Genetic Networks in Plant Vascular Development

Raili Ruonala [1,2,*], Donghwi Ko [2,*], and Ykä Helariutta [1,2]

[1]Institute of Biotechnology and Department of Biosciences, University of Helsinki, 00014 Helsinki, Finland
[2]Sainsbury Laboratory, University of Cambridge, Bateman Street, Cambridge CB2 1LR, UK

[*]These authors contributed equally to this work.

raili.ruonala@slcu.cam.ac.uk
donghwi.ko@slcu.cam.ac.uk
yrjo.helariutta@slcu.cam.ac.uk

Author for correspondence: Ykä Helariutta (yrjo.helariutta@slcu.cam.ac.uk)

Keywords:
Plant vascular tissue patterning, cambial development, xylem and phloem differentiation, phytohormone signalling, transcriptional network, genetic approach

ABSTRACT

Understanding the development of vascular tissues in plants is crucial since the evolution of vasculature enabled land 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

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 (130). From an evolutionary perspective, the invention of vasculature has been crucial to enable plants thrive on land (79; 151) since these tissues provide rapid, controlled delivery paths throughout the plant, as well as mechanical support. The plant vascular system is complex, with a vascular cylinder that consists of three cell types arranged in a highly organized manner, each with 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 cells give rise to cells that differentiate into xylem towards the inner side of the vascular cylinder and phloem towards the outer side. Xylem and phloem cells are specialized for water and nutrient transport, respectively, and also facilitate the transport of various signalling 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 signalling mechanisms that have been recently reviewed in detail (16; 25; 75; 105). We therefore start with a historical overview of the major approaches that have been valuable in this important research field.

Diversity of plant vascular developmental systems and genetic approaches

Classical descriptive and experimental studies on plant vascular development established the anatomical and physiological foundations of the research field (133). 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 (41; 140). The importance of plant hormones in the regulation of vascular patterning was highlighted during the same period. In the 1930s, treatment with auxin was shown to induce cambial growth in various plant species, including *Coleus*, *Vicia faba*, and *Tradescantia* (134). Young and coworkers 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 (155). Later, cytokinins were suggested to also be important signalling molecules in vascular patterning based on phenotypic changes observed in the vascular tissue of pea epicotyls upon cytokinin treatment (135).

Before 1980, most studies of xylem formation used entire multicellular organisms, which did not allow investigation of the stages of xylem cell differentiation. In order to overcome this problem, Fukuda and coworkers established a method to isolate single mesophyll cells from Zinnia leaves and initiate differentiation of the mesophyll cells into xylem vessels (tracheary elements, TEs) (43). 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; 44; 108). In addition, the Zinnia cell culture system was crucial in identifying signalling peptides important for cambial proliferation, known as Tracheary Differentiation Inhibiting Factor (TDIF)

(58; 63). In 2005, microarray analysis of xylem vessel formation using a newly established in vitro Arabidopsis system 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 (74). The Vascular cell Induction culture System Using Arabidopsis Leaves (VISUAL) was established more recently and has been used to investigate genome-wide expression pattern of genes during phloem differentiation (73).

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 (116). 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, accelerating studies of the genetic networks governing vascular development in plants (8; 46; 90). For instance, *MONOPTEROS* (*MP*), an auxin-inducible transcription factor which plays a crucial role in vascular patterning in both embryonic and post-embryonic organs was identified from an EMS-mutagenized mutant pool (90). In order to elaborate the genetic networks involved in the *MP* pathway, genome-wide transcriptional changes in the *mp* mutant and related transgenic plants were analysed, revealing several TARGET OF MONOPTEROS (TMO) genes (132). 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; 66; 146). In addition to the forward genetic approach, a study of natural genetic variation in root growth identified *BREVIS RADIX* (*BRX*) as an important factor in protophloem differentiation in Arabidopsis. Of the tree species, Populus is currently the best understood, thanks to the availability of its full genome sequence and organ/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 (56; 153). In 1998, expressed sequence tags (ESTs) were constructed from the wood-forming tissues of poplar to help identify genes involved in controlling the development of vascular tissues in this tree (137).

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; 48). Genes identified via transcriptomics were investigated further by reverse genetics approaches (74), which have been facilitated by the availability of a genome-wide T-DNA insertional mutant library (116). More recently, a yeast one-hybrid network was used to establish the presence of a feedback loop in the transcriptional regulation of secondary cell wall formation (139). The genome-wide approaches have accumulated massive amounts of data and, together with the emergence of mathematical modelling (ideally with the capacity to predict gene regulatory networks), have begun to expand our understanding of these developmental processes (23; 33; 93; 102). The major approaches described here are illustrated in Figure 2 in consecutive order.

GENETIC NETWORKS IN PLANT VASCULAR DEVELOPMENT

Patterning of the vascular tissues in diverse vascular meristems

Provascular development during embryogenesis. The Arabidopsis zygote undergoes a series of cell divisions to give rise to an embryo comprised of cells with designated, specific fates. The establishment of the provascular tissue in the root, which is the origin of the post-embryonic vascular cylinder, is initiated by periclinal division of the four central cells in the lower-tier domain of the early globular stage embryo (24; 130). The *monopteros* (*mp*) mutant, which displays severe defects in root-pole formation and vascular development, was first isolated by a forward genetic screen of an EMS-generated mutant pool (90). *MONOPTEROS/AUXIN RESPONSE FACTOR 5* (*MP/ARF5*) is an auxin-dependent transcription factor which plays an important role in the specification of the provascular tissue in the embryo, as well as in post-embryonic vascular patterning (9; 53; 148). In the early stages of embryogenesis, *MP/ARF5* is broadly expressed, but its expression is gradually confined to the provascular tissue (53), 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 (42; 136). Examination of expression profiles revealed that *MP/ARF5* act 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***) (110; 132; 147; 148). Consistent with this, mutation of either *MP/ARF5* or *PIN1* abolishes the auxin maximum, as well as provascular patterning in embryo, indicating that MP/ARF5-mediated auxin maximum formation and signalling is essential for provascular establishment (9; 42; 53).

