whereas lower auxin concentrations nearer the roots would be associated with the lower cell division activity seen in mature wood. High auxin concentrations in the cambium could also translate into a broader radial auxin gradient and extend the duration of the cell expansion phase while shortening the secondary wall deposition phase, producing the typical characteristics of juvenile wood. Notably, juvenile and early wood are similar in nature, as are mature and late wood. Comparatively little evidence supports the involvement of regulators other than auxin in the early to late wood transition. However, independent proteomics and transcriptomics studies of *Pinus* sp. have tentatively identified ethylene as a potential regulator of the transition (46, 73). ACO accumulates in juvenile wood (46), which could explain its greater radial growth compared with mature wood. Alternative hypotheses to regulation by auxin and ethylene include regulation based on the distribution of

photosynthates from young bark (47), induction of mature wood formation by mechanical cues (112), and induction by an intrinsic cue based on the developmental age of the cambium.

Functional studies of the transition between juvenile and mature are hampered by the long time required for the induction of mature wood formation. Descriptive studies in this area have focused on wood quality rather than cambial properties (46, 73). Knowledge about juvenile wood derived from greenhouse-grown young trees may not be directly relevant to mature wood formation. However, in the absence of a model describing the transition from early to mature wood formation, knowledge about developmental cambial plasticity will necessarily depend solely on descriptive work.

4.2. Seasonality and Cambial Dormancy

Unlike annuals, perennials have annual growth cycles that are temporally coordinated with seasonal changes. These seasonally synchronized growth cycles constitute a key adaptive developmental program that protects the meristems from harsh environmental conditions such as extreme cold during winter in temperate and boreal regions or drought in the tropics and subtropics. Accordingly, meristems undergo the transition from active growth to growth cessation and induction of dormancy prior to the initiation of winter in boreal or temperate regions of the world. In the dormant state, meristematic activity is arrested by transitory nonresponsiveness to growth-promoting signals. After dormancy and favorable conditions return, meristematic activity resumes. Annual, seasonally synchronized activity-dormancy cycles result in the formation of distinct annual rings in perennials. In recent years, some progress has been made toward understanding the molecular basis of seasonal control over vascular cambium activity in woody plants, particularly in the model tree hybrid aspen (5, 35, 114). The perception of short days heralding winter causes cambial activity to cease and eventually induces dormancy. Whereas induction of dormancy upon short-day perception is mediated by phytohormone abscisic acid (133), modulation of auxin response mediates photoperiodic control of the cambial activity-dormancy transition. It was initially assumed that auxin levels in the cambium regulated both growth arrest and dormancy induction. However, careful high-resolution analyses of active and dormant cambial cells indicated that both cell types had very similar auxin levels (137). Moreover, Little & Bonga (75) observed that treatment with exogenous auxin did not stimulate cambial activity in dormant cells and that this nonresponsiveness could be reversed by exposure to dormancy-breaking low temperatures. These results suggest that some other mechanism, most likely a shift in the cambium's responsiveness to growth-promoting auxin, may underpin the activity-dormancy transition in the vascular cambium. Baba et al. (5) reported a temporal analysis of transcriptomic auxin responsiveness in hybrid aspen stem tissues following short-day treatment, revealing a progressive change in transcriptomic auxin responsiveness. Two distinct phases of auxin responsiveness loss were observed, the first of which coincided with cessation of cambial activity (growth arrest) and the second with the transition to dormancy. Interestingly, the second phase correlated with downregulation of the tree homolog of the *Arabidopsis* auxin receptor TIR (32, 66) and reduced ubiquitination of AUX/IAA repressors. This reduced AUX/IAA ubiquitination was tentatively attributed to downregulation of *TIR1*, which would be expected to stabilize these repressors and suppress the cambium's auxin response, inducing the dormant state. Interestingly, Baba et al. (5) also showed that some genes retained their auxin responsiveness even during cambial dormancy. These results may indicate that auxin has two roles: maintaining cambial meristem identity and stimulating cambial cell division. During dormancy, cambial cell division activity must be arrested, but cambial meristem identity must be preserved even during the dormant state. Therefore, although the genes involved in the cambium's cell division activity are switched off in response

to short days, the genes that determine cambial meristem identity remain auxin responsive even during dormancy. In summary, perennials possess conserved mechanisms that maintain cambial identity, but they have also evolved regulatory pathways that mediate seasonal control over cambial activity and that are not present in annuals. Although induction of cambial dormancy is now reasonably well understood, the release of dormancy and the restoration of auxin responsiveness will have to be studied further to explain fully the unique cambial properties of perennials.

