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Genetic Networks in Plant Vascular Development

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Abstract

Understanding the development of vascular tissues in plants is crucial because the evolution of vasculature enabled plants to thrive on land. Various systems and approaches have been used to advance our knowledge about the genetic regulation of vasculature development, from the scale of single genes to networks. In this review, we provide a perspective on the major approaches used in studying plant vascular development, and we cover the mechanisms and genetic networks underlying vascular tissue specification, patterning, and differentiation.

INTRODUCTION

Vascular bundle: an assembly of xylem, phloem, and (pro)cambial cell files; also known as the vascular cylinder in roots

Searches for information about vascular development typically yield an impressive number of results from medical research. A cardiovascular network consisting of a heart and blood vessels is formed very early during organogenesis and is essential in supporting vertebrate life. In plants, an equally crucial vascular system of connective tubing is specified during early embryogenesis; at the early globular stage, cells that will develop as vascular initials can already be identified (135). From an evolutionary perspective, the invention of vasculature has been crucial to enabling plants to thrive on land (84, 158) because these tissues provide rapid, controlled delivery paths throughout the plant, as well as mechanical support. The plant vascular system is complex: each vascular bundle that consists of three cell types is arranged in a highly organized manner, and each cell type has a specialized function (**Figure 1**). The middle layer, the (pro)cambium, is composed of cells with meristematic capacity, which remain inactive at the early procambial stage. These pluripotent

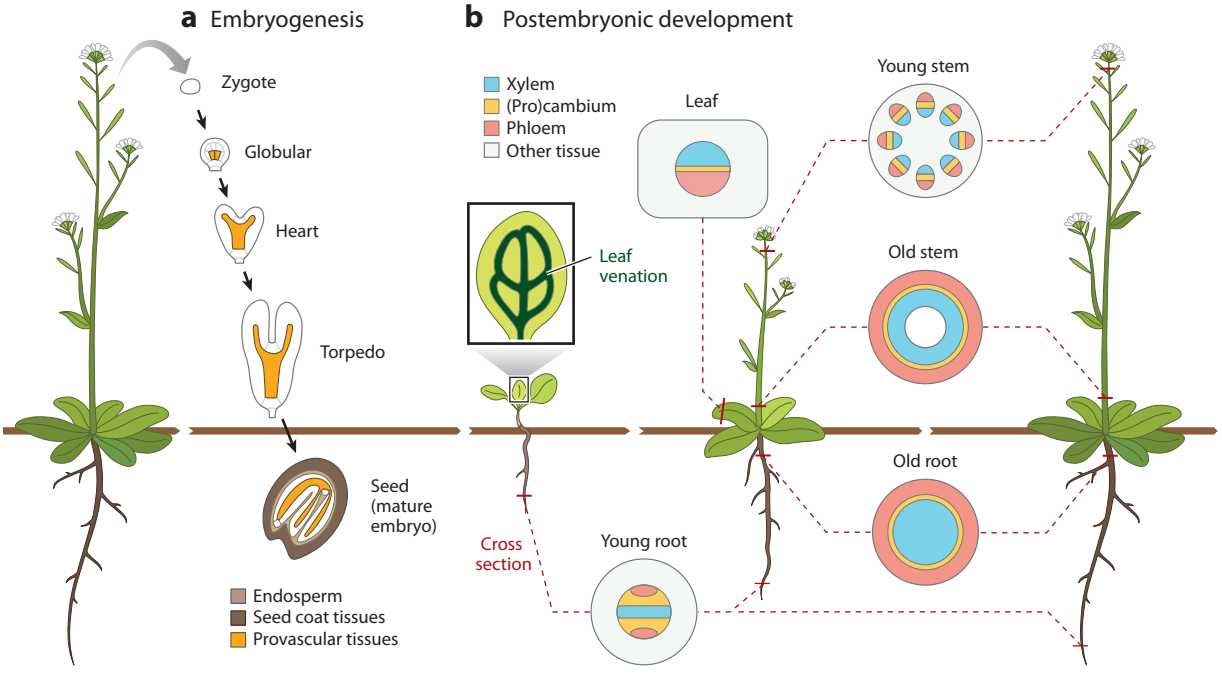

Figure 1

Vascular development in dicot angiosperms. (*a*) Vascular development begins with the periclinal division of the lower tier of cells (*orange*) in globular-stage embryos. Throughout embryogenesis, controlled division of these cells forms the well-organized provascular tissue in the center of the developing embryo; embryogenesis can be divided into five stages: zygote, globular, heart, torpedo, and mature embryo. (*b*) The vascular bundles in newly developing postembryonic organs originate from the shoot apical meristem or the root apical meristem. Intriguingly, despite the structural differences among vascular bundles in different organs and growth stages, the vascular system tightly connects all of the organs, indicating that vascular patterning is highly organized at the whole-plant level. Newly emerging leaves develop a reticulate vein structure (leaf venation) starting from the mid-vein, which is connected to the rest of the plant body; phloem develops on the abaxial side (under, or facing away from the shoot meristem), and xylem develops on the adaxial side (upper, or facing toward the meristem). A uniform structure is seen not only in the leaf vascular bundles but also in other plant organs. In the young root, the central xylem axis is sandwiched by intervening procambial cells and phloem; it will later develop to have a central core of xylem tissue surrounded by cambium and phloem tissue. In the young stem, individual vascular bundles composed of procambial cells between xylem (*inner*) and phloem (*outer*) are disconnected, but they become connected in older stems and give rise to continuous xylem tissue on the inside that is surrounded by cambium and then phloem tissue on the outside. Data from References 24, 46, 69, and 84; figure adapted from References 25 and 138 with permission.

cells give rise to cells that differentiate into xylem toward the inner side of the vascular bundle and phloem toward the outer side. Xylem and phloem cells are specialized for, respectively, water and nutrient transport, and they also facilitate the transport of various signaling molecules. Later in development, the plant may also expand laterally, when the cambial cells are activated, and divide periclinally; this is typically referred to as secondary vascular development. In this review, we discuss the processes of vascular specification and differentiation and touch briefly on secondary vascular development and lateral growth. We aim to emphasize the various genetic approaches and plant systems that have improved our understanding of plant vascular development and to provide an overview of signaling mechanisms that have been recently reviewed in detail (16, 25, 80, 110). We start with a historical overview of the major approaches that have been valuable in this important research field.

Classical descriptive and experimental studies on plant vascular development established the anatomical and physiological foundations of the research field (137). For instance, the reticulated vein networks in the foliage leaves of various angiosperms and the well-organized vascular cylinder of roots were described between the 1850s and early 1900s (42, 146). The importance of plant hormones in regulating vascular patterning was highlighted during the same period. In the 1930s, treatment with auxin was shown to induce cambial growth in various flowering plant species, including sunflower, *Coleus*, *Vicia faba*, and *Tradescantia* (139). Young (162) provided experimental evidence that the young leaves of *Lupinus albus* are a source of auxin for the stem, inducing procambial development as well as xylem differentiation, implying that auxin transport is crucial for vascular patterning. Later, cytokinins were suggested to also be important signaling molecules in vascular patterning on the basis of phenotypic changes observed in the vascular tissue of pea epicotyls upon cytokinin treatment (140).

Before 1980, most studies of xylem formation used entire multicellular organisms, which made it difficult to investigate the stages of xylem cell differentiation. To overcome this problem, Fukuda & Komamine (44) established a method to isolate single mesophyll cells from *Zinnia* leaves and initiate differentiation of the mesophyll cells into xylem vessels [tracheary elements (TEs)]. Since then, the *Zinnia* cell culture system has been used to investigate the morphogenesis of xylem cells in detail, including secondary cell wall formation, programmed cell death, and genome-wide changes in expression during xylogenesis (29, 45, 113). In addition, the *Zinnia* cell culture system was crucial in identifying the signaling peptides important for cambial proliferation, known as tracheary differentiation inhibiting factor (TDIF) (61, 67). In 2005, microarray analysis of xylem vessel formation using *Arabidopsis* suspension cells identified key transcription factors involved in *Arabidopsis* xylem differentiation, VASCULAR-RELATED NAC DOMAIN 6 (VND6) and VND7. The in vivo physiological significance of these transcription factors was validated by reverse genetics approaches in *Arabidopsis* and *Populus* (79). The vascular cell induction culture system using *Arabidopsis* leaves (VISUAL) was established more recently and has been used to investigate the genome-wide expression pattern of genes during phloem differentiation (78).

Since the 1980s, *Arabidopsis thaliana* has served as a prominent model plant not only for investigating vascular development but also for other research fields due to its relatively short life span, small genome size, ease of crossing, and amenability to saturation mutagenesis screens in the laboratory (121). Indeed, in the early 1990s, various mutants were isolated from forward genetic screens of *Arabidopsis* mutant pools produced by a variety of methods, including ethyl methanesulfonate (EMS) treatment, gene traps, T-DNA insertions, and transposon insertions, thus accelerating studies of the genetic networks governing vascular development in plants (8, 48, 95). For instance, *MONOPTEROS* (*MP*), an auxin-inducible transcription factor that has a crucial role in vascular patterning in both embryonic and postembryonic organs, was identified from an EMS-mutagenized mutant pool (95). To elucidate the genetic networks involved in the *MP*

Cambium: lateral meristem that generates secondary xylem and secondary phloem during secondary growth in stem and root

Procambium: undifferentiated tissue with narrow and cytoplasm-dense cells that differentiate to primary xylem and primary phloem, and later develop into cambium

Meristem: zone of plant tissue containing nondifferentiated cells that can divide

Tracheary element (TE): in the xylem, the conductive vessel element characterized by secondary cell walls and perforation plates that connect TEs into a continuous file; TE cells undergo autolysis as part of their differentiation program

Secondary cell wall: multilayered polymer structure formed inside the primary cell wall of differentiated cells in some tissues, such as xylem and fibers

Reverse genetics: targeted mutation, deletion, inhibition, or expression of a gene thought to function in a given process, followed by phenotypic analysis

pathway, genome-wide transcriptional changes in the *mp* mutant and related transgenic plants were analyzed, revealing several *TARGET OF MONOPTEROS* (*TMO*) genes (136). In addition, the *MP* pathway was scrutinized further using multiple biochemical approaches, including chromatin immunoprecipitation and co-immunoprecipitation followed by liquid chromatography–tandem mass spectrometry (26, 71, 152). In addition to the forward genetics approach, a study of natural genetic variation in root growth identified *BREVIS RADIX* (*BRX*) as an important factor in protophloem differentiation (103).

Of the tree species, *Populus* is currently the best understood, thanks to the availability of its full genome sequence and organ- and tissue-specific gene expression databases created by whole genome transcriptome profiling, as well as the ability to routinely generate transgenic trees for the functional characterization of genes (59, 160). In 1998, expressed sequence tags were constructed from the wood-forming tissues of poplar to help identify the genes involved in controlling the development of vascular tissues (142).

During recent decades, a comprehensive picture of the gene regulatory machinery involved in vascular development has started to emerge from genome-wide approaches. In the context of the *Arabidopsis* primary root, global transcriptional profiling in a spatiotemporal manner, facilitated by fluorescence-activated cell sorting and microarray techniques, has provided the first atlases of gene expression, covering many root cell types at different development stages (11, 14). These atlases have laid the foundations for network analyses and systems biology approaches, which have been used to study secondary cell wall formation, for example. In addition to cell sorting, microdissection was also used to collect specific vascular tissues for transcriptome analysis (3, 27, 50). Genes identified via transcriptomics were investigated further by reverse genetics approaches (79), which have been facilitated by the availability of a genome-wide T-DNA insertional mutant library (121). More recently, a yeast one-hybrid network allowing the binding of transcription factors to defined promoters was used to establish the presence of a feedback loop in the transcriptional regulation of secondary cell wall formation (145). The genome-wide approaches have accumulated massive amounts of data and, together with the emergence of mathematical modeling (ideally with the capacity to predict gene regulatory networks), have begun to expand our understanding of these developmental processes (23, 34, 98, 107). The major approaches described here are illustrated in **Figure 2** chronologically, based on the time when each technique began to be used.

T-DNA: a portion of *Agrobacterium*-derived Ti plasmid that is inserted into the host genome and used as an insertional mutagen

Forward genetics: gene discovery by untargeted mutation (or overexpression), followed by screening of mutants (or transgenics) for a phenotype of interest

Protophloem: phloem tissues arising from procambium. Cell divisions first give rise to protophloem, metaphloem develops later, and both differentiate into sieve elements

Provascular tissue: embryonic tissue from which the future vascular bundle originates

Periclinal division: cell divisions that occur parallel to the surface of the plant body, resulting in radial growth

GENETIC NETWORKS IN PLANT VASCULAR DEVELOPMENT

Patterning of the Vascular Tissues in Diverse Vascular Meristems

Development of plant vascular tissue initiates with specification of cells into certain fates: xylem, phloem, and cambium. For example, cells localized in the central axis of the vascular cylinder of young roots are designated as xylem cells and, afterward, differentiate into conducting cells with a specialized structure for their function. The specification of cell fates generates uniform patterns of vascular tissues in plants. In this section, genetic and hormonal signal networks involved in the specification and patterning of vascular tissues in different plant organs are described.