Auxin signalling initiates upon the binding of auxin to TRANSPORT INHIBITOR RESPONSE 1 (TIR1) in the SKP1-CUL1-F-box $(SCF)^{TIR1/AFB}$ 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 (125). Another EMS-generated mutant screen isolated the *bodenlos* (*bdl*) mutant, which shows phenotypes similar with those of the *mp* mutant and harbours a point mutation in the conserved ubiquitination domain of the *AUX/IAA12* transcriptional repressor (50; 51). 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 *mp* mutation (50) (**Figure 3*a***). Transcriptomic analysis of the *mp* mutant and seedlings expressing dexamethasone-inducible *bdl* revealed that MP/ARF5 directly upregulates the transcription of *TARGET OF MONOPEROS* (*TMO*) *3*, *5*, *6* and *7* (132). *TMO5* encodes a basic helix-loop-helix (bHLH) transcription factor which is sufficient to rescue the provascular initiation defects in *mp* (26). Furthermore, co-immunoprecipitation followed by liquid chromatography tandem mass spectrometry analysis revealed that TMO5 forms a heterodimer with LONESOME HIGWAY (LHW), an atypical bHLH transcription factor, to promote the periclinal division of provascular and procambial cells (**Figure 3*a,b***) (26; 111; 112). High-order knockout mutants of either *TMO5* or *LHW* with their corresponding paralogue, *TMO5-LIKE1* (*T5L1*) and *LHW-LIKE1* (*LL1*), exhibit a severe reduction in the number of periclinal divisions of the provascular and procambial cells in the embryo and root, respectively, indicating that these bHLH transcription factors are crucial components in *MP/ARF5*-mediated provascular and procambial patterning (26; 111). *TMO3* encodes an AP2 transcription factor which is also known as *CYTOKININ RESPONSE FACTOR 2* (*CRF2*), a cytokinin-inducible gene which is crucial for embryonic development (119), indicating that auxin and cytokinin signalling converge in provascular patterning.

Cytokinins, like auxin, are key signalling molecules implicated in vascular development. The strong expression of the cytokinin receptor *WOODENLEG* and the synthetic cytokinin reporter

Two Component signalling Sensor new (TCSn)::GFP in embryo provascular tissue suggests that cytokinin functions in provascular patterning (81; 160). 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 signalling mediated by plasma-membrane localized cytokinin receptors (160). 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 will be further discussed below.

Hormonal signalling 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). *WOODENLEG* (*WOL*), *SHORTROOT* (*SHR*), and *SCARECROW* (*SCR*), key regulators involved in patterning the root vascular cylinder, were isolated from mutant pools generated by EMS-mediated mutagenesis in the early 1990s (8; 131). 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 (81; 129). *WOL* encodes a cytokinin receptor, a two-component histidine kinase also known as *CYTOKININ RESPONSE1* (*CRE1*) and *ARABIDOPSIS HISTIDINE KINASE4* (*AHK4*) (62; 81; 129; 143). 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 (60). Interestingly, the *wol* mutant harbours 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 (82). The phenotypes of *wol* and the *ahk2ahk3ahk4* triple mutant, together with the procambium-specific expression of cytokinin-inducible *ARR5*, indicate that cytokinin signalling is restricted to procambial cells and mediates procambial proliferation and maintenance (12; 62; 81). Consistent with this, treatment with exogenous cytokinin suppresses the differentiation of protoxylem, while overexpression of the cytokinin oxidases, which degrade cytokinins, phenocopies the *wol* mutant (80; 149). A screen for suppressors of *wol* isolated *AHP6*, a negative regulator of cytokinin signalling which is primarily expressed in the protoxylem. *AHP6* inhibits cytokinin signalling in the developing protoxylem and stimulates protoxylem differentiation at the marginal position of the central xylem axis in roots (Figure 3*b*) (80).

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*-naphathylphtalamic (NPA) and the absence of xylem cells in the auxin-signalling-resistant mutant *auxin-resistant1* (*axr1*) (12). Auxin response reporters, such as *DR5* and *IAA2*, are primarily expressed in the xylem axis, but their expression expands into the procambial cells following changes in cytokinin signalling, suggesting that cytokinin confines the auxin maximum to the xylem axis of the root (12). On the other hand, auxin-induced *LHW* induces expression of *AHP6* in the protoxylem and adjacent pericycle cells (112), where it inhibits cytokinin signalling, demonstrating that there is a mutual negative feedback loop between auxin and cytokinin signalling which generates the distinct domains of the hormones 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, *3* and *7* but also for the appropriate subcellular localization of PIN1 in procambial cells (12; 83; 84). Likewise, the *pin1pin3* mutant displays distorted development of protoxylem (12), indicating that cytokinin signalling regulates the PINs in order to steer auxin flow toward the xylem axis. Recently, it has been established that local cytokinin biosynthesis by *LOG3* and *4*, which are directly upregulated by auxin-induced *TMO5/LHW* in the xylem axis, plays an important role in 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 signalling domains (23; 33; 93; 102).

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 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 vs. spiral in protoxylem) or molecularly by cell-type specific markers, such as *ACAULIS5* for metaxylem (101) and *AHP6* for protoxylem (80). Combined with the consistent organization of the xylem axis, with metaxylem cells in the centre 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–microRNA 165/166*–HD-ZIP III pathway in a reciprocal manner; while a high dose of *miR165/166* and the consequent 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* (55; 104), to activate *miR165/166* outside the stele (20). Upon moving back into the stele, *miR165/166* degrades HD-ZIP III transcripts, such as *PHABULOSA* (*PHB*), producing a gradient that determines the vessel type. Recently, *PHB* has been reported to upregulate *IAA20/30* and *MP*, stabilizing the auxin response in the xylem axis, which suggests that there may also be a feedback loop between hormone signalling and the *SHR–SCR–miRNA165/6*–HD-ZIP III pathway (Figure 3*b*) (100). Complementing the experimental evidence, a computational model suggests that degradation of *miRNA165/166* itself, in addition to its degradation of the HD-ZIP III transcripts, is necessary for correct vascular patterning (102).