4.3. Regeneration Potency of Cambia

Cambial stem cells are pluripotent, with the capacity to form a limited number of different phloem and xylem cell types. Like animal stem cells, cambial stem cells contribute to both growth and the repair of damaged tissue. In this respect, they may differ from apical meristems, which are replaced by lateral independent stem cell populations if lost. Consequently, many dicots can be efficiently propagated from stem/internode cuttings even in the absence of exogenous phytohormones because callus formation is initiated at the cut surface, from which adventitious roots or shoots can develop, and damaged stem tissue can be repaired by wound tissue. During repair, cambial stem cells may become more versatile and produce cell types that are normally contributed by other stem cell populations. It is not clear whether the increased potency of cambial stem cells involves dedifferentiation or transdifferentiation (63). In some angiosperms, the wound tissue that closes the cambial cylinder originates from the xylem parenchyma and developing xylem cells (98, 123, 151). Early wound tissue typically consists of a callus that differentiates in later vasculature, establishing a new cambium. Therefore, some xylem parenchyma or developing xylem cells reinitiate cell division activity. It has not been established whether these cells should be considered quiescent stem cells or dedifferentiated cells. In *Populus* cuttings, adventitious root formation from calli at the cut surfaces is promoted by auxin and repressed by GA signaling (84, 118). Repression of the TIR1-like auxin receptor *PagFBL1* delays adventitious root formation in *Populus* ssp. (118), whereas its overexpression increases the number and size of the regenerates. *PagFBL1* is expressed in the cambium and the secondary phloem during the initiation of adventitious roots from cuttings, suggesting that cambial stem cells may be involved in this regeneration. It would be interesting to determine whether auxin and GA are involved in a potential dedifferentiation or transdifferentiation process or regulate only growth rates. In the gymnosperm *Pinus canariensis*, wound healing occurs from the margins of the wounds and probably involves cambial stem cells. Global transcript analysis revealed that during wound healing, genes associated with earlywood formation are induced and latewood genes are repressed (22).

Although graft surfaces are commonly cut so as to expose large portions of the cambia in both scion and stock, we are far from understanding how the cambium contributes to the formation of wound tissue and the formation of joint vasculature in graft unions. It is tempting to suggest that cambial stem cells contribute to the wound tissue in graft unions. For functional axial union, it is more important that vessel elements of the scion and the stock connect than that coordinated growth is established. Data from primary tissues in pea and *Arabidopsis* support the hypothesis that auxin plays a role in vascular union (86, 88, 107), with PIN proteins rapidly making auxin available to the stock. Although auxin transport in secondary tissue probably also depends on the activity of PIN proteins expressed in the cambium, the role of auxin in the union of woody structures remains to be determined. Interestingly, there are only a few reports of successful grafting from monocots, which may be related to the observation that the primary vasculature (i.e., veins) of monocots cannot join in leaf blades (87). It is further tempting to speculate that the same mechanism prevents lateral union of individual vascular bundles in monocot stems and thus prevents cambial cylinder formation.