Provascular development during embryogenesis. The *Arabidopsis* zygote undergoes a series of cell divisions to give rise to an embryo composed of cells with designated, specific fates. The establishment of the provascular tissue in the root, which is the origin of the postembryonic vascular bundle, is initiated by periclinal division of the four central cells in the lower-tier domain of the early globular-stage embryo (24, 135). The *mp* mutant, which displays severe defects in root-pole formation and vascular development, was first isolated by a forward genetic screen of an

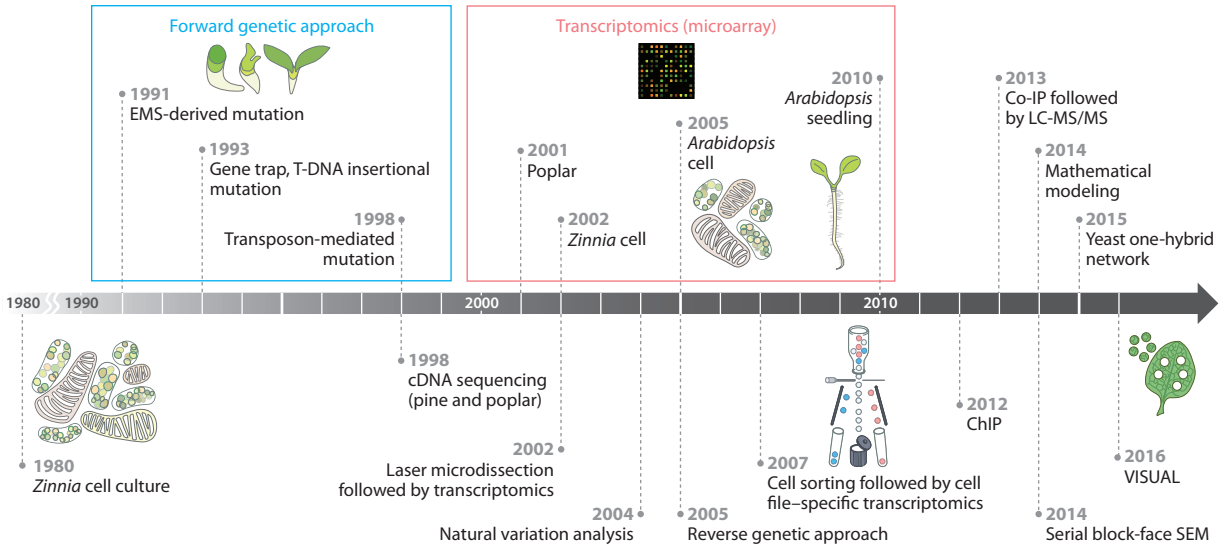

Figure 2

Major approaches used to study plant vascular development. Approaches are shown chronologically, that is, based on the time when each technique started to be used for research on vascular development. Abbreviations: cDNA, complementary DNA; ChIP, chromatin immunoprecipitation; Co-IP, co-immunoprecipitation; EMS, ethyl methanesulfonate; LC-MS/MS, liquid chromatography–tandem mass spectrometry; SEM, scanning electron microscopy; T-DNA, transfer DNA; VISUAL, vascular cell induction culture system using *Arabidopsis* leaves.

EMS-generated mutant pool (95). *MONOPTEROS* or *AUXIN RESPONSE FACTOR 5* (*MP/ARF5*) is an auxin-dependent transcription factor that plays an important part in the specification of the provascular tissue in the embryo, as well as in postembryonic vascular patterning (9, 55, 154). In the early stages of embryogenesis, *MP/ARF5* is broadly expressed, but its expression is gradually confined to the provascular tissue (55), which coincides with the auxin response maximum produced by the concentration of auxin in those tissues by the PIN proteins, which export auxin from cells (43, 141). The examination of expression profiles revealed that *MP/ARF5* acts as a positive regulator of *PIN1* expression, thereby forming a positive feedback loop to generate an auxin maximum in the provascular tissue during embryogenesis (**Figure 3*a***) (115, 136, 153, 154). Consistent with this, mutation of either *MP/ARF5* or *PIN1* abolishes the auxin maximum, as well as provascular patterning in the embryo, indicating that MP/ARF5-mediated auxin maximum formation and signaling is essential for provascular establishment (9, 43, 55).

Auxin signaling initiates upon the binding of auxin to TRANSPORT INHIBITOR RESPONSE 1 (TIR1) in the SKP1–CUL1–F-box$^{TIR1/AFB}$ ($SCF^{TIR1/AFB}$) ubiquitin ligase complex, which triggers the 26S proteasome-dependent degradation of the AUXIN/INDOLE-3-ACETIC ACID (AUX/IAA) transcriptional repressors, releasing the ARFs to act (130). The *bodenlos* (*bdl*) mutant, which shows phenotypes similar to those of the *mp* mutant, was identified in a separate EMS-generated mutant screen. BDL encodes the *AUX/IAA12* transcriptional repressor, and the *bdl* mutation is in the conserved ubiquitination domain (52, 53). The *mp*-like phenotypes of *bdl* are attributed to this gain-of-function mutation of *AUX/IAA12*, which causes it to constitutively inhibit MP activity, thus mimicking the loss-of-function *mp* mutation (**Figure 3*a***) (52). Transcriptomic analysis of the *mp* mutant and of seedlings expressing dexamethasone-inducible *bdl* revealed that MP/ARF5 directly upregulates *TMO3*, *5*, *6*, and 7 (136). *TMO5* encodes a basic helix-loop-helix (bHLH) transcription factor that is sufficient to rescue the provascular initiation defects in

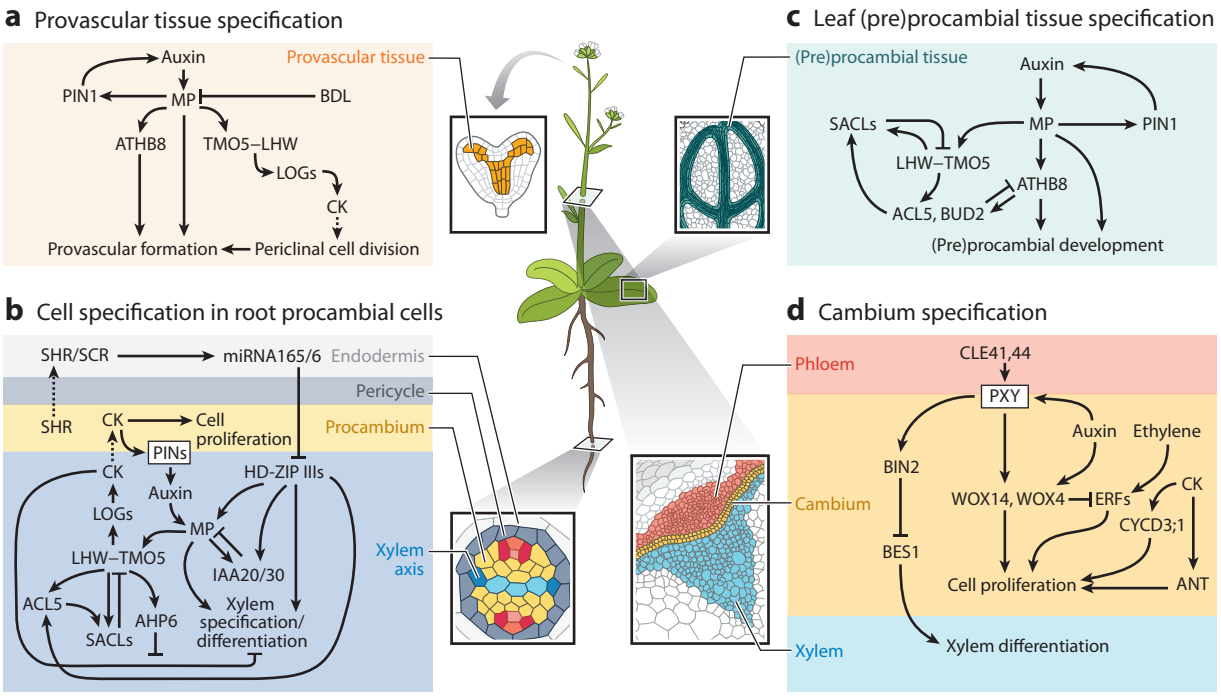

Figure 3

Genetic networks underlying vascular tissue specification. Vascular patterning in plants is controlled by complex genetic networks involving multiple transcription factors and phytohormones. (*a*) Provascular tissue specification. Auxin-inducible MP positively regulates *PIN1* expression, which, in turn, forms a positive feedback loop resulting in an auxin maximum in the provascular tissue. *BDL* encodes AUX/IAA12, which represses MP-mediated transcriptional activation of target genes. MP upregulates the expression of *ATHB8*, which has a crucial role in vascular patterning. Another direct target of MP is *TMO5*, which forms a heterodimer with LHW and induces expression of the LOGs to produce bioactive cytokinins (CK) that stimulate periclinal cell division. The expression pattern of *MP* in heart-stage embryo is shown in orange. (*b*) Cell specification in root procambial cells. SHR, a transcription factor expressed in the stele, moves to the endodermis and activates the transcription factor *SCR*. Together, SHR and SCR induce expression of *miRNA165* and *miRNA166* in the endodermis, which diffuse toward the center of the stele to form a gradient. miRNA165 and miRNA166 target the HD-ZIP IIIs for degradation, resulting in an HD-ZIP III gradient toward the center of the xylem, which specifies metaxylem versus protoxylem. In addition, the HD-ZIP III PHABULOSA upregulates *MP* and *IAA20* and *IAA30* to stabilize the auxin response in the xylem axis of the root. TMO5/T5L1–LHW induces expression of *ACL5*, a thermospermine synthase, as well as the *SACL* genes. Thermospermine generated by ACL5 induces translation of SACLs, which inhibit TMO5/T5L1 and LHW heterodimerization by binding to LHW. Furthermore, T5L1–LHW is shown to induce the expression of AHP6, which suppresses cytokinin signaling in the xylem. (*c*) Leaf (pre)procambial tissue specification. The auxin–MP pathway also has key roles in leaf (pre)procambial development. *BUD2* encodes *S*-adenosylmethionine decarboxylase, which is involved in the synthesis of polyamines, such as thermospermine. *BUD2* and *ACL5* are directly induced by ATHB8, but they negatively affect expression of *ATHB8*, forming a negative feedback loop. (*d*) Cambial specification. PXY, a leucine-rich repeat receptor–like kinase, is activated by its ligands, CLE41 and CLE44, and upregulates the expression of *WOX4* and *WOX14*, resulting in cell proliferation in the cambium. In addition, the PXY pathway activates BIN2, a GSK3 protein that inhibits the expression of the transcription factor *BES1*, stimulating xylem differentiation. Ethylene-induced ERFs and cytokinin-induced ANT and CYCD3;1 are also involved in cambial cell proliferation. Data from References 24, 25, 46, and 115; figure adapted from References 69 and 138 with permission.

mp (26). Furthermore, co-immunoprecipitation followed by liquid chromatography–tandem mass spectrometry analyses revealed that TMO5 forms a heterodimer with LONESOME HIGHWAY (LHW), an atypical bHLH transcription factor, to promote the periclinal division of provascular and procambial cells (**Figure 3*a*,*b***) (26, 116, 117). High-order knockout mutants of either *TMO5* or *LHW* with their corresponding paralogs, *TMO5-LIKE 1* (*T5L1*) and *LHW-LIKE 1* (*LL1*), exhibit a severe reduction in the number of periclinal divisions of the provascular and procambial cells, respectively in the embryo and root. This indicates that these bHLH transcription factors are crucial components in *MP/ARF5*-mediated provascular and procambial patterning (26, 116). *TMO3* encodes an AP2 transcription factor–encoding gene that is also known as *CYTOKININ RESPONSE FACTOR 2* (*CRF2*), a cytokinin-inducible gene that is crucial for embryonic development (124), indicating that auxin and cytokinin signaling converge in provascular patterning.

Cytokinins, like auxin, are key signaling molecules implicated in vascular development. The strong expression of the cytokinin receptor *WOODENLEG* (*WOL*) and the synthetic cytokinin reporter known as two component signaling sensor new (TCSn)::green fluorescent protein in embryo provascular tissue suggests that cytokinin functions in provascular patterning (86, 167). In addition, impaired embryonic root pole formation results from the overexpression of *PURINE PERMEASE 14* (*PUP14*), which takes up bioactive cytokinins from the apoplast and inhibits cytokinin signaling mediated by plasma-membrane-localized cytokinin receptors (167). Local biosynthesis of active cytokinins by the LONELY GUY (LOG) genes induced by TMO5–LHW has also been revealed to promote periclinal divisions to establish the provascular and procambial tissues (**Figure 3**) (23). The crosstalk between auxin and cytokinin regulating vascular development is discussed in the next section.