The players in this dynamic pathway were identified in mutant screens for altered vascular development in the Arabidopsis primary root (8; 20; 55; 131). 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 (55), while the HD-ZIP III genes redundantly restrict procambium proliferation (20).

In addition to their activity in the root, the HD-ZIP III transcription factors play crucial roles in radial vascular patterning in the shoot (34; 92). Antagonistic regulation between the HD-ZIP IIIs and the KANADI (KAN) genes, a family of GARP transcription factors primarily expressed in developing phloem, has been reported to regulate radial vascular pattering in the shoot (34;

67). The *phv phb rev* mutant displays abaxialized cotyledons and a distorted radial vascular pattern in which the xylem is surrounded by phloem (34). 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 (34; 36). 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 (54). In Populus, transgenic trees misexpressing the HD-ZIP III transcription factor *REVOLUTA* showed ectopic formation of cambium and severe patterning defects with reversed polarity; secondary xylem was produced towards the outside and phloem towards the inside of the stem (120). Other HD-ZIP III genes have been shown to affect the rate of secondary xylem and phloem differentiation in Populus (32; 159), further indicating that cambial expression of the HD-ZIP IIIs is important in vascular patterning as well as 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*) (141) was discovered in a systematic survey for genes expressed during early procambial (103) 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; 141). 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 (141). *OPS* is a plant-specific plasma membrane protein that was recently shown to regulate brassinosteroid signalling via direct repression of the GLYCOGEN SYNTHASE KINASE 3 (GSK3) family member *BRASSINOSTEROID-INSENSITIVE 2* (*BIN2*) (2) (Figure 4a). Further work has confirmed that brassinosteroid signalling, which was initially described in vascular differentiation of the inflorescence stem (17), also plays a role in Arabidopsis root protophloem differentiation (65; 124).

An interesting study on natural variation in root growth among 44 Arabidopsis accessions prompted the discovery of a natural loss-of-function allele *brevis radix* (*brx*) in the accession Umkirch-1 (98); later, *BRX* was linked to early protophloem development in a manner similar to *OPS* (121). In the short-rooted *brx* mutant, the levels of brassinosteroids are down and auxin responsiveness is reduced, indicating feedback regulation between these two hormones at an early stage in Arabidopsis root growth (98; 99). A second-site suppressor screen in the *brx* mutant background added more dimensions to the regulatory network related to phloem differentiation (Figure 4a). Depuydt and coworkers (30) reported that *BRX* restricts the expression of *BARELY ANY MERISTEM 3* (*BAM3*), the receptor of a 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* (65). Given that phloem files, though 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; 121; 141). *COTYLEDON VASCULAR PATTERN 2* (*CVP2*) was also isolated from a *brx* suppressor screen (122); a double mutant with its close homolog *CVP2-like 1* has discontinuous cell files in the root protophloem and abnormal level of phosphoinositides. These genes had also been characterized earlier from a mutant screen for discontinuous vein patterns in the cotyledons, suggesting that phosphoinositides may act as signalling molecules for vascular strand propagation (18; 19).

Leaf vein patterning. Leaf primordia develop preprocambial tissue composed of subepidermal cells which are morphologically inconspicuous but are marked by auxin response genes (41; 145). The preprocambial cells give rise to procambia which consist of narrow cells arranged in continuous strands and which differentiate into the vascular cylinder of leaves (5; 41; 127; 128). Since an early 1950s study which showed that exogenous auxin treatment mimics the endogenous signal from young leaf primordia in inducing vascular development (64), numerous studies have supported the role of auxin as a positional cue in the formation of the vascular system in leaves (87; 123; 128; 145). The expression profile of the synthetic auxin reporter *DR5* shows an auxin maximum at the site where veins will form (87). 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 (87). 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 preprocambium (128). Accordingly, the *pin1* mutant exhibits reduced auxin transport and a distorted vascular pattern which 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 (87; 88; 113). 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 (126). 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 (114). In addition, defective auxin signalling in the *mp* mutant results in impaired vascular patterning in leaves, as well as in embryos (9; 117). 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) (148).

In 1995, studies of Arabidopsis homeobox genes containing a leucine-zipper motif (HD-ZIP) identified the class III HD-ZIP gene *ARABIDOPSIS THALIANA HOMEOBOX 8* (*ATHB8*) as an auxin-inducible preprocambial and procambial marker specifically expressed in the vascular tissues of the embryo and post-embryonic 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 essential for the gene's specific expression in the vascular tissues (31; 132). Expression of *ATHB8* is dramatically diminished in the *mp* mutant and increased by overexpression of *MP*, suggesting that *MP* is a direct transcriptional activator of *ATHB8* in the preprocambial and procambial cells (Figure 3*c*) (31). Recently, Baima and coworkers identified *ACAULIS5* (*ACL5*) and *BUSHY AND DWARF2* (*BUD2*) as downstream targets of *ATHB8* (4). The *acl5* mutant was originally identified from an EMS mutant screen because of its dwarf phenotype and distorted xylem development (4; 52; 101). An in vitro enzymatic activity analysis revealed that *ACL5* encodes thermospermine synthase, which produces thermospermine, a polyamine which is important in various plant developmental processes (68). The *bud2* mutant was isolated from a mutant pool produced by a sense/antisense RNA expression system (97); it shows retarded shoot growth and impaired vascular patterning similar to the *acl5* mutant (47). *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 role in vascular patterning (47). Treatment with exogenous polyamines inhibits auxin-induced