4.4. Tension Wood

Reaction wood, known as tension wood in angiosperms and compression wood in gymnosperms, is commonly characterized by asymmetric cambial growth, changes in cell wall ultrastructure and chemical composition, and modified xylem anatomy (41, 48). The tension wood of angiosperms has recently attracted interest because of its low lignin content, which is attractive for pulping and bioethanol production (20, 108). Less attention has been paid to how cambial activity is regulated during tension wood formation. Formation of tension wood in angiosperms and compression wood in gymnosperms is induced upon displacement of a woody branch or stem from its original growth axis. Both mechanical and gravitropic stimuli can contribute to tension wood formation. However, as demonstrated by the effect of bending woody branches into loops, the gravitational cue may override the mechanical stimulus because tension wood forms only in the upper and lower parts of such loops rather than along their entire length as would be expected if mechanical stimulus alone were sufficient to induce its formation (60). Induction of tension wood formation in response to positional displacement may involve a mechanism similar to that established for other tropic responses, whereby an asymmetric distribution of auxin develops and causes the growth rate of tension wood to differ from that of opposite wood. In keeping with this hypothesis, opposite wood exhibits lower radial growth rates and auxin concentrations than tension wood (53), and the induction of tension wood formation alters the expression of several auxin transporter and signaling genes (4, 90, 100). Significantly, PIN3, which redirects auxin flow in response to gravistimulation in primary organs of *Arabidopsis*, localizes to plasma membranes oriented toward the cambium in tension wood and toward the cortex in opposite wood (153). Consequently, auxin concentrations in the two wood types may differ, resulting in asymmetric growth.

The reported effects of exogenous auxin treatment on the regulation of tension wood formation are inconsistent (41), but treatment with GA induces upward bending and increased asymmetric cambial activity in the branches of several tree species (62, 93). Moreover, application of GA to the main stem increases cambial activity (64) and induces ultrastructural changes of cell walls typical for tension wood (43).

In *Populus* tension wood, expression of ethylene biosynthesis genes is strongly induced, and elevated ethylene levels were detected during reaction wood formation in angiosperm and gymnosperm trees (3, 116). Moreover, treatment with ethylene or ACC stimulated local cambial activity in *Populus* (78), and expression of several *ERF*s was upregulated during tension wood formation. Significantly, overexpression of *ERF18*, *ERF34*, and *ERF35* increases stem diameter (139). Together, these results suggest that differences in ethylene signaling between tension and opposite wood may contribute to asymmetric cambial activity and stem growth.

5. PERSPECTIVES IN THE STUDY OF VASCULAR CAMBIA

In comparison to primary vasculature, much remains unknown about the molecular regulation of the development of secondary vasculature. Future research should focus on closing this knowledge gap and will likely involve important developments in technology to make secondary growth more accessible to molecular and analytical tools.

Live imaging has greatly added to our understanding of developmental processes. Temporally highly resolved subcellular spatial resolution and live-cell tracking have helped to unravel molecular mechanisms underlying complex developmental processes as patterning, tropisms or organ size homeostasis (30, 33, 141). Because other tissues cover the vascular cambium, live imaging by conventional confocal microscopy is impossible. Other methods for nonintrusive live imaging are currently not in reach. Mathematical models using anatomic, gene expression, and protein

localization data could be potentially useful in simulating growth and underlying molecular processes. Rapid tissue sectioning and processing methods and automated image analysis, which are required for such an approach, have been developed for the *Arabidopsis* hypocotyl (50). A future challenge will be to apply this technology to tree models. Tree models may also become increasingly important thanks to the advent of genome editing. Bi-allelic recessive mutations generated by genome editing will further facilitate functional studies of regulators of cambial activity in trees.

SUMMARY POINTS

1. The vascular cambium—a secondary lateral meristem—contains the stem cells and transient amplifying cells that produce the secondary phloem (bast) and secondary xylem (wood).
2. Regulation of cambial activity is complex, involving hormones, peptides, and environmental and mechanical cues.
3. Shoot-derived auxin forms a concentration gradient with a maximum in the cambium. Auxin is important for cambial activity and may also regulate radial cell expansion and differentiation of cambial derivatives.
4. Plant hormone cytokinin has the highest concentration in the developing phloem tissue. Cytokinin is a positive regulator of cambial activity and necessary for the cambium development.
5. Active gibberellic acid (GA) concentration peaks in young xylary cambial derivatives. GA promotes cambial activity and cell elongation of cambial derivatives.
6. The TDIF/CLE41-PXY/TDR ligand-receptor system serves as a major hub in the control of cambial cell division activity, cell differentiation, and vascular patterning.
7. In perennials, i.e., trees, cambium displays seasonally synchronized cycles of cell division and is mediated by modulation of auxin response of the cambial meristem.
8. Ethylene and abscisic acid play important roles in the regulation of cambial plasticity in response to mechanical and environmental stimuli.

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