Secondary growth: lateral growth of stem and root by division of cambial cells

Protoxylem: primary xylem that develops from procambium earlier than metaxylem, and is characterized by spiral cell wall thickenings

Hormonal signaling governing procambial patterning of the root. The root vascular cylinder is a uniform structure composed of a central xylem axis flanked by phloem and intervening procambial cells; during secondary growth, the procambial cells give rise to xylem, phloem, and the lateral meristem cambium (**Figure 3**). In the early 1990s, *WOL*, *SHORT-ROOT* (*SHR*), and *SCARECROW* (*SCR*), key regulators involved in patterning the root vascular cylinder, were isolated from mutant pools generated by EMS-mediated mutagenesis (8, 134). The *wol* mutant exhibits a significant reduction in the number of vascular cell files in the root, which is attributed to defects in periclinal cell division in the procambium, and the abnormal differentiation of all of the vascular cell files as protoxylem (86, 134). *WOL* encodes a cytokinin receptor, a two-component histidine kinase, also known as *CYTOKININ RESPONSE 1* (*CRE1*), and *ARABIDOPSIS HISTIDINE KINASE 4* (*AHK4*) (66, 86, 134, 149). Cytokinins are perceived by three homologous receptors, AHK2, 3, and 4, which initiate a phosphorelay involving the ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEINs (AHPs) and ARABIDOPSIS RESPONSE REGULATORs (ARRs) to regulate target genes (63). Interestingly, the *wol* mutant harbors an amino acid substitution (T278I) in the cytokinin-binding domain of AHK4, locking the receptor in a form with reduced kinase activity but constitutive phosphatase activity, resulting in an impaired cytokinin response and changes in vascular patterning similar to the triple cytokinin receptor mutant (87). The phenotypes of *wol* and the *ahk2ahk3ahk4* triple mutant, together with the procambium-specific expression of cytokinin-inducible *ARR5*, indicate that cytokinin signaling is restricted to procambial cells and it mediates procambial proliferation and maintenance (12, 66, 86). Consistent with this, treatment with exogenous cytokinin suppresses the differentiation of protoxylem, and overexpression of the cytokinin oxidases, which degrade cytokinins, induces phenocopies of the *wol* mutant (85, 155). A screen for suppressors of *wol* isolated *AHP6*, a negative regulator of cytokinin signaling that is primarily expressed in the protoxylem. *AHP6* inhibits

cytokinin signaling in the developing protoxylem and stimulates protoxylem differentiation at the marginal position of the central xylem axis in roots (**Figure 3*b***) (85).

Metaxylem: primary xylem that develops from the procambium, characterized by shorter and broader cells than the protoxylem and by pitted cell wall thickenings

In contrast to cytokinins, auxin promotes the differentiation of xylem cells, as evinced by the repression of protoxylem formation following treatment with the auxin transport inhibitor 1-*N*-naphthylphthalamic acid (NPA) and the absence of xylem cells in the auxin-signaling-resistant mutant *auxin-resistant 1* (*axr1*) (12). Auxin response promoters, such as *DR5* and *IAA2*, are primarily expressed in the xylem axis, but their expression expands into the procambial cells following changes in cytokinin signaling, suggesting that cytokinin confines the auxin maximum to the xylem axis of the root (12). However, auxin-induced LHW–T5L1 induces expression of *AHP6* in the protoxylem and adjacent pericycle cells (117), where it inhibits cytokinin signaling, demonstrating that there is a mutual negative feedback loop between auxin and cytokinin signaling that generates the distinct domains of the hormone responses in the procambium and xylem axis to pattern the vascular cylinder (12). Indeed, detailed analysis of the expression and subcellular localization of the PIN proteins in the *wol* mutant or upon cytokinin treatment revealed that cytokinin is required not only for the proper expression of *PIN1*, *PIN3*, and *PIN7* but also for the appropriate subcellular localization of PIN1 in procambial cells (12, 88, 89). Likewise, the *pin1pin3* mutant displays distorted development of protoxylem (12), indicating that cytokinin signaling regulates the PINs to steer auxin flow toward the xylem axis. Recently, it has been established that local cytokinin biosynthesis by *LOG3* and *LOG4*, which are directly upregulated by auxin-induced *TMO5* and *LHW* in the xylem axis, plays an important part in inducing cytokinin response in procambial cells and forming the asymmetric hormone response domain in the root vascular cylinder (23). Using the key components described above, three distinct mathematical models have examined the mechanisms underlying the formation and maintenance of these asymmetric hormone signaling domains (23, 34, 98, 107).

Radial patterning of xylem during root development. As discussed above, the role of plant hormones, such as cytokinin and auxin, in vascular cell specification is evident and is closely linked to root patterning and cell differentiation. These processes are often mediated by transcriptional regulation; for example, several members of the MYB, NAC, and class III HOMEOBOX CONTAINING A LEUCINE ZIPPER MOTIF (HD-ZIP III) transcription factor families are well-known players in vascular differentiation. In the *Arabidopsis* root, xylem cell types can be distinguished morphologically by the pattern of their secondary cell wall thickening (pitted in metaxylem vessels versus spiral in protoxylem) or molecularly by cell type–specific markers, such as *ACAULIS5* for metaxylem (106) and *AHP6* for protoxylem (85). Combined with the consistent organization of the xylem axis, with metaxylem cells in the center and protoxylem in the periphery, this has allowed the detailed tracing of xylem identity determination by traditional phenotype-to-genotype mutant analyses.

Following the independent identification of a set of key transcription factors, a fascinating mechanism that balances metaxylem-versus-protoxylem identity in the *Arabidopsis* primary root was discovered. In the primary root, the identity of the two types of xylem vessels is determined by the *SHR–SCR–miRNA165/166*–HD-ZIP III pathway in a bidirectional manner; although a high dose of miRNA165/166 and the consequently low level of the HD-ZIP III genes promote protoxylem identity, metaxylem is specified by high levels of the HD-ZIP III genes (**Figure 3*b***) (20). This finely tuned transcriptional regulation involves non–cell autonomous activity of the mobile transcription factor SHR together with SCR (58, 109) to activate miRNA165/166 outside the stele (20). Upon moving back into the stele, miRNA165/166 targets HD-ZIP III transcripts, such as *PHABULOSA* (*PHB*), for degradation, and produces a gradient that determines the vessel type. Recently, PHB has been reported to upregulate *IAA20*, *IAA30*, and *MP*, stabilizing the

auxin response in the xylem axis, which suggests that there may also be a feedback loop between hormone signaling and the *SHR–SCR–miRNA165/6*–HD-ZIP III pathway (**Figure 3*b***) (105). Complementing the experimental evidence, a computational model suggests that degradation of miRNA165/166, in addition to its degradation of the HD-ZIP III transcripts, is necessary for correct vascular patterning (107).

The players in this dynamic pathway were identified in mutant screens for altered vascular development in the *Arabidopsis* primary root (8, 20, 58, 134). Phenotypes of the short-rooted *shr* and *phb* mutants share similarities in many aspects, including the formation of ectopic metaxylem in the protoxylem position and delayed phloem formation (20). Loss-of-function of all five HD-ZIP III genes completely abolishes the differentiation of procambial cells into xylem. SHR, together with SCR, is also involved in controlling cell division to form the cortex and endodermis cell layers in the ground tissue of the primary root (58), and the HD-ZIP III genes redundantly restrict procambium proliferation (20).

In addition to their activity in the root, the HD-ZIP III transcription factors have crucial roles in radial vascular patterning in the shoot (35, 97). Antagonistic regulation between the HD-ZIP IIIs and the KANADI (KAN) genes, a family of genes encoding GARP transcription factors primarily expressed in developing phloem, has been reported to regulate radial vascular pattering in the shoot (35, 72). The *phvphbrev* mutant displays abaxialized cotyledons and a distorted radial vascular pattern in which the xylem is surrounded by phloem (35). By contrast, mutation of the KAN genes in the *kan1kan2kan3* mutant results in a phloem-surrounded-by-xylem pattern in the shoot, similar to the gain-of-function HD-ZIP III mutants (35, 37). However, *kan1kan2kan3* does not exhibit an apparent phenotype in root vascular patterning, suggesting that the KAN genes are not required for radial pattern formation in the root vascular cylinder (56). In *Populus*, transgenic trees misexpressing *REVOLUTA* showed ectopic formation of cambium and severe patterning defects with reversed polarity; secondary xylem was produced toward the outside, and phloem toward the inside, of the stem (125). Other HD-ZIP III genes have been shown to affect the rate of secondary xylem and phloem differentiation in *Populus* (33, 166), further indicating that cambial expression of these genes is important in vascular patterning as well as in differentiation.

Phloem specification in the root. As for early phloem specification and differentiation in the *Arabidopsis* root, a few important genes have been identified using different approaches. *OCTOPUS* (*OPS*) (147) was discovered in a systematic survey for genes expressed during early procambial (108) and protophloem (7) development. *OPS* thus represents an example of reverse genetics in identifying novel genes involved in vascular development. The initial expression domain of *OPS* in the embryonic provascular cells narrows later in development to only the phloem lineage (7, 147). The *ops-1* mutant, which originated from a promoter trap collection, is characterized by a short primary root and adventitious roots growing out from the root hypocotyl junction at an early age, as well as reduced venation in the cotyledons. Phloem development is discontinuous in the mutant root; so-called gap cells that do not differentiate into protophloem interrupt the cell file (147). OPS is a plant-specific plasma membrane protein that was recently shown to regulate brassinosteroid signaling via direct repression of the GLYCOGEN SYNTHASE KINASE 3 (GSK3) family member *BRASSINOSTEROID-INSENSITIVE 2* (*BIN2*) (**Figure 4*a***) (2). Further work has confirmed that brassinosteroid signaling, which was initially described in the vascular differentiation of the inflorescence stem (17), also plays a part in *Arabidopsis* root protophloem differentiation (70, 129).

An interesting study on natural variation in root growth among 44 *Arabidopsis* accessions prompted the discovery of a natural loss-of-function allele, *brx*, in the accession Umkirch-1 (103); later, *BRX* was linked to early protophloem development in a manner similar to *OPS* (126). In

Cotyledons: the embryonic leaves of the plant; postembryonically, the first true leaves are formed from the shoot apical meristem