xylem differentiation in leaves and prolongs xylem differentiation in Zinnia cell cultures (101; 154). Interestingly, overexpression of *POPACAULIS5*, the orthologue of *ACL5* in poplar, suppresses biosynthesis of auxin, while *PttHB8*, the poplar orthologue of *ATHB8*, increases expression of *POPACAULIS5*, suggesting that there is a negative feedback loop between thermospermine biosynthesis and auxin signalling (94). Indeed, chromatin immunoprecipitation (ChIP) 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 inhibiting the expression of HD-ZIP III and auxin signalling genes (**Figure 3*c***) (4; 101). More recently, two studies have established the existence of a negative feedback network involving *ACL5*, four bHLH transcription factors belonging to the SUPPRESSOR OF ACACULIS51 LIKE (SACL) clade, and *TMO5/LHW*, all of which are specifically expressed in the vascular cylinder of the leaf, root and stem (66; 146). The LHW heterodimer with either TMO5 or T5L1 increases the activity of the SACLs either by 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***) (66; 146).

Vascular cambium: establishment and patterning of the secondary meristem. Vascular cambium, a layer of meristematic cells, typically separates the differentiated tissues xylem and phloem in the vasculature; such an organized pattern requires coordinated and properly oriented division of the cambial cells, as well as timely differentiation processes of the specialized cell types. In a mutant screen for irregular vascular organization in the Arabidopsis inflorescence stem, Fisher and Turner (40) identified an LRR kinase which 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 since the cambial cell division plane was abnormal; in addition, the ratio of vessels compared to 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 (43; 58). In Arabidopsis, two genes, *CLE41* and *CLE44*, encode the TDIF peptide (63), which is found in phloem, while *PXY* is expressed in dividing cambial cells (40; 58). This implies that *PXY-CLE41/44* signalling 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 *CLE41/44*-dependent interaction of PXY with BIN2, a GSK3 protein that transcriptionally downregulates the transcription factor *BES1* in brassinosteroid signalling (**Figure 3*d***) (72). *PXY-CLE41/44* promotes cambial proliferation during secondary growth of the Arabidopsis hypocotyl and inflorescence stem by activating *WUSCHEL-related HOMEOBOX 4* (*WOX4*) and *WOX14* (**Figure 3*d***) (38; 57). Interestingly, *PXY-CLE41/44* also controls secondary growth in the model tree species Populus; transgenic trees overexpressing the Populus orthologues of *PXY* and *CLE41* specifically in the cambium and phloem, respectively, showed an increased rate of cambial cell division, leading to a dramatic increase in biomass (37). Thus, the *PXY-CLE41/44* signalling pathway may affect vascular organization in many ways, and manipulation of this conserved pathway might even be considered as a means for improved 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 positive and negative roles in cambium activity, respectively, have been identified through spatiotemporal transcriptome analysis of tissues collected via laser microdissection following in vitro induction of secondary growth (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 a decrease and increase in interfascicular cambium-derived tissue, respectively, suggesting that they mediate opposing signals regulating cambium activity (1). More recently, Gursanscky and coworkers suggested that *MOL1* negatively regulates ethylene and jasmonic acid signalling, which induce lateral growth (49).

Alongside *PXY-CLE41/44*, several other factors relevant for 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 direct continuous xylem differentiation by promoting cell-cell interactions in Zinnia and Arabidopsis (96). 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 (43; 74; 109; 115; 152); these hormones may also act as important regulators of cambial activity.

Hormone signalling and networks regulating cambial activity. Much of our current understanding of secondary growth arises from research on the herbaceous species Arabidopsis, which can be regarded as a valid model since there is evidence of conserved mechanisms controlling cambial activity across species. However, 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 (134); since then, numerous studies have supported the role of basipetally transported auxin as one of the major regulators of cambial activity (70; 77). For instance, the auxin-signalling-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; 144). In the Arabidopsis stem, expression of the auxin response reporter *DR5* is upregulated in parenchyma cells differentiating into interfascicular cambium (91). Furthermore, auxin activates expression of *WOX4*, a transcription factor downstream of *PXY* which is involved in cambial cell proliferation (**Figure 3*d***) (138). Accumulation of auxin in the Arabidopsis stem after a 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***) (138). In Populus, functional studies have shown that reduced auxin responsiveness reduces cambial activity (107).

Cytokinins are also well-known to stimulate cell division in meristems. The high-order Arabidopsis mutant of the ISOPENTENYLADENINE TRANSFERASE (IPT) genes, which are rate-limiting enzymes in cytokinin biosynthesis, has a severe reduction in the size of the cambium (86). Consistent with this, cytokinin signalling 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 (106), while suppression of CKX genes in Arabidopsis generates a thicker inflorescence stem (6). In Populus, overexpression of *IPT3* increased auxin levels and greatly enhanced radial growth. 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 (61) 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, as evinced by the severe growth retardation in the stem of the *abcg14* mutant, which is defective in cytokinin transport (69; 156). 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; 118). Accordingly, mutants of *ant* and *cycd3;1* exhibit impaired cambial activity similar to that of cytokinin biosynthesis and signalling mutants. Compared with the single mutant phenotypes, the additive phenotype of the *ant cycd3* double mutant indicates that they are both regulated by cytokinins but contribute independently to cell proliferation (**Figure 3*d***) (118).

Another example of the hormonal regulation of cambial activity comes from Populus, where gibberellin levels are positively correlated with biomass accumulation (35) and gibberellin signalling appears to stimulate secondary xylem differentiation, as well as biomass accumulation in terms of plant height and fibre length (89). Furthermore, exogenously applied ethylene or overexpression of an ethylene biosynthesis gene stimulates cambial growth and wood formation in Populus (78). Ethylene appears to function in parallel with the *PXY* pathway to induce cambial proliferation (39; 78). The mild reduction in vascular cell number by the mutation of *PXY* is attributed to compensatory upregulation of ETHYLENE RESPONSE FACTORs (ERFs), such as *ERF018* and *ERF109*, in Arabidopsis. The expression of *ERF018*/*109* is increased in both the *pxy* and *wox4* mutants, and the phenotype of *pxy* is exaggerated by mutation of *ERF018/109*, suggesting that both the *PXY* pathway and ethylene signalling positively regulate cambial activity, while the *PXY* pathway also represses ethylene signalling (**Figure 3*d***) (39).