Hypocotyl: the embryonic stem connecting the cotyledons with the embryonic root

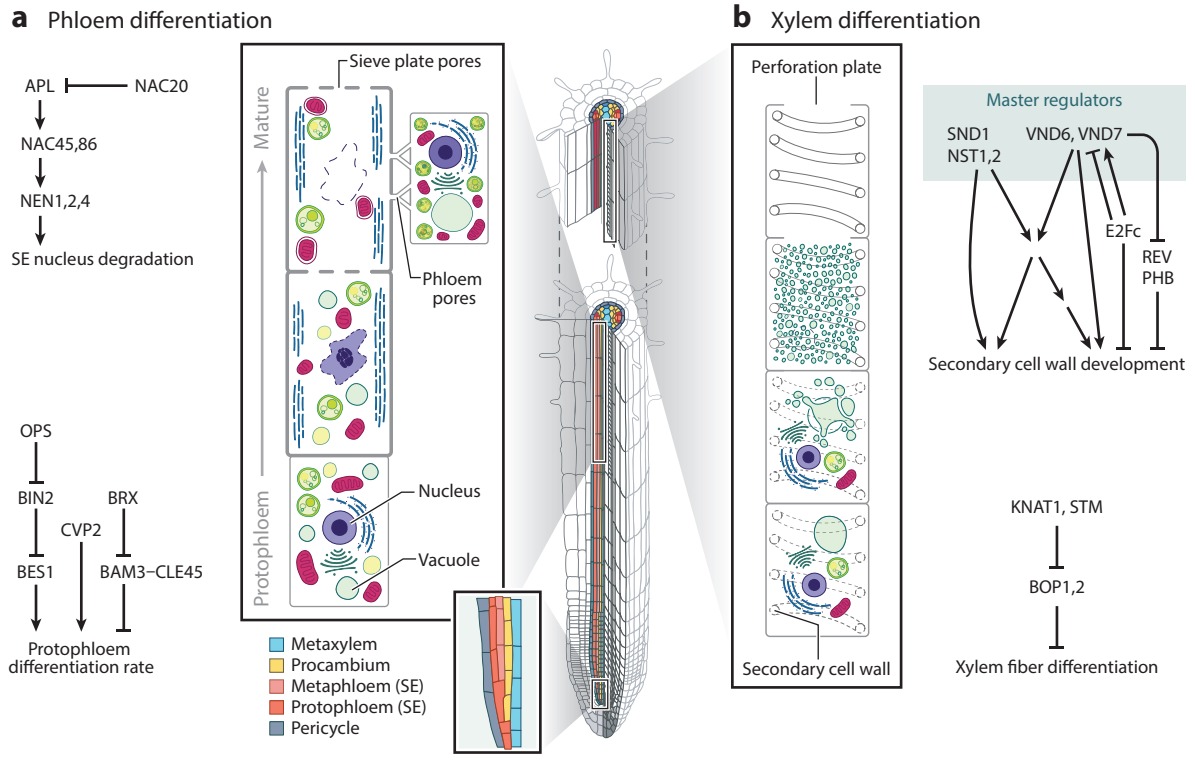

Figure 4

Morphogenetic events and transcriptional networks involved in vascular tissue differentiation. (*a*) During the course of phloem differentiation from protophloem to mature sieve elements (SE), cell walls thicken and the cells undergo cellular rearrangements during which organelles are modified and realigned. The process culminates in the breakdown of the nucleus and the formation of sieve plate pores that connect SEs in a cell file. Via phloem pores, the mature SE is associated with a companion cell that supports the enucleated SE. OPS, BRX, and CVP2 are positive regulators of protophloem specification and early SE differentiation. OPS represses BIN2, a suppressor of BES1 in the brassinosteroid signaling pathway that promotes xylem differentiation. BRX represses BAM3, which is a receptor of CLE45 that inhibits differentiation. CVP2 is involved in balancing phosphoinositide levels, which also affect differentiation. The *ops* and *brx* mutants (as well as combinations with *cvp2*) are characterized by discontinuous protophloem files, suggesting that these genes have a role in regulating the protophloem differentiation rate. APL is required in the later stages of SE differentiation. It regulates *NAC45* and *NAC86* expression and is downregulated by NAC20. NEN1, NEN2, and NEN4 are key regulators of SE nuclear degradation that act downstream of NAC45 and NAC86. (*b*) Xylem vessel elements undergo programmed cell death, and their secondary cell walls develop pitted or spiral deposition patterns. The nuclear contents are degraded, and perforation plates that connect the cells are formed. Secondary cell wall development is controlled by a complex transcriptional network involving multiple steps, presented here schematically by arrows. The arrows also represent a multitude of feedforward loops that are important in this process. The NAC transcription factors VND6, VND7, SND1, NST1, and NST2 are sufficient to regulate xylem formation and, therefore, are considered master regulators (*green shaded area*). Two recently identified novel pathways are also indicated; upstream of *VND7*, E2Fc may have either a positive or a negative effect, and the HD-ZIP III transcription factors *REV* and *PHB* are suppressed by VND7. In a separate pathway, KNAT1 and STM, which are typically associated with shoot meristem maintenance, were found to repress *BOP1* and *BOP2*, which suppress xylem fiber differentiation. Data from References 46 and 115; figure adapted from References 25, 47, 57, and 84 with permission.

the short-rooted *brx* mutant, the levels of brassinosteroids and auxin responsiveness are reduced, indicating feedback regulation between these two hormones at an early stage in *Arabidopsis* root growth (103, 104). A second-site suppressor screen in the *brx* mutant background added more dimensions to the regulatory network related to phloem differentiation (**Figure 4*a***). Depuydt and coworkers (30) reported that *BRX* restricts the expression of *BARELY ANY MERISTEM 3*

(*BAM3*), the receptor of the small peptide ligand CLAVATA3/ENDOSPERM SURROUNDING REGION 45 (CLE45). CLE45 suppresses protophloem differentiation (30); this signal is amplified by MEMBRANE-ASSOCIATED KINASE REGULATOR 5, which acts downstream of BAM3 (70). Given that phloem files, although discontinuous, form in the *ops* and *brx* mutants, these genes are not required for phloem differentiation but rather seem to be involved in regulating the rate of this process (30, 126, 147). *COTYLEDON VASCULAR PATTERN 2* (*CVP2*) was also isolated from a *brx* suppressor screen (127); a double mutant with its close homolog *CVP2-like 1*, it has discontinuous cell files in the root protophloem and abnormal levels of phosphoinositides. These genes had been characterized earlier from a mutant screen for discontinuous vein patterns in the cotyledon, suggesting that phosphoinositides may act as signaling molecules for vascular strand propagation (18, 19).

Preprocambium: undifferentiated leaf tissue consisting of cells that are morphologically indistinguishable but genetically distinct from mesophyll cells and that develop into procambium

Leaf vein patterning. As described in the previous sections, various studies have characterized genetic and hormonal networks implicated in the patterning of (pro)vascular tissue of embryo and root. Similarly, mechanisms underlying leaf vein patterning have been actively investigated and common signaling components involved in (pro)vascular tissue specification of embryo, root, and leaf have been identified. For instance, MP and HD-ZIP IIIs, which mediate auxin signaling, and the cytokinin receptors, AHKs, were shown to be crucial for vascular patterning and development in leaves (5, 9, 87, 122), as well as in embryo and root. However, it seems that the common signaling components do not always play identical roles in vascular patterning of distinct organs. For example, the three KAN transcription factors, KAN1, KAN2, and KAN3, which act antagonistically to the HD-ZIP IIIs, are expressed in vascular tissues of both root and shoot but the *kan1kan2kan3* triple mutant exhibits distorted vascular patterning only in the shoot but not in the root (54). In addition, the *wol* mutant exhibits a reduced number of vascular cells and abnormal differentiation of all of the vascular cell files as protoxylem in the primary root but only shows reduction of cell division in leaf petiole (87). These observations suggest that there are common signaling components involved in vascular patterning of different organs and that their activity or function might be differently regulated via unknown mechanisms, depending on the organ. It requires further investigations to clarify how the common components differently regulate vascular patterning among distinct organs. In this section, major components involved in leaf vein patterning are described.

Leaf primordia develop preprocambial tissue composed of subepidermal cells that are morphologically indistinguishable but are marked by auxin response genes (42, 151). The preprocambial cells give rise to procambia consisting of narrow cells arranged in continuous strands that differentiate into the vascular bundle of leaves (5, 42, 132, 133). Since a study in the early 1950s showed that exogenous auxin treatment mimics the endogenous signal from young leaf primordia in inducing vascular development (68), numerous studies have supported the role of auxin in defining the position of vein formation in leaves (92, 128, 133, 151). The expression profile of the synthetic auxin promoter *DR5* shows an auxin maximum at the site where veins will form (92). In addition, treatment with the auxin transport inhibitor NPA restricted the auxin maximum and vascular formation to the primordium margin, suggesting that appropriate endogenous auxin transport toward the site of the preprocambium is a prerequisite for proper leaf venation (92). Indeed, analysis of the expression profiles and subcellular polarity of the PIN proteins demonstrated that *PIN1* is expressed prior to preprocambial cell markers and localized on the plasma membrane in order to steer auxin flow toward the site of the preprocambium (133). Accordingly, the *pin1* mutant exhibits reduced auxin transport and a distorted vascular pattern that can be reproduced by NPA treatment, indicating that PIN1 is the major auxin exporter involved in forming the auxin maximum necessary for correct leaf venation (92, 93, 118). The defects in leaf venation of the *pin1*

mutant are exacerbated in higher order mutants that include *pin6* or *pin6pin8*, the endoplasmic reticulum–localized PINs, indicating that intracellular auxin transport is also important for leaf vein patterning (131). Distorted leaf vein formation in the cotyledons of mutants of the auxin importer *LIKE AUXIN RESISTANT 2* (*LAX2*) suggests that auxin import is also necessary for formation of the maximum and veins (119). In addition, defective auxin signaling in the *mp* mutant results in impaired vascular patterning in leaves, as well as in embryos (9, 122). The spatiotemporal expression pattern profiles of *MP* and *PIN1* in developing leaf primordia revealed that auxin upregulates the expression of *MP*, which, in turn, induces the expression of *PIN1* to form a positive feedback loop generating auxin maxima at the preprocambial sites of leaf primordia, similar to the feedback loop observed in the embryo (**Figure 3**) (154).

In 1995, HD-ZIP III gene *ARABIDOPSIS THALIANA HOMEOBOX 8* (*ATHB8*) was identified as an auxin-inducible preprocambial and procambial marker specifically expressed in the vascular tissues of the embryo and postembryonic organs, as well as during the regeneration of vascular strands (5). Expression profiling of *ATHB8* promoter fragments revealed that the sequence TGTCTG in the promoter is a functional auxin response element and is important for the gene's expression in vascular tissues (31, 136). Expression of *ATHB8* is dramatically diminished in the *mp* mutant and is increased by overexpression of *MP*, suggesting that *MP* is a direct transcriptional activator of *ATHB8* in preprocambial and procambial cells (**Figure 3*c***) (31). Recently, Baima and coworkers (4) identified *ACAULIS5* (*ACL5*) and *BUSHY AND DWARF 2* (*BUD2*) as downstream targets of *ATHB8*. The *acl5* mutant was originally identified from an EMS mutant screen because of its dwarf phenotype and distorted xylem development (4, 54, 106). An in vitro enzymatic activity analysis revealed that *ACL5* encodes a thermospermine synthase; thermospermine is a polyamine that is important in various plant developmental processes (73, 144). It has been demonstrated that the thermospermine positively regulates translation of four bHLH transcription factors belonging to the SUPPRESSOR OF ACAULIS51-LIKE (SACL) clade: SAC51, SACL1, SACL2, and SACL3, which inhibit auxin signaling and xylem differentiation (64, 71, 152). Spermine, a structural isomer of the thermospermine, binds to ribosomes and plays bimodal roles in translation; it stimulates peptide bond formation at low concentration but inhibits it at high concentration (32, 157). This suggests that the thermospermine might bind to ribosomes and modulate translation of proteins, but further investigation is required to clarify the thermospermine-mediated regulatory mechanism. The *bud2* mutant shows retarded shoot growth and impaired vascular patterning, similar to the *acl5* mutant (49, 102). *BUD2* encodes *S*-adenosylmethionine decarboxylase, an enzyme necessary for polyamine synthesis. The mutation decreases the production of polyamines, including spermine; together, these phenotypes suggest that the polyamine produced by *ACL5* and *BUD2* plays an important part in vascular patterning (49). Treatment with exogenous polyamines inhibits auxin-induced xylem differentiation in leaves and prolongs xylem differentiation in *Zinnia* cell cultures (106, 161). Interestingly, overexpression of *POPACAULIS5*, the ortholog of *ACL5* in poplar, suppresses biosynthesis of auxin, but *PttHB8*, the poplar ortholog of *ATHB8*, increases expression of *POPACAULIS5*, suggesting that there is a negative feedback loop between thermospermine biosynthesis and auxin signaling (99). Indeed, chromatin immunoprecipitation of ATHB8 showed that it directly binds to the promoters of *ACL5* and *BUD2* to upregulate their expression in *Arabidopsis*; in turn, these genes suppress premature xylem differentiation from the procambium by negatively affecting the expression of HD-ZIP III and auxin signaling genes (**Figure 3*c***) (4, 106). More recently, two studies have established the existence of a negative feedback network involving *ACL5*, *SACL*s, and *TMO5* and *LHW*, all of which are specifically expressed in the vascular bundle of the root and hypocotyl (71, 152). The LHW heterodimer with either TMO5 or T5L1 increases the activity of the SACLs by either directly increasing transcription or via ACL5 thermospermine-mediated translational

activation, which, in turn, represses the TMO5–LHW interaction because the SACLs bind to LHW (**Figure 3*c***) (71, 152).

Interfascicular cambium: cambial cells arising between vascular bundles of stem to produce a ring structure

The vascular cambium: establishment and patterning of the secondary meristem. The vascular cambium, a layer of meristematic cells, typically separates the differentiated xylem and phloem tissues in the vasculature; such an organized pattern requires the coordinated and properly oriented division of the cambial cells, as well as the timely differentiation processes of the specialized cell types. In a mutant screen for irregular vascular organization in the *Arabidopsis* inflorescence stem, Fisher & Turner (41) identified an LRR kinase that they named *PHLOEM INTERCALATED WITH XYLEM* (*PXY*). In the vascular bundles of the *pxy* mutant, phloem and xylem were not separated by a distinct cambial layer because the cambial cell division plane was abnormal; in addition, the ratio of vessels compared with other xylem cell types was reduced. *PXY* was also discovered in an independent set of experiments because of the mutant's insensitivity to TDIF, a CLE peptide that was originally purified from a *Zinnia* cell culture medium because of its capacity to suppress TE differentiation (44, 61). In *Arabidopsis*, two genes, *CLE41* and *CLE44*, encode the TDIF peptide (67), which is found in phloem, and *PXY* is expressed in dividing cambial cells (41, 61). This implies that *PXY–CLE41/44* signaling regulates cell fate in a non–cell autonomous manner (**Figure 3*d***). In the *Arabidopsis* hypocotyl, PXY–CLE41/44 was reported to suppress TE differentiation through the CLE41/44-dependent interaction of PXY with BIN2, a GSK3 protein that transcriptionally downregulates the transcription factor *BES1* in brassinosteroid signaling (**Figure 3*d***) (77). PXY–CLE41/44 promotes cambial proliferation during secondary growth of the *Arabidopsis* hypocotyl and inflorescence stem by activating *WUSCHEL-related HOMEOBOX* (*WOX*) *4* and *WOX14* (**Figure 3*d***) (39, 60). Interestingly, PXY–CLE41/44 also controls secondary growth in the model tree species *Populus*; transgenic trees overexpressing the *Populus* orthologs of *PXY* and *CLE41* in, respectively, the cambium and phloem showed an increased rate of cambial cell division, leading to a dramatic increase in biomass (38). Thus, the PXY–CLE41/44 signaling pathway may affect vascular organization in many ways, and manipulation of this conserved pathway might even be considered as a means for improving the production of sustainable energy.

In addition to PXY, two other LRR-RLKs, REDUCED IN LATERAL GROWTH 1 (RUL1) and MORE LATERAL GROWTH 1 (MOL1), which play, respectively, positive and negative roles in cambium activity, have been identified through spatiotemporal transcriptome analysis of tissues collected via laser microdissection following in vitro induction of secondary growth (i.e., stem fragment treated with auxin and NPA) (1). In contrast to the *pxy* mutant, which displays defects in interfascicular cambium formation, the *rul1* and *mol1* mutants do not exhibit impaired interfascicular cambium formation. However, *rul1* and *mol1* show, respectively, a decrease and increase in interfascicular cambium–derived tissue, suggesting that they mediate the opposing signals that regulate cambium activity (1). More recently, Gursanscky and coworkers (51) suggested that MOL1 negatively regulates ethylene and jasmonic acid signaling, which induce lateral growth.

Alongside PXY–CLE41/44, several other factors relevant to vascular development have emerged from *Zinnia* and *Arabidopsis* cell culture studies. For example, xylogen was purified from *Zinnia* cultures and described as a polar-localized proteoglycan-like factor essential to directing continuous xylem differentiation by promoting cell–cell interactions in *Zinnia* and *Arabidopsis* (101). The involvement of various hormones in xylem vessel differentiation—such as auxin, cytokinins, brassinosteroids, and ethylene—has also been convincingly demonstrated in cell culture studies (44, 79, 114, 120, 159); these hormones may also act as important regulators of cambial activity.

Hormone signaling and networks regulating cambial activity. Much of our current understanding of secondary growth arises from research on the herbaceous species *Arabidopsis*, which is regarded as a valid model because there is evidence of conserved mechanisms controlling cambial activity across species. For example, in the previous section the PXY–CLE41/44 signaling pathway was described; this pathway regulates cambial cell division in *Arabidopsis* as well as in *Populus* (38, 39, 60). Secondary development is most evident in the secondary xylem of a tree trunk (also known as wood), and *Populus* has emerged as a popular forest tree model. An increasing amount of data indicates that cambial activity is regulated by several plant hormones, such as auxin, cytokinin, ethylene, gibberellic acid, strigolactone, and jasmonic acid. The role of auxin in the cambium was highlighted in the early 1930s, as treatment with exogenous auxin stimulated cambial growth in the petiole and hypocotyl (139); since then, numerous studies have supported the role of basipetally transported auxin as one of the major regulators of cambial activity (75, 82). For instance, the auxin-signaling-resistant mutant *axr1* displays reduced interfascicular cambium activity in the *Arabidopsis* stem, and auxin concentration peaks in the cambium of *Populus* and Scots pine (10, 150). In the *Arabidopsis* stem, expression of the auxin response reporter *DR5* is upregulated in parenchyma cells differentiating into interfascicular cambium (96). Furthermore, auxin activates the expression of *WOX4*, a transcription factor downstream of PXY that is involved in cambial cell proliferation (**Figure 3*d***) (143). The accumulation of auxin in the *Arabidopsis* stem after 1-day treatment with NPA induces the expression of *WOX4* in wild-type plants and in the *pxy* mutant. However, prolonged auxin accumulation following 7 days of NPA treatment results in high expression of *WOX4* in wild-type but not in *pxy*, indicating that the induction of *WOX4* by auxin is independent of *PXY*, but stable, auxin-mediated *WOX4* induction requires *PXY* (**Figure 3*d***) (143). In *Populus*, functional studies have shown that reduced auxin responsiveness reduces cambial activity (112).

Cytokinins are also well known to stimulate cell division in meristems. The high-order *Arabidopsis* mutant of the *ADENOSINE PHOSPHATE ISOPENTENYLTRANSFERASE* (*IPT*) gene leads to a severe reduction in the size of the cambium (91); IPTs are rate-limiting enzymes in cytokinin biosynthesis. Consistent with this, cytokinin signaling genes are primarily expressed in cambial cells, and overexpression of the cytokinin-degrading enzyme *CYTOKININ OXIDASE 2* (*CKX2*) results in a reduction of cambial size in poplar (111), and suppression of CKX genes in *Arabidopsis* generates a thicker inflorescence stem (6). In *Populus*, overexpression of *IPT7* increased cytokinin and auxin levels and greatly enhanced radial growth (65). High-resolution profiling of the cambial region by RNA sequencing revealed distinct profiles for auxin and cytokinin response, indicating a hormonal gradient in the cambium (65) and further supporting the role of these two hormones as critical regulators of secondary growth. Just as basipetal auxin transport is important for cambial function, cytokinin transport is also crucial for cambial activity. It was demonstrated that the *abcg14* mutant, which is defective in cytokinin transport, exhibits severe growth retardation in the stem (74, 163). Recently, it has also been demonstrated that cytokinin induces the expression of the APETALA2-like transcription factor *AINTEGUMENTA* (*ANT*) and the cell cycle factor *CYCLIN D3;1* (*CYCD3;1*) to promote cell proliferation in the cambium (21, 123). Accordingly, mutants of *ant* and *cycd3;1* exhibit impaired cambial activity similar to that of mutants for cytokinin biosynthesis and signaling. Compared with the single mutant phenotypes, the additive phenotype of the *antcycd3;1* double mutant indicates that they are both regulated by cytokinins but contribute independently to cell proliferation (**Figure 3*d***) (123).

Another example of the hormonal regulation of cambial activity comes from *Populus*, in which gibberellin levels are positively correlated with biomass accumulation (36), and gibberellin signaling appears to stimulate secondary xylem differentiation as well as biomass accumulation in terms of plant height and fiber length (94). Furthermore, exogenously applied ethylene or overexpression of an ethylene biosynthesis gene stimulates cambial growth and wood formation

in *Populus* (83). Ethylene appears to function in parallel with the PXY pathway to induce cambial proliferation (40, 83). The mild reduction in vascular cell numbers by the mutation of *PXY* in *Arabidopsis* is attributed to compensatory upregulation of ETHYLENE RESPONSE FACTORs (ERFs) such as *ERF018* and *ERF109*. The expression of *ERF018* and *ERF109* is increased in both the *pxy* and *wox4* mutants, and the phenotype of *pxy* is exaggerated by mutation of *ERF018* and *ERF109*, suggesting that both the PXY pathway and ethylene signaling positively regulate cambial activity, and the PXY pathway also represses ethylene signaling (**Figure 3*d***) (40).

Differentiation of Phloem and Xylem

In the previous sections, we focused on the establishment and patterning of various meristems in plant vascular development. Meristematic cambial tissues in the stem, as well as in the hypocotyl and root, can be activated to produce secondary phloem and xylem by periclinal cell divisions and subsequent cell differentiation processes. Specialization of xylem and phloem cell fate culminates in the development of the secondary cell wall and programmed cell death once the final size of the cell has been reached (**Figure 4**). All of these processes are important for generating tissues in organs, such as the *Arabidopsis* inflorescence stem or tree trunks, that can support upright growth and facilitate the flow of essential fluids and compounds throughout the growing plant. Because much of a plant's biomass derives from the development of the secondary cell walls of xylem vessels and fibers, these major components of wood are also important natural sources of support for human life.

Transcriptional regulation in xylem secondary cell wall development. The NAC family of transcription factors appears to be important in activating the secondary cell wall regulatory network. Various approaches have identified five NACs that are sufficient to regulate xylem vessel formation and, therefore, are regarded as master regulators. Ectopic overexpression of *VND6*, *VND7*, *NAC SECONDARY WALL THICKENING PROMOTING FACTOR 1* (*NST1*), *NST2*, or *SECONDARY WALL–ASSOCIATED NAC DOMAIN PROTEIN* (*SND1*) results in ectopic secondary cell wall formation in various cell types (79, 100, 164). *VND6* and *VND7* emerged from comprehensive transcriptomics analysis of in vitro *Arabidopsis* xylogenesis cell suspension cultures, and overexpression studies subsequently linked them to transdifferentiation into, respectively, metaxylem- and protoxylem-like cells in *Arabidopsis* roots and *Populus* leaves (79). In the *Arabidopsis* inflorescence stem, *VND6* expression is restricted to the metaxylem vessels, but the *VND7* expression domain includes both metaxylem and protoxylem vessels (165). *NST1* and *NST2* were discovered because of their roles in anther dehiscence in *Arabidopsis* by dominant EAR-motif-mediated repression analysis of the NAC genes (100). *NST1* and *NST2* function redundantly; in the double knockout *nst1nst2*, secondary walls are not formed in the endothecium, which results in anther indehiscence. Promoter analysis of *NST1* and *NST2* indicated strong expression in tissues other than the anthers; in particular, *NST1* is strongly expressed in the xylem vessels and fibers of the *Arabidopsis* inflorescence stem (100). The fifth master regulator, *SND1*, was discovered as a highly expressed NAC transcription factor in xylem fiber cells in the *Arabidopsis* inflorescence stem (164).

Transcriptional regulatory networks down- and upstream of the five master regulator NACs in secondary cell wall formation have been extensively studied, and a comprehensive view of the gene regulatory machinery has started to emerge from genome-wide approaches (62, 80, 145). Recently, interesting new connections were made in a large-scale yeast one-hybrid screen and subsequent network analyses that studied protein interactions with transcription factors expressed in the root xylem (**Figure 4*b***) (14, 15, 145, 165). For example, the *E2Fc* transcription factor, which is a negative regulator of endoreduplication that may occur upon cell differentiation (28, 90), was

placed upstream of *VND7* by the network analysis, and it had either a positive or a negative effect depending on the dose (145). This interaction was confirmed by monitoring luciferase activity under the *VND7* promoter in tobacco leaves co-infiltrated with transiently overexpressed *E2Fc* at different ratios. Furthermore, VND7 was found to act as an upstream regulator of the HD-ZIP III genes *REV* and *PHB*, adding a third major transcription family to the secondary cell wall transcriptional regulatory network alongside the NACs and MYBs. Several other feedforward loops that contribute to xylem differentiation were also highlighted in the transcriptional network (145). Thus, a systems biology approach conducted in a heterologous yeast system appears to have greatly enhanced our understanding of xylem development, pending validation in planta.

Sieve element (SE): in the phloem, conductive cells characterized by thick cell walls; sieve plates with pores connect SEs into a continuous file, and SE cells undergo enucleation as part of their differentiation program

In the case of xylem fiber differentiation, perhaps surprisingly, Liebsch and coworkers (81) discovered a redundant function for the class I KNOX transcription factors SHOOT MERISTEMLESS (STM) and KNAT1, via transcriptional repression of the meristem boundary genes *BLADE-ON-PETIOLE 1* (*BOP1*) and *BOP2* in the *Arabidopsis* hypocotyl. In the weak mutant alleles *stm* and *knat1*, *SND1* and *NST1* expression levels were significantly reduced, correlating with the reduced formation of fibers in the xylem. This is an interesting finding of important regulators in shoot meristem maintenance having an opposite role in secondary growth by promoting cell differentiation.

Phloem sieve element differentiation in the *Arabidopsis* primary root. Although many pathways leading to xylem differentiation have been established, less is known about phloem differentiation. The first identified gene to mark phloem identity emerged from a mutant screen for compromised root growth: *ALTERED PHLOEM DEVELOPMENT* (*APL*), an MYB transcription factor–encoding gene that is required for the formation of phloem poles (13). APL also represses xylem formation; in the *apl* loss-of-function mutant, the cells positioned in the expected phloem poles are characterized by lignified cell walls that are characteristic of TEs rather than sieve elements (SEs). The *apl* mutant was selected from a transposon-tagged pool of *Arabidopsis* (156) because of its *wol*-like phenotype: a short, determinate primary root and arrested shoot development. The severe developmental effects of the recessive, seedling-lethal *apl* mutant could not be rescued, suggesting that *APL* function is required for phloem identity throughout the plant. Because protophloem can develop in a normal manner in *apl* embryos (148), APL appears necessary for the later stages of phloem development. More recently, the molecular regulatory pathway downstream of APL has been studied, providing a detailed view of SE maturation in the *Arabidopsis* root and highlighting the involvement of NAC transcription factors and their targets, the NAC-DEPENDENT EXONUCLEASEs (NENs), in this process (**Figure 4*a***) (47). *NAC45* and *NAC86* were identified by comparing transcript profiles of the *apl* mutant and wild-type plants; the downstream NENs were found by comparing cell type–specific transcript profiles from the *nac45/86* double mutant, wild-type plants, and *NAC45*-overexpressing plants. Analysis of the NAC double mutant plants revealed that these genes were involved in the breakdown of the nucleus during SE differentiation, which was imaged at high resolution using serial block-face scanning electron microscopy (47).