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 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 to generate 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. Since much of plant biomass derives from the development of the secondary cell walls of xylem vessels and fibres, 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 very important in the activation of the secondary cell wall regulatory network. Various approaches have identified five NACs that are sufficient to regulate xylem vessel formation and are therefore regarded as master regulators. Ectopic overexpression of *VASCULAR-RELATED NAC-DOMAIN 6* (*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 (74; 95; 157). *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 metaxylem and protoxylem-like cells, respectively, in Arabidopsis roots and Populus leaves (74). In the Arabidopsis inflorescence stem, *VND6* expression is restricted to the metaxylem vessels, while the *VND7* expression domain includes both meta- and protoxylem vessels (158). *NST1* and *NST2* were discovered because of their role in anther dehiscence in Arabidopsis by dominant EAR-motif-mediated repression analysis of the NAC

genes (95). *NST1* and *NST2* function redundantly; in the double knockout *nst1 nst2*, 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 fibres of the Arabidopsis inflorescence stem (95). Finally, the fifth master regulator, *SND1* was discovered as a highly expressed NAC transcription factor in xylem fibre cells that were isolated from Arabidopsis inflorescence stems by laser microdissection (157).

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 (59; 75; 139). Recently, interesting new connections were made in a large-scale yeast one-hybrid (Y1H) screen and subsequent network analysis to study protein interactions with transcription factors expressed in the root xylem (**Figure 4*b***) (14; 15; 139; 158). For example, the *E2Fc* transcription factor, which is a negative regulator of endoreduplication (28; 85), was placed upstream of *VND7* by the network analysis, with either a positive or negative effect, depending on dosage (139). 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 feed-forward loops that contribute to xylem differentiation were also highlighted in the transcriptional network (139). 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.

In the case of xylem fibre differentiation, perhaps surprisingly, Liebsch and coworkers (76) discovered a redundant function for the class I KNOX transcription factors *SHOOT MERISTEMLESS* (*STM*) and *KNAT 1*, 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 fibres 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. While 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*), a MYB transcription factor 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 characteristic of tracheary elements rather than sieve elements (SEs). The *apl* mutant was selected from a transposon-tagged pool of Arabidopsis (150) because of its *wol*-like phenotype: a short, determinate primary root and arrested shoot development. In contrast to *wol*, 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. Since protophloem can develop in a normal manner in *apl* embryos (142), *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 (45) (**Figure 4*a***). *NAC45/86* were identified by

comparing transcript profiles of the *apl* mutant and wild-type plants, while the downstream NENs were found by comparison of 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 (45).

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 (71; 73). In this system, named VISUAL (Vascular cell Induction culture System Using Arabidopsis Leaves), 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 identified *NAC20* as an upstream negative regulator of *APL* (73). 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.

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 signalling molecules.

2. Various systems and approaches have been invented and used to study the mechanisms governing plant vascular patterning and identify multiple genetic components and 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 signalling and transcriptional regulation. The interaction between auxin and cytokinin plays 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. Signalling mediated by LRR-RLKs and their ligands interacts with hormonal signalling to regulate cell specification and proliferation during vascular development.

5. Several members of the MYB and NAC transcription factor families play important roles in vascular tissue differentiation processes during vasculature maturation.

FUTURE ISSUES

1. Cell fate is determined by specific proteins, which can be regulated transcriptionally, post-transcriptionally, translationally, post-translationally 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. This suggests that translational regulation is indeed a crucial step 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 which reported that trimethylation of histone by *POLYCOMB REPRESSIVE COMPLEX 2* has a role in vascular cell proliferation and differentiation (22). However, the detailed mechanisms underlying translational and epigenetic regulation of vascular-specific genes remain elusive. Research on translational and epigenetic regulation may identify novel factors and further elaborate the processes governing vascular patterning.

2. Network construction and analysis based on bioinformatics and statistics, combined with dynamic and predictive modelling, may become even more powerful tools once data about the translatome and protein interactions are incorporated. This may also be extended to explore evolutionary aspects of plant vascular development and connect the disparate genetic networks.

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 in order to support the ever-growing human population. After all, in crop species, the storage organs we eat consist mostly of vascular tissues, and wood is composed of secondary xylem, the water-conducting tissue of the vasculature.

ACKNOWLEDGEMENTS

We sincerely thank Bonghee Heo for assistance with graphical illustrations, Sedeer el-Showk for helpful comments while proof-reading the manuscript and Ari Pekka Mähönen for discussions. The Y.H. lab is generously supported by the Gatsby Foundation, the European Research Council Advanced Investigator Grant Symdev (No. 323052), the Academy of Finland Centre of Excellence programme, the University of Helsinki, Tekes (the Finnish Funding Agency for Technology and Innovation) and the Biotechnology and Biological Sciences Research Council.

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*. *Current Biology* 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. *The Plant Journal* 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. *Molecular 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. *The 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 and Cell Physiology* 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? *Physiologia plantarum* 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 (New York, N.Y.)* 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. *Current Biology* 21:917-26
13. Bonke M, Thitamadee S, Mähönen AP, Hauser M-T, Helariutta Y. 2003. APL regulates vascular tissue identity in *Arabidopsis*. *Nature* 426:181-6
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 (New York, N.Y.)* 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. *The Plant Cell* 17:2281-95
16. Campbell L, Turner S. 2017. Regulation of vascular cell division. *Journal of Experimental Botany* 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 F, Nelson T. 2009. CVP2- and CVL1-mediated phosphoinositide signaling as a regulator of the ARF GAP SFC/VAN3 in establishment of foliar vein patterns. *The Plant journal : for cell and molecular biology* 59:895-907
19. 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
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. *Journal of experimental botany* 66:4595-606
22. de Lucas M, Pu L, Turco GM, Gaudinier A, Marao AK, et al. 2016. Transcriptional Regulation of Arabidopsis Polycomb Repressive Complex 2 Coordinates Cell Type Proliferation and Differentiation. *The Plant Cell*:tpc-00744
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 (New York, N.Y.)* 345:1255215
24. De Rybel B, Breda AS, Weijers D. 2014. Prenatal plumbing—vascular tissue formation in the plant embryo. *Physiologia plantarum* 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. *Developmental 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. *The Plant Journal* 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-SCFSKP2A pathway in *Arabidopsis*. *The 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. *Proceedings of the National Academy of Sciences* 99:15794-9
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. *Proceedings of the National Academy of Sciences* 110:7074-9
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. 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
33. 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
34. 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. *Current Biology* 13:1768-74
35. 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* 18:784-8
36. Eshed Y, Baum SF, Perea JV, Bowman JL. 2001. Establishment of polarity in lateral organs of plants. *Curr Biol* 11:1251-60
37. Etchells J P, Mishra Laxmi S, Kumar M, Campbell L, Turner Simon R. 2015. Wood formation in trees is increased by manipulating PXY-regulated cell division. *Current Biology* 25:1050-5
38. 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
39. 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
40. Fisher K, Turner S. 2007. PXY, a receptor-like kinase essential for maintaining polarity during plant vascular-tissue development. *Current Biology* 17:1061-6
41. Foster AS. 1952. Foliar venation in angiosperms from an ontogenetic standpoint. *American Journal of Botany*:752-66
42. 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
43. 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 physiology* 65:57-60
44. 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

45. Furuta KM, Yadav SR, Lehesranta S, Belevich I, Miyashima S, et al. 2014. *Arabidopsis* NAC45/86 direct sieve element morphogenesis culminating in enucleation. *Science (New York, N.Y.)* 345:933
46. 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 (New York, N.Y.)* 282:2226-30
47. 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 research* 16:446-56
48. Goué N, Noël-Boizot N, Vallance M, Magel E, Label P. 2012. Microdissection to isolate vascular cambium cells in poplar. *Silva Fennica* 46:5-16
49. Gursanscky NR, Jouannet V, Grünwald K, Sanchez P, Laaber-Schwarz M, Greb T. 2016. MOL1 is required for cambium homeostasis in Arabidopsis. *The Plant Journal* 86:210-20
50. 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 & development* 16:1610-5
51. 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
52. Hanzawa Y, Takahashi T, Komeda Y. 1997. ACL5: an Arabidopsis gene required for internodal elongation after flowering. *The Plant Journal* 12:863-74
53. Hardtke CS, Berleth T. 1998. The Arabidopsis gene MONOPTEROS encodes a transcription factor mediating embryo axis formation and vascular development. *The EMBO journal* 17:1405-11
54. Hawker NP, Bowman JL. 2004. Roles for Class III HD-Zip and KANADI genes in Arabidopsis root development. *Plant Physiology* 135:2261-70
55. 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
56. Hertzberg M, Aspeborg H, Schrader J, Andersson A, Erlandsson R, et al. 2001. A transcriptional roadmap to wood formation. *Proceedings of the National Academy of Sciences* 98:14732-7
57. Hirakawa Y, Kondo Y, Fukuda H. 2010. TDIF peptide signaling regulates vascular stem cell proliferation via the *WOX4* homeobox gene in *Arabidopsis*. *The Plant Cell* 22:2618-29
58. 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. *Proceedings of the National Academy of Sciences* 105:15208-13
59. Hussey S, Mizrachi E, Creux N, Myburg A. 2013. Navigating the transcriptional roadmap regulating plant secondary cell wall deposition. *Frontiers in Plant Science* 4
60. Hwang I, Sheen J, Müller B. 2012. Cytokinin signaling networks. *Annual review of plant biology* 63:353-80
61. 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* 26:1990-7
62. Inoue T, Higuchi M, Hashimoto Y, Seki M, Kobayashi M, et al. 2001. Identification of CRE1 as a cytokinin receptor from Arabidopsis. *Nature* 409:1060-3
63. Ito Y, Nakanomyo I, Motose H, Iwamoto K, Sawa S, et al. 2006. Dodeca-CLE peptides as suppressors of plant stem cell differentiation. *Science (New York, N.Y.)* 313:842
64. Jacobs WP. 1952. The role of auxin in differentiation of xylem around a wound. *American Journal of Botany*:301-9
65. Kang YH, Hardtke CS. 2016. *Arabidopsis* MAKR5 is a positive effector of BAM3-dependent CLE45 signaling. *EMBO reports* 17:1145-54