An upstream regulator of APL was recently identified through an interesting in vitro system. Ectopic differentiation of xylem and phloem was induced in *Arabidopsis* leaf discs cultured in a medium containing auxin, cytokinin, and the GSK3 inhibitor bikinin; mesophyll cells were converted into procambial cells prior to differentiation in a highly synchronized manner (76, 78). In this system, named VISUAL, an increased number of phloem-marker-expressing cells was recorded in the bikinin-induced leaf discs, as quantified by flow cytometry. Transcriptomics analysis coupled with cell sorting of the induced SE-like cells using a fluorescently labeled SE-specific marker identified *NAC20* as an upstream negative regulator of *APL* (78). Although not all of the

cellular events related to SE morphogenesis were observed, VISUAL appears to have significant potential for identifying novel regulatory elements in TE and SE differentiation. Outside of the intact plant, it may not be possible to develop a thorough understanding of gene regulatory networks during SE differentiation from protophloem to mature, functional SEs, however, and this remains a challenge to be tackled.

SUMMARY POINTS

1. The plant vascular system is highly organized at the whole-plant level, and it supports the coordinated growth of organs in response to environmental changes by providing a pipeline for the delivery of nutrients and signaling molecules.
2. Various systems and approaches have been used to study the mechanisms governing plant vascular patterning and to identify multiple genetic components and the networks important for vascular patterning and development.
3. The development of the vascular system starts with cell specification, which involves numerous positional cues based on hormone signaling and transcriptional regulation. The interaction between auxin and cytokinin has crucial roles in determining cell fates in the vascular cylinder. In addition, mobile transcription factors and miRNAs generate gradients of HD-ZIP IIIs along the vascular tissues to pattern the vasculature. Furthermore, antagonistic regulation between two groups of transcription factors, HD-ZIP IIIs and KAN, is crucial for adaxial and abaxial patterning.
4. Signaling mediated by LRR-RLKs and their ligands interacts with hormonal signaling to regulate cell specification and proliferation during vascular development.
5. Several members of the MYB and NAC transcription factor families play important parts in vascular tissue differentiation processes during vasculature maturation.

FUTURE ISSUES

1. Cell fate is determined by specific proteins, which can be regulated transcriptionally, posttranscriptionally, translationally, posttranslationally, and epigenetically. Studies during recent decades have characterized not only (post)transcriptional regulation by transcription factors and miRNAs but also protein–protein interactions involved in vascular patterning. Recently, it has been reported that the expression of SACL genes is positively regulated in translation through thermospermine generated by *ACL5*, which is important for appropriate vascular patterning (64, 71, 152). This suggests that translational regulation is indeed a crucial step in controlling the specific expression of genes involved in vascular patterning. Furthermore, the importance of epigenetic regulation in vascular development has been suggested by a recent study reporting that the trimethylation of histone by *POLYCOMB REPRESSIVE COMPLEX 2* has a role in vascular cell proliferation and differentiation (22). However, the detailed mechanisms underlying the translational and epigenetic regulation of vascular-specific genes remain elusive. Research on translational and epigenetic regulation may identify novel factors and further elucidate the processes governing vascular patterning.

2. Network construction and analysis based on bioinformatics and statistics, combined with dynamic and predictive modeling, may become even more powerful tools once data about the translatome and protein interactions are incorporated. This may also be extended to explore the evolutionary aspects of plant vascular development and connect the disparate genetic networks emerging from different plant organs, and even between species.
3. Understanding the mechanisms of plant vascular development is important not only for the sake of advancing basic knowledge but also to improve agriculture and forestry to support the ever-growing human population. After all, in crop species, such as cassava, sweet potato, and carrot, the storage organs we eat consist mostly of vascular tissues, and wood is composed of secondary xylem, the water-conducting tissue of the vasculature.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Agusti J, Lichtenberger R, Schwarz M, Nehlin L, Greb T. 2011. Characterization of transcriptome remodeling during cambium formation identifies *MOL1* and *RUL1* as opposing regulators of secondary growth. *PLOS Genet*. 7:e1001312
2. Anne P, Azzopardi M, Gissot L, Beaubiat S, Hématy K, Palauqui J-C. 2015. OCTOPUS negatively regulates BIN2 to control phloem differentiation in *Arabidopsis thaliana*. *Curr. Biol*. 25:2584–90
3. Asano T, Masumura T, Kusano H, Kikuchi S, Kurita A, et al. 2002. Construction of a specialized cDNA library from plant cells isolated by laser capture microdissection: toward comprehensive analysis of the genes expressed in the rice phloem. *Plant J*. 32:401–8
4. Baima S, Forte V, Possenti M, Peñalosa A, Leoni G, et al. 2014. Negative feedback regulation of auxin signaling by ATHB8/ACL5–BUD2 transcription module. *Mol. Plant* 7:1006–25
5. Baima S, Nobili F, Sessa G, Lucchetti S, Ruberti I, Morelli G. 1995. The expression of the *Athb-8* homeobox gene is restricted to provascular cells in *Arabidopsis thaliana*. *Development* 121:4171–82
6. Bartrina I, Otto E, Strnad M, Werner T, Schmülling T. 2011. Cytokinin regulates the activity of reproductive meristems, flower organ size, ovule formation, and thus seed yield in *Arabidopsis thaliana*. *Plant Cell* 23:69–80
7. Bauby H, Divol F, Truernit E, Grandjean O, Palauqui J-C. 2007. Protophloem differentiation in early *Arabidopsis thaliana* development. *Plant Cell Physiol*. 48:97–109
8. Benfey PN, Linstead PJ, Roberts K, Schiefelbein JW, Hauser M-T, Aeschbacher RA. 1993. Root development in *Arabidopsis*: four mutants with dramatically altered root morphogenesis. *Development* 119:57–70

9. Berleth T, Jurgens G. 1993. The role of the *monopteros* gene in organising the basal body region of the *Arabidopsis* embryo. *Development* 118:575–87
10. Bhalerao RP, Fischer U. 2014. Auxin gradients across wood—instructive or incidental? *Physiol. Plant.* 151:43–51
11. Birnbaum K, Shasha DE, Wang JY, Jung JW, Lambert GM, et al. 2003. A gene expression map of the *Arabidopsis* root. *Science* 302:1956–60
12. Bishopp A, Help H, el-Showk S, Weijers D, Scheres B, et al. 2011. A mutually inhibitory interaction between auxin and cytokinin specifies vascular pattern in roots. *Curr. Biol.* 21:917–26
13. Bonke M, Thitamadee S, Mähönen AP, Hauser M-T, Helariutta Y. 2003. APL regulates vascular tissue identity in *Arabidopsis. Nature* 426:181–86
14. Brady SM, Orlando DA, Lee J-Y, Wang JY, Koch J, et al. 2007. A high-resolution root spatiotemporal map reveals dominant expression patterns. *Science* 318:801–6
15. Brown DM, Zeef LAH, Ellis J, Goodacre R, Turner SR. 2005. Identification of novel genes in *Arabidopsis* involved in secondary cell wall formation using expression profiling and reverse genetics. *Plant Cell* 17:2281–95
16. Campbell L, Turner S. 2017. Regulation of vascular cell division. *J. Exp. Bot.* 68:27–43
17. Caño-Delgado A, Yin Y, Yu C, Vafeados D, Mora-García S, et al. 2004. BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in *Arabidopsis. Development* 131:5341
18. Carland FM, Berg BL, FitzGerald JN, Jinamornphongs S, Nelson T, Keith B. 1999. Genetic regulation of vascular tissue patterning in *Arabidopsis. Plant Cell* 11:2123–37
19. Carland FM, Nelson T. 2009. CVP2- and CVL1-mediated phosphoinositide signaling as a regulator of the ARF GAP SFC/VAN3 in establishment of foliar vein patterns. *Plant J.* 59:895–907
20. Carlsbecker A, Lee J-Y, Roberts CJ, Dettmer J, Lehesranta S, et al. 2010. Cell signalling by microRNA165/6 directs gene dose-dependent root cell fate. *Nature* 465:316–21
21. Collins C, Maruthi NM, Jahn CE. 2015. CYCD3 D-type cyclins regulate cambial cell proliferation and secondary growth in *Arabidopsis. J. Exp. Bot*. 66:4595–606
22. de Lucas M, Pu L, Turco GM, Gaudinier A, Morao AK, et al. 2016. Transcriptional regulation of *Arabidopsis* Polycomb Repressive Complex 2 coordinates cell type proliferation and differentiation. *Plant Cell* 28:2616–31
23. De Rybel B, Adibi M, Breda AS, Wendrich JR, Smit ME, et al. 2014. Integration of growth and patterning during vascular tissue formation in *Arabidopsis*. *Science* 345:1255215
24. De Rybel B, Breda AS, Weijers D. 2014. Prenatal plumbing—vascular tissue formation in the plant embryo. *Physiol. Plant*. 151:126–33
25. De Rybel B, Mähönen AP, Helariutta Y, Weijers D. 2016. Plant vascular development: from early specification to differentiation. *Nat. Rev. Mol. Cell Biol.* 17:30–40
26. De Rybel B, Möller B, Yoshida S, Grabowicz I, de Reuille PB, et al. 2013. A bHLH complex controls embryonic vascular tissue establishment and indeterminate growth in *Arabidopsis. Dev. Cell* 24:426–37
27. Deeken R, Ache P, Kajahn I, Klinkenberg J, Bringmann G, Hedrich R. 2008. Identification of *Arabidopsis thaliana* phloem RNAs provides a search criterion for phloem-based transcripts hidden in complex datasets of microarray experiments. *Plant J.* 55:746–59
28. del Pozo JC, Diaz-Trivino S, Cisneros N, Gutierrez C. 2006. The balance between cell division and endoreplication depends on E2FC–DPB, transcription factors regulated by the ubiquitin–SCF^{SKP2A} pathway in *Arabidopsis*. *Plant Cell* 18:2224–35
29. Demura T, Tashiro G, Horiguchi G, Kishimoto N, Kubo M, et al. 2002. Visualization by comprehensive microarray analysis of gene expression programs during transdifferentiation of mesophyll cells into xylem cells. *PNAS* 99:15794–99
30. Depuydt S, Rodriguez-Villalon A, Santuari L, Wyser-Rmili C, Ragni L, Hardtke CS. 2013. Suppression of *Arabidopsis* protophloem differentiation and root meristem growth by CLE45 requires the receptor-like kinase BAM3. *PNAS* 110:7074–79
31. Donner TJ, Sherr I, Scarpella E. 2009. Regulation of preprocambial cell state acquisition by auxin signaling in *Arabidopsis* leaves. *Development* 136:3235–46
32. Drainas D, Kalpaxis DL. 1994. Bimodal action of spermine on ribosomal peptidyltransferase at low concentration of magnesium ions. *Biochim Biophys Acta*. 1208:55–64