66. 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. *Current Biology* 25:3144-50
67. Kerstetter RA, Bollman K, Taylor RA, Bomblies K, Poethig RS. 2001. KANADI regulates organ polarity in Arabidopsis. *Nature* 411:706-9
68. Knott JM, Römer P, Sumper M. 2007. Putative spermine synthases from Thalassiosira pseudonana and Arabidopsis thaliana synthesize thermospermine rather than spermine. *FEBS letters* 581:3081-6
69. 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. *Proceedings of the National Academy of Sciences* 111:7150-5
70. 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 Physiology* 135:1069-83
71. Kondo Y, Fujita T, Sugiyama M, Fukuda H. 2015. A novel system for xylem cell differentiation in *Arabidopsis thaliana*. *Molecular Plant* 8:612-21
72. 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. *Nature Communications* 5:3504
73. 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. *The Plant Cell* 28:1250-62
74. Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, et al. 2005. Transcription switches for protoxylem and metaxylem vessel formation. *Genes & development* 19:1855-60
75. Kumar M, Campbell L, Turner S. 2015. Secondary cell walls: biosynthesis and manipulation. *Journal of Experimental Botany* 67:515-31
76. 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* 141:4311-9
77. 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. *International Journal of Plant Sciences* 163:519-29
78. 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. *Proceedings of the National Academy of Sciences* 106:5984-9
79. Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav S-R, et al. 2013. The plant vascular system: Evolution, development and functions. *Journal of Integrative Plant Biology* 55:294-388
80. 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 (New York, N.Y.)* 311:94-8
81. 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 & development* 14:2938-43
82. Mähönen AP, Higuchi M, Törmäkangas K, Miyawaki K, Pischke MS, et al. 2006. Cytokinins regulate a bidirectional phosphorelay network in Arabidopsis. *Current Biology* 16:1116-22
83. 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. *Developmental cell* 21:796-804

84. 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. *Current Biology* 24:1031-7
85. Mariconti L, Pellegrini B, Cantoni R, Stevens R, Bergounioux C, et al. 2002. The E2F family of transcription factors from *Arabidopsis thaliana*. *Journal of Biological Chemistry* 277:9911-9
86. Matsumoto-Kitano M, Kusumoto T, Tarkowski P, Kinoshita-Tsujimura K, Václavíková K, et al. 2008. Cytokinins are central regulators of cambial activity. *Proceedings of the National Academy of Sciences* 105:20027-31
87. Mattsson J, Ckurshumova W, Berleth T. 2003. Auxin signaling in Arabidopsis leaf vascular development. *Plant Physiology* 131:1327-39
88. Mattsson J, Sung ZR, Berleth T. 1999. Responses of plant vascular systems to auxin transport inhibition. *Development* 126:2979-91
89. 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. *The Plant Journal* 67:805-16
90. Mayer U, Ruiz RAT, Berleth T, Miseéra S, Juürgens G. 1991. Mutations affecting body organization in the Arabidopsis embryo. *Nature* 353:402-7
91. Mazur E, Kurczyńska EU, Friml J. 2014. Cellular events during interfascicular cambium ontogenesis in inflorescence stems of Arabidopsis. *Protoplasma* 251:1125-39
92. 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
93. 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. *Journal of Experimental Botany* 68:5-16
94. 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. *The Plant Journal* 75:685-98
95. 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. *The Plant Cell* 17:2993-3006
96. Motose H, Sugiyama M, Fukuda H. 2004. A proteoglycan mediates inductive interaction during plant vascular development. *Nature* 429:873-8
97. 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. *The Plant Cell* 14:2031-43
98. 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 & Development* 18:700-14
99. Mouchel CF, Osmont KS, Hardtke CS. 2006. *BRX* mediates feedback between brassinosteroid levels and auxin signalling in root growth. *Nature* 443:458-61
100. Müller CJ, Valdés AE, Guodong W, Ramachandran P, Beste L, et al. 2015. PHABULOSA mediates an auxin signaling loop to regulate vascular patterning in Arabidopsis. *Plant physiology*:pp-01204
101. 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
102. 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. *Proceedings of the National Academy of Sciences* 111:857-62
103. Nagawa S, Sawa S, Sato S, Kato T, Tabata S, Fukuda H. 2006. Gene trapping in *Arabidopsis* reveals genes involved in vascular development. *Plant and Cell Physiology* 47:1394-405

104. Nakajima K, Sena G, Nawy T, Benfey PN. 2001. Intercellular movement of the putative transcription factor SHR in root patterning. *Nature* 413:307-11
105. Nieminen K, Blomster T, Helariutta Y, Mähönen AP. 2015. Vascular cambium development. *The Arabidopsis Book*:e0177
106. Nieminen K, Immanen J, Laxell M, Kauppinen L, Tarkowski P, et al. 2008. Cytokinin signaling regulates cambial development in poplar. *Proceedings of the National Academy of Sciences* 105:20032-7
107. 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. *The Plant Cell* 20:843-55
108. 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 physiology* 125:615-26
109. 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 Physiology* 137:1027-36
110. Ohashi-Ito K, Fukuda H. 2010. Transcriptional regulation of vascular cell fates. *Current opinion in plant biology* 13:670-6
111. Ohashi-Ito K, Matsukawa M, Fukuda H. 2013. An atypical bHLH transcription factor regulates early xylem development downstream of auxin. *Plant and Cell Physiology*:pct013
112. 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. *Current Biology* 24:2053-8
113. 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. *The Plant Cell* 3:677-84
114. 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. *The Plant Cell* 24:2874-85
115. Pesquet E, Tuominen H. 2011. Ethylene stimulates tracheary element differentiation in *Zinnia elegans* cell cultures. *New Phytologist* 190:138-49
116. Provart NJ, Alonso J, Assmann SM, Bergmann D, Brady SM, et al. 2016. 50 years of Arabidopsis research: highlights and future directions. *New Phytologist* 209:921-44
117. 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
118. 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. *Biology open*:bio-013128
119. 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. *Proceedings of the National Academy of Sciences* 103:11081-5
120. 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 Physiology* 155:1214-25
121. Rodriguez-Villalon A, Gujas B, Kang YH, Breda AS, Cattaneo P, et al. 2014. Molecular genetic framework for protophloem formation. *Proceedings of the National Academy of Sciences* 111:11551-6
122. 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
123. Sachs T. 1981. The control of the patterned differentiation of vascular tissues. *Advances in botanical research* 9:151-262

124. 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. *Journal of Experimental Botany* 67:4951-61
125. Salehin M, Bagchi R, Estelle M. 2015. SCFTIR1/AFB-based auxin perception: mechanism and role in plant growth and development. *The Plant Cell* 27:9-19
126. Sawchuk MG, Edgar A, Scarpella E. 2013. Patterning of leaf vein networks by convergent auxin transport pathways. *PLoS Genet* 9:e1003294
127. Sawchuk MG, Head P, Donner TJ, Scarpella E. 2007. Time-lapse imaging of Arabidopsis leaf development shows dynamic patterns of procambium formation. *New Phytologist* 176:560-71
128. Scarpella E, Marcos D, Friml J, Berleth T. 2006. Control of leaf vascular patterning by polar auxin transport. *Genes & development* 20:1015-27
129. Scheres B, 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
130. Scheres B, 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
131. Scheres BJG, Di Laurenzio L, Willemsen V, Hauser MT, Janmaat K, Benfey PN. 1995. Mutations affecting the radial organisation of the Arabidopsis root display specific defects throughout the embryonic axis. *Development* 121:53-62
132. 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* 464:913-6
133. Shininger TL. 1979. The control of vascular development. *Annual Review of Plant Physiology* 30:313-37
134. Snow R. 1935. Activation of cambial growth by pure hormones. *New Phytologist* 34:347-60
135. Sorokin HP, Mathur SN, Thimann KV. 1962. The effects of auxins and kinetin on xylem differentiation in the pea epicotyl. *American Journal of Botany*:444-54
136. 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 (New York, N.Y.)* 286:316-8
137. 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. *Proceedings of the National Academy of Sciences* 95:13330-5
138. Suer S, Agusti J, Sanchez P, Schwarz M, Greb T. 2011. WOX4 imparts auxin responsiveness to cambium cells in Arabidopsis. *The Plant Cell* 23:3247-59
139. 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* 517:571-5
140. Torrey JG. 1965. Physiological bases of organization and development in the root. In *Differentiation and Development/Differenzierung und Entwicklung*:1256-327: Springer. Number of 1256-327 pp.
141. 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
142. 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*. *The Plant Cell* 20:1494-503
143. Ueguchi C, Koizumi H, Suzuki T, Mizuno T. 2001. Novel family of sensor histidine kinase genes in Arabidopsis thaliana. *Plant and Cell Physiology* 42:231-5
144. Uggla C, Mellerowicz EJ, Sundberg B. 1998. Indole-3-acetic acid controls cambial growth in Scots pine by positional signaling. *Plant Physiology* 117:113-21

145. Uggla C, Moritz T, Sandberg G, Sundberg B. 1996. Auxin as a positional signal in pattern formation in plants. *Proceedings of the national academy of sciences* 93:9282-6
146. 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. *Developmental cell* 35:432-43
147. Weijers D, Schlereth A, Ehrismann JS, Schwank G, Kientz M, Jürgens G. 2006. Auxin triggers transient local signaling for cell specification in Arabidopsis embryogenesis. *Developmental cell* 10:265-70
148. Wenzel CL, Schuetz M, Yu Q, Mattsson J. 2007. Dynamics of MONOPTEROS and PIN-FORMED1 expression during leaf vein pattern formation in Arabidopsis thaliana. *The Plant Journal* 49:387-98
149. Werner T, Motyka V, Strnad M, Schmülling T. 2001. Regulation of plant growth by cytokinin. *Proceedings of the National Academy of Sciences* 98:10487-92
150. 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
151. Xu B, Ohtani M, Yamaguchi M, Toyooka K, Wakazaki M, et al. 2014. Contribution of NAC transcription factors to plant adaptation to land. *Science (New York, N.Y.)* 343:1505-8
152. 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* 38:980-3
153. Ye Z-H, Zhong R. 2015. Molecular control of wood formation in trees. *Journal of experimental botany* 66:4119-31
154. 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 and Cell Physiology* 53:635-45
155. Young BS. 1954. The effects of leaf primordia on differentiation in the stem. *New Phytologist* 53:445-60
156. Zhang K, Novak O, Wei Z, Gou M, Zhang X, et al. 2014. Arabidopsis ABCG14 protein controls the acropetal translocation of root-synthesized cytokinins. *Nature communications* 5
157. 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*. *The Plant Cell* 18:3158-70
158. 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*. *The Plant Cell* 20:2763-82
159. 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
160. Zürcher E, Liu J, di Donato M, Geisler M, Müller B. 2016. Plant development regulated by cytokinin sinks. *Science (New York, N.Y.)* 353:1027-30

TERMS AND DEFINITIONS

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

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

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

Provascular tissue: Embryonic tissue which originates the future vascular cylinder

Vascular bundle (cylinder): Assembly of xylem, phloem and (pro)cambial cell files

Preprocambium: Undifferentiated leaf tissue consisting of cells that are morphologically inconspicuous but genetically distinct from mesophyll cells, and develop to procambium.

Procambium: Undifferentiated tissue with narrow and cytoplasm-dense cells which differentiate to primary xylem and primary phloem, and later develop to cambium.

Cambium: Lateral meristem which generates secondary xylem and secondary phloem during secondary growth in stem and root

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

Interfascicular cambium (IFC) and fascicular cambium (FC): Cambial cells arising between (IFC) and within (FC) vascular bundles of stem; IFC connects FC in a ring structure.

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

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

Tracheary element (TE): Xylem conductive vessel element characterized by secondary cell walls, perforation plates that connect TEs into a continuous file, and autolysis.

Protophloem (PP) and metaphloem (MP): Phloem tissues arising from procambium. Cell divisions first give rise to PP while MP develops later; both differentiate into SEs.

Sieve element (SE): Phloem conductive cell characterized by thick cell walls, sieve plates with pores connecting SEs into a continuous file, and enucleation.

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

Secondary cell wall: Multi-layered polymer structure formed inside the primary cell wall of differentiated cells in some tissues such as xylem and fibres

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

Cotyledons: The embryonic leaves of the plant; post-embryonically, the first true leaves are formed from the shoot apical meristem.

Abaxial and adaxial: Refers to the under and upper sides of leaves, respectively

FIGURE CAPTIONS