33. Du J, Miura E, Robischon M, Martinez C, Groover A. 2011. The *Populus* Class III HD ZIP transcription factor *POPCORONA* affects cell differentiation during secondary growth of woody stems. *PLOS ONE* 6:e17458
34. el-Showk S, Blomster T, Siligato R, Marée AFM, Mähönen AP, Grieneisen VA. 2015. Parsimonious model of vascular patterning links transverse hormone fluxes to lateral root initiation: auxin leads the way, while cytokinin levels out. *PLOS Comput. Biol.* 11:e1004450
35. Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, et al. 2003. Radial patterning of *Arabidopsis* shoots by class III HD-ZIP and KANADI genes. *Curr. Biol.* 13:1768–74
36. Eriksson ME, Israelsson M, Olsson O, Moritz T. 2000. Increased gibberellin biosynthesis in transgenic trees promotes growth, biomass production and xylem fiber length. *Nat. Biotechnol.* 18:784–88
37. Eshed Y, Baum SF, Perea JV, Bowman JL. 2001. Establishment of polarity in lateral organs of plants. *Curr. Biol.* 11:1251–60
38. Etchells JP, Mishra LS, Kumar M, Campbell L, Turner SR. 2015. Wood formation in trees is increased by manipulating PXY-regulated cell division. *Curr. Biol.* 25:1050–55
39. Etchells JP, Provost CM, Mishra L, Turner SR. 2013. *WOX4* and *WOX14* act downstream of the PXY receptor kinase to regulate plant vascular proliferation independently of any role in vascular organisation. *Development* 140:2224
40. Etchells JP, Provost CM, Turner SR. 2012. Plant vascular cell division is maintained by an interaction between PXY and ethylene signalling. *PLOS Genet.* 8:e1002997
41. Fisher K, Turner S. 2007. PXY, a receptor-like kinase essential for maintaining polarity during plant vascular-tissue development. *Curr. Biol.* 17:1061–66
42. Foster AS. 1952. Foliar venation in angiosperms from an ontogenetic standpoint. *Am. J. Bot* 39:752–66
43. Friml J, Vieten A, Sauer M, Weijers D, Schwarz H, et al. 2003. Efflux-dependent auxin gradients establish the apical–basal axis of *Arabidopsis. Nature* 426:147–53
44. Fukuda H, Komamine A. 1980. Establishment of an experimental system for the study of tracheary element differentiation from single cells isolated from the mesophyll of *Zinnia elegans. Plant Physiol.* 65:57–60
45. Fukuda H, Komamine A. 1982. Lignin synthesis and its related enzymes as markers of tracheary-element differentiation in single cells isolated from the mesophyll of *Zinnia elegans*. *Planta* 155:423–30
46. Furuta KM, Hellmann E, Helariutta Y. 2014. Molecular control of cell specification and cell differentiation during procambial development. *Annu. Rev. Plant Biol.* 65:607–38
47. Furuta KM, Yadav SR, Lehesranta S, Belevich I, Miyashima S, et al. 2014. *Arabidopsis* NAC45/86 direct sieve element morphogenesis culminating in enucleation. *Science* 345:933
48. Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, et al. 1998. Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissue. *Science* 282:2226–30
49. Ge C, Cui X, Wang Y, Hu Y, Fu Z, et al. 2006. *BUD2*, encoding an *S*-adenosylmethionine decarboxylase, is required for *Arabidopsis* growth and development. *Cell Res.* 16:446–56
50. Goué N, Noël-Boizot N, Vallance M, Magel E, Label P. 2012. Microdissection to isolate vascular cambium cells in poplar. *Silva Fenn.* 46:5–16
51. Gursanscky NR, Jouannet V, Grünwald K, Sanchez P, Laaber-Schwarz M, Greb T. 2016. *MOL1* is required for cambium homeostasis in *Arabidopsis. Plant J.* 86:210–20
52. Hamann T, Benkova E, Bäurle I, Kientz M, Jürgens G. 2002. The *Arabidopsis BODENLOS* gene encodes an auxin response protein inhibiting MONOPTEROS-mediated embryo patterning. *Genes Dev.* 16:1610–15
53. Hamann T, Mayer U, Jurgens G. 1999. The auxin-insensitive *bodenlos* mutation affects primary root formation and apical-basal patterning in the *Arabidopsis* embryo. *Development* 126:1387–95
54. Hanzawa Y, Takahashi T, Komeda Y. 1997. *ACL5*: an *Arabidopsis* gene required for internodal elongation after flowering. *Plant J.* 12:863–74
55. Hardtke CS, Berleth T. 1998. The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. *EMBO J.* 17:1405–11
56. Hawker NP, Bowman JL. 2004. Roles for Class III HD-Zip and KANADI genes in *Arabidopsis* root development. *Plant Physiol.* 135:2261–70

57. Heo JO, Blob B, Helariutta Y. 2017. Differentiation of conductive cells: a matter of life and death. *Curr. Opin. Plant Biol.* 35:23–29
58. Helariutta Y, Fukaki H, Wysocka-Diller J, Nakajima K, Jung J, et al. 2000. The *SHORT-ROOT* gene controls radial patterning of the *Arabidopsis* root through radial signaling. *Cell* 101:555–67
59. Hertzberg M, Aspeborg H, Schrader J, Andersson A, Erlandsson R, et al. 2001. A transcriptional roadmap to wood formation. *PNAS* 98:14732–37
60. Hirakawa Y, Kondo Y, Fukuda H. 2010. TDIF peptide signaling regulates vascular stem cell proliferation via the *WOX4* homeobox gene in *Arabidopsis*. *Plant Cell* 22:2618–29
61. Hirakawa Y, Shinohara H, Kondo Y, Inoue A, Nakanomyo I, et al. 2008. Non-cell-autonomous control of vascular stem cell fate by a CLE peptide/receptor system. *PNAS* 105:15208–13
62. Hussey S, Mizrachi E, Creux N, Myburg A. 2013. Navigating the transcriptional roadmap regulating plant secondary cell wall deposition. *Front. Plant Sci.* 4:325
63. Hwang I, Sheen J, Müller B. 2012. Cytokinin signaling networks. *Annu. Rev. Plant Biol.* 63:353–80
64. Imai A, Hanzawa Y, Komura M, Yamamoto KT, Komeda Y, Takahashi T. 2006. The dwarf phenotype of the *Arabidopsis acl5* mutant is suppressed by a mutation in an upstream ORF of a bHLH gene. *Development* 133:3575–85
65. Immanen J, Nieminen K, Smolander OP, Kojima M, Alonso Serra J, et al. 2016. Cytokinin and auxin display distinct but interconnected distribution and signaling profiles to stimulate cambial activity. *Curr. Biol.* 26:1990–97
66. Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, et al. 2001. Identification of CRE1 as a cytokinin receptor from *Arabidopsis*. *Nature* 409:1060–63
67. Ito Y, Nakanomyo I, Motose H, Iwamoto K, Sawa S, et al. 2006. Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science* 313:842
68. Jacobs WP. 1952. The role of auxin in differentiation of xylem around a wound. *Am. J. Bot.* 39:301–9
69. Jouannet V, Brackmann K, Greb T. 2015. (Pro)cambium formation and proliferation: two sides of the same coin? *Curr. Opin. Plant Biol.* 23:54–60
70. Kang YH, Hardtke CS. 2016. *Arabidopsis* MAKR5 is a positive effector of BAM3-dependent CLE45 signaling. *EMBO Rep.* 17:1145–54
71. Katayama H, Iwamoto K, Kariya Y, Asakawa T, Kan T, et al. 2015. A negative feedback loop controlling bHLH complexes is involved in vascular cell division and differentiation in the root apical meristem. *Curr. Biol.* 25:3144–50
72. Kerstetter RA, Bollman K, Taylor RA, Bomblies K, Poethig RS. 2001. *KANADI* regulates organ polarity in *Arabidopsis*. *Nature* 411:706–9
73. Knott JM, Römer P, Sumper M. 2007. Putative spermine synthases from *Thalassiosira pseudonana* and *Arabidopsis thaliana* synthesize thermospermine rather than spermine. *FEBS Lett.* 581:3081–86
74. Ko D, Kang J, Kiba T, Park J, Kojima M, et al. 2014. *Arabidopsis* ABCG14 is essential for the root-to-shoot translocation of cytokinin. *PNAS* 111:7150–55
75. Ko J-H, Han K-H, Park S, Yang J. 2004. Plant body weight–induced secondary growth in *Arabidopsis* and its transcription phenotype revealed by whole-transcriptome profiling. *Plant Physiol.* 135:1069–83
76. Kondo Y, Fujita T, Sugiyama M, Fukuda H. 2015. A novel system for xylem cell differentiation in *Arabidopsis thaliana*. *Mol. Plant* 8:612–21
77. Kondo Y, Ito T, Nakagami H, Hirakawa Y, Saito M, et al. 2014. Plant GSK3 proteins regulate xylem cell differentiation downstream of TDIF–TDR signalling. *Nat. Commun.* 5:3504
78. Kondo Y, Nurani AM, Saito C, Ichihashi Y, Saito M, et al. 2016. Vascular cell induction culture system using *Arabidopsis* leaves (VISUAL) reveals the sequential differentiation of sieve element–like cells. *Plant Cell* 28:1250–62
79. Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, et al. 2005. Transcription switches for protoxylem and metaxylem vessel formation. *Genes Dev.* 19:1855–60
80. Kumar M, Campbell L, Turner S. 2015. Secondary cell walls: biosynthesis and manipulation. *J. Exp. Bot.* 67:515–31
81. Liebsch D, Sunaryo W, Holmlund M, Norberg M, Zhang J, et al. 2014. Class I KNOX transcription factors promote differentiation of cambial derivatives into xylem fibers in the *Arabidopsis* hypocotyl. *Development* 141:4311–19

82. Little CHA, MacDonald JE, Olsson O. 2002. Involvement of indole-3-acetic acid in fascicular and interfascicular cambial growth and interfascicular extraxylary fiber differentiation in *Arabidopsis thaliana* inflorescence stems. *Int. J. Plant Sci.* 163:519–29
83. Love J, Björklund S, Vahala J, Hertzberg M, Kangasjärvi J, Sundberg B. 2009. Ethylene is an endogenous stimulator of cell division in the cambial meristem of *Populus*. *PNAS* 106:5984–89
84. Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav S-R, et al. 2013. The plant vascular system: evolution, development and functions. *J. Integr. Plant Biol.* 55:294–388
85. Mähönen AP, Bishopp A, Higuchi M, Nieminen KM, Kinoshita K, et al. 2006. Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development. *Science* 311:94–98
86. Mähönen AP, Bonke M, Kauppinen L, Riikonen M, Benfey PN, Helariutta Y. 2000. A novel two-component hybrid molecule regulates vascular morphogenesis of the *Arabidopsis* root. *Genes Dev.* 14:2938–43
87. Mähönen AP, Higuchi M, Törmäkangas K, Miyawaki K, Pischke MS, et al. 2006. Cytokinins regulate a bidirectional phosphorelay network in *Arabidopsis*. *Curr. Biol.* 16:1116–22
88. Marhavý P, Bielach A, Abas L, Abuzeineh A, Duclercq J, et al. 2011. Cytokinin modulates endocytic trafficking of PIN1 auxin efflux carrier to control plant organogenesis. *Dev. Cell* 21:796–804
89. Marhavý P, Duclercq J, Weller B, Feraru E, Bielach A, et al. 2014. Cytokinin controls polarity of PIN1-dependent auxin transport during lateral root organogenesis. *Curr. Biol.* 24:1031–37
90. Mariconti L, Pellegrini B, Cantoni R, Stevens R, Bergounioux C, et al. 2002. The E2F family of transcription factors from *Arabidopsis thaliana*. *J. Biol. Chem.* 277:9911–19
91. Matsumoto-Kitano M, Kusumoto T, Tarkowski P, Kinoshita-Tsujimura K, Václavíková K, et al. 2008. Cytokinins are central regulators of cambial activity. *PNAS* 105:20027–31
92. Mattsson J, Ckurshumova W, Berleth T. 2003. Auxin signaling in *Arabidopsis* leaf vascular development. *Plant Physiol.* 131:1327–39
93. Mattsson J, Sung ZR, Berleth T. 1999. Responses of plant vascular systems to auxin transport inhibition. *Development* 126:2979–91
94. Mauriat M, Sandberg LG, Moritz T. 2011. Proper gibberellin localization in vascular tissue is required to control auxin-dependent leaf development and bud outgrowth in hybrid aspen. *Plant J.* 67:805–16
95. Mayer U, Ruiz RAT, Berleth T, Miseéra S, Juürgens G. 1991. Mutations affecting body organization in the *Arabidopsis* embryo. *Nature* 353:402–7
96. Mazur E, Kurczyńska EU, Friml J. 2014. Cellular events during interfascicular cambium ontogenesis in inflorescence stems of *Arabidopsis*. *Protoplasma* 251:1125–39
97. McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK. 2001. Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots. *Nature* 411:709–13
98. Mellor N, Adibi M, el-Showk S, De Rybel B, King J, et al. 2017. Theoretical approaches to understanding root vascular patterning: a consensus between recent models. *J. Exp. Bot.* 68:5–16
99. Milhinhos A, Prestele J, Bollhöner B, Matos A, Vera-Sirera F, et al. 2013. Thermospermine levels are controlled by an auxin-dependent feedback loop mechanism in *Populus* xylem. *Plant J.* 75:685–98
100. Mitsuda N, Seki M, Shinozaki K, Ohme-Takagi M. 2005. The NAC transcription factors NST1 and NST2 of *Arabidopsis* regulate secondary wall thickenings and are required for anther dehiscence. *Plant Cell* 17:2993–3006
101. Motose H, Sugiyama M, Fukuda H. 2004. A proteoglycan mediates inductive interaction during plant vascular development. *Nature* 429:873–78
102. Mou Z, Wang X, Fu Z, Dai Y, Han C, et al. 2002. Silencing of phosphoethanolamine *N*-methyltransferase results in temperature-sensitive male sterility and salt hypersensitivity in *Arabidopsis*. *Plant Cell* 14:2031–43
103. Mouchel CF, Briggs GC, Hardtke CS. 2004. Natural genetic variation in *Arabidopsis* identifies *BREVIS RADIX*, a novel regulator of cell proliferation and elongation in the root. *Genes Dev.* 18:700–14
104. Mouchel CF, Osmont KS, Hardtke CS. 2006. *BRX* mediates feedback between brassinosteroid levels and auxin signalling in root growth. *Nature* 443:458–61
105. Müller CJ, Valdés AE, Guodong W, Ramachandran P, Beste L, et al. 2016. PHABULOSA mediates an auxin signaling loop to regulate vascular patterning in *Arabidopsis*. *Plant Physiol.* 170:956–70

106. Muñiz L, Minguet EG, Singh SK, Pesquet E, Vera-Sirera F, et al. 2008. ACAULIS5 controls *Arabidopsis* xylem specification through the prevention of premature cell death. *Development* 135:2573–82
107. Muraro D, Mellor N, Pound MP, Lucas M, Chopard J, et al. 2014. Integration of hormonal signaling networks and mobile microRNAs is required for vascular patterning in *Arabidopsis* roots. *PNAS* 111:857–62
108. Nagawa S, Sawa S, Sato S, Kato T, Tabata S, Fukuda H. 2006. Gene trapping in *Arabidopsis* reveals genes involved in vascular development. *Plant Cell Physiol.* 47:1394–405
109. Nakajima K, Sena G, Nawy T, Benfey PN. 2001. Intercellular movement of the putative transcription factor SHR in root patterning. *Nature* 413:307–11
110. Nieminen K, Blomster T, Helariutta Y, Mähönen AP. 2015. Vascular cambium development. *Arabidopsis Book* 13:e0177
111. Nieminen K, Immanen J, Laxell M, Kauppinen L, Tarkowski P, et al. 2008. Cytokinin signaling regulates cambial development in poplar. *PNAS* 105:20032–37
112. Nilsson J, Karlberg A, Antti H, Lopez-Vernaza M, Mellerowicz E, et al. 2008. Dissecting the molecular basis of the regulation of wood formation by auxin in hybrid aspen. *Plant Cell* 20:843–55
113. Obara K, Kuriyama H, Fukuda H. 2001. Direct evidence of active and rapid nuclear degradation triggered by vacuole rupture during programmed cell death in *Zinnia*. *Plant Physiol.* 125:615–26
114. Oda Y, Mimura T, Hasezawa S. 2005. Regulation of secondary cell wall development by cortical microtubules during tracheary element differentiation in *Arabidopsis* cell suspensions. *Plant Physiol.* 137:1027–36
115. Ohashi-Ito K, Fukuda H. 2010. Transcriptional regulation of vascular cell fates. *Curr. Opin. Plant Biol.* 13:670–76
116. Ohashi-Ito K, Matsukawa M, Fukuda H. 2013. An atypical bHLH transcription factor regulates early xylem development downstream of auxin. *Plant Cell Physiol.* 54:398–405
117. Ohashi-Ito K, Saegusa M, Iwamoto K, Oda Y, Katayama H, et al. 2014. A bHLH complex activates vascular cell division via cytokinin action in root apical meristem. *Curr. Biol.* 24:2053–58
118. Okada K, Ueda J, Komaki MK, Bell CJ, Shimura Y. 1991. Requirement of the auxin polar transport system in early stages of *Arabidopsis* floral bud formation. *Plant Cell* 3:677–84
119. Péret B, Swarup K, Ferguson A, Seth M, Yang Y, et al. 2012. *AUX/LAX* genes encode a family of auxin influx transporters that perform distinct functions during *Arabidopsis* development. *Plant Cell* 24:2874–85
120. Pesquet E, Tuominen H. 2011. Ethylene stimulates tracheary element differentiation in *Zinnia elegans* cell cultures. *N. Phytologist* 190:138–49
121. Provart NJ, Alonso J, Assmann SM, Bergmann D, Brady SM, et al. 2016. 50 years of Arabidopsis research: Highlights and future directions. *New Phytol.* 209:921–44
122. Przemeck GKH, Mattsson J, Hardtke CS, Sung ZR, Berleth T. 1996. Studies on the role of the *Arabidopsis* gene *MONOPTEROS* in vascular development and plant cell axialization. *Planta* 200:229–37
123. Randall RS, Miyashima S, Blomster T, Zhang J, Elo A, et al. 2015. AINTEGUMENTA and the D-type cyclin CYCD3;1 regulate root secondary growth and respond to cytokinins. *Biol. Open* 4:1229–36
124. Rashotte AM, Mason MG, Hutchison CE, Ferreira FJ, Schaller GE, Kieber JJ. 2006. A subset of *Arabidopsis* AP2 transcription factors mediates cytokinin responses in concert with a two-component pathway. *PNAS* 103:11081–85
125. Robischon M, Du J, Miura E, Groover A. 2011. The *Populus* Class III HD ZIP, *popREVOLUTA*, influences cambium initiation and patterning of woody stems. *Plant Physiol.* 155:1214–25
126. Rodriguez-Villalon A, Gujas B, Kang YH, Breda AS, Cattaneo P, et al. 2014. Molecular genetic framework for protophloem formation. *PNAS* 111:11551–56
127. Rodriguez-Villalon A, Gujas B, van Wijk R, Munnik T, Hardtke CS. 2015. Primary root protophloem differentiation requires balanced phosphatidylinositol-4,5-biphosphate levels and systemically affects root branching. *Development* 142:1437
128. Sachs T. 1981. The control of the patterned differentiation of vascular tissues. *Adv. Bot. Res.* 9:151–262
129. Salazar-Henao JE, Lehner R, Betegón-Putze I, Vilarrasa-Blasi J, Caño-Delgado AI. 2016. BES1 regulates the localization of the brassinosteroid receptor BRL3 within the provascular tissue of the *Arabidopsis* primary root. *J. Exp. Bot.* 67:4951–61
130. Salehin M, Bagchi R, Estelle M. 2015. $SCF^{TIR1/AFB}$-based auxin perception: mechanism and role in plant growth and development. *Plant Cell* 27:9–19

131. Sawchuk MG, Edgar A, Scarpella E. 2013. Patterning of leaf vein networks by convergent auxin transport pathways. *PLOS Genet*. 9:e1003294
132. Sawchuk MG, Head P, Donner TJ, Scarpella E. 2007. Time-lapse imaging of *Arabidopsis* leaf development shows dynamic patterns of procambium formation. *New Phytol*. 176:560–71
133. Scarpella E, Marcos D, Friml J, Berleth T. 2006. Control of leaf vascular patterning by polar auxin transport. *Genes Dev*. 20:1015–27
134. Scheres BJG, Di Laurenzio L, Willemsen V, Hauser M-T, Janmaat K, et al. 1995. Mutations affecting the radial organisation of the *Arabidopsis* root display specific defects throughout the embryonic axis. *Development* 121:53–62
135. Scheres BJG, Wolkenfelt H, Willemsen V, Terlouw M, Lawson E, et al. 1994. Embryonic origin of the *Arabidopsis* primary root and root meristem initials. *Development* 120:2475–87
136. Schlereth A, Moller B, Liu W, Kientz M, Flipse J, et al. 2010. MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature* 464:913–16
137. Shininger TL. 1979. The control of vascular development. *Annu. Rev. Plant Physiol.* 30:313–37
138. Smet W, De Rybel B. 2016. Genetic and hormonal control of vascular tissue proliferation. *Curr. Opin. Plant Biol.* 29:50–56
139. Snow R. 1935. Activation of cambial growth by pure hormones. *New Phytol.* 34:347–60
140. Sorokin HP, Mathur SN, Thimann KV. 1962. The effects of auxins and kinetin on xylem differentiation in the pea epicotyl. *Am. J. Bot.* 49:444–54
141. Steinmann T, Geldner N, Grebe M, Mangold S, Jackson CL, et al. 1999. Coordinated polar localization of auxin efflux carrier PIN1 by GNOM ARF GEF. *Science* 286:316–18
142. Sterky F, Regan S, Karlsson J, Hertzberg M, Rohde A, et al. 1998. Gene discovery in the wood-forming tissues of poplar: analysis of 5,692 expressed sequence tags. *PNAS* 95:13330–35
143. Suer S, Agusti J, Sanchez P, Schwarz M, Greb T. 2011. *WOX4* imparts auxin responsiveness to cambium cells in *Arabidopsis*. *Plant Cell* 23:3247–59
144. Takano A, Kakehi JI, Takahashi T. 2012. Thermospermine is not a minor polyamine in the plant kingdom. *Plant Cell Physiol.* 53:606–16
145. Taylor-Teeples M, Lin L, de Lucas M, Turco G, Toal TW, et al. 2015. An *Arabidopsis* gene regulatory network for secondary cell wall synthesis. *Nature* 517:571–75
146. Torrey JG. 1965. Physiological bases of organization and development in the root. In *Encyclopedia of Plant Physiology*, Vol. 15: *Differentiation and Development*, ed. FT Addicott, A Lang, W Ruhland, pp. 1256–327. Berlin: Springer
147. Truernit E, Bauby H, Belcram K, Barthélémy J, Palauqui J-C. 2012. OCTOPUS, a polarly localised membrane-associated protein, regulates phloem differentiation entry in *Arabidopsis thaliana*. *Development* 139:1306
148. Truernit E, Bauby H, Dubreucq B, Grandjean O, Runions J, et al. 2008. High-resolution whole-mount imaging of three-dimensional tissue organization and gene expression enables the study of phloem development and structure in *Arabidopsis*. *Plant Cell* 20:1494–503
149. Ueguchi C, Koizumi H, Suzuki T, Mizuno T. 2001. Novel family of sensor histidine kinase genes in *Arabidopsis thaliana*. *Plant Cell Physiol.* 42:231–35
150. Uggla C, Mellerowicz EJ, Sundberg B. 1998. Indole-3-acetic acid controls cambial growth in Scots pine by positional signaling. *Plant Physiol.* 117:113–21
151. Uggla C, Moritz T, Sandberg G, Sundberg B. 1996. Auxin as a positional signal in pattern formation in plants. *PNAS* 93:9282–86
152. Vera-Sirera F, De Rybel B, Urbez C, Kouklas E, Pesquera M, et al. 2015. A bHLH-based feedback loop restricts vascular cell proliferation in plants. *Dev. Cell* 35:432–43
153. Weijers D, Schlereth A, Ehrismann JS, Schwank G, Kientz M, Jürgens G. 2006. Auxin triggers transient local signaling for cell specification in *Arabidopsis* embryogenesis. *Dev. Cell* 10:265–70
154. Wenzel CL, Schuetz M, Yu Q, Mattsson J. 2007. Dynamics of *MONOPTEROS* and PIN-FORMED1 expression during leaf vein pattern formation in *Arabidopsis thaliana*. *Plant J.* 49:387–98
155. Werner T, Motyka V, Strnad M, Schmülling T. 2001. Regulation of plant growth by cytokinin. *PNAS* 98:10487–92

156. Wisman E, Cardon GH, Fransz P, Saedler H. 1998. The behaviour of the autonomous maize transposable element *En/Spm* in *Arabidopsis thaliana* allows efficient mutagenesis. *Plant Mol. Biol.* 37:989–99
157. Xaplanteri MA, Petropoulos AD, Dinos GP, Kalpaxis DL. 2005. Localization of spermine binding sites in 23S rRNA by photoaffinity labeling: parsing the spermine contribution to ribosomal 50S subunit functions. *Nucleic Acids Res.* 33:2792–805
158. Xu B, Ohtani M, Yamaguchi M, Toyooka K, Wakazaki M, et al. 2014. Contribution of NAC transcription factors to plant adaptation to land. *Science* 343:1505–8
159. Yamamoto R, Demura T, Fukuda H. 1997. Brassinosteroids induce entry into the final stage of tracheary element differentiation in cultured *Zinnia* cells. *Plant Cell Physiol.* 38:980–83
160. Ye Z-H, Zhong R. 2015. Molecular control of wood formation in trees. *J. Exp. Bot.* 66:4119–31
161. Yoshimoto K, Noutoshi Y, Hayashi K-I, Shirasu K, Takahashi T, Motose H. 2012. A chemical biology approach reveals an opposite action between thermospermine and auxin in xylem development in *Arabidopsis thaliana*. *Plant Cell Physiol.* 53:635–45
162. Young BS. 1954. The effects of leaf primordia on differentiation in the stem. *New Phytol.* 53:445–60
163. Zhang K, Novak O, Wei Z, Gou M, Zhang X, et al. 2014. *Arabidopsis* ABCG14 protein controls the acropetal translocation of root-synthesized cytokinins. *Nat. Commun.* 5:3274
164. Zhong R, Demura T, Ye Z-H. 2006. SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of *Arabidopsis*. *Plant Cell* 18:3158–70
165. Zhong R, Lee C, Zhou J, McCarthy RL, Ye Z-H. 2008. A battery of transcription factors involved in the regulation of secondary cell wall biosynthesis in *Arabidopsis*. *Plant Cell* 20:2763–82
166. Zhu Y, Song D, Sun J, Wang X, Li L. 2013. *PtrHB7*, a class III HD-Zip gene, plays a critical role in regulation of vascular cambium differentiation in *Populus*. *Mol. Plant* 6:1331–43
167. Zürcher E, Liu J, di Donato M, Geisler M, Müller B. 2016. Plant development regulated by cytokinin sinks. *Science* 353:1027–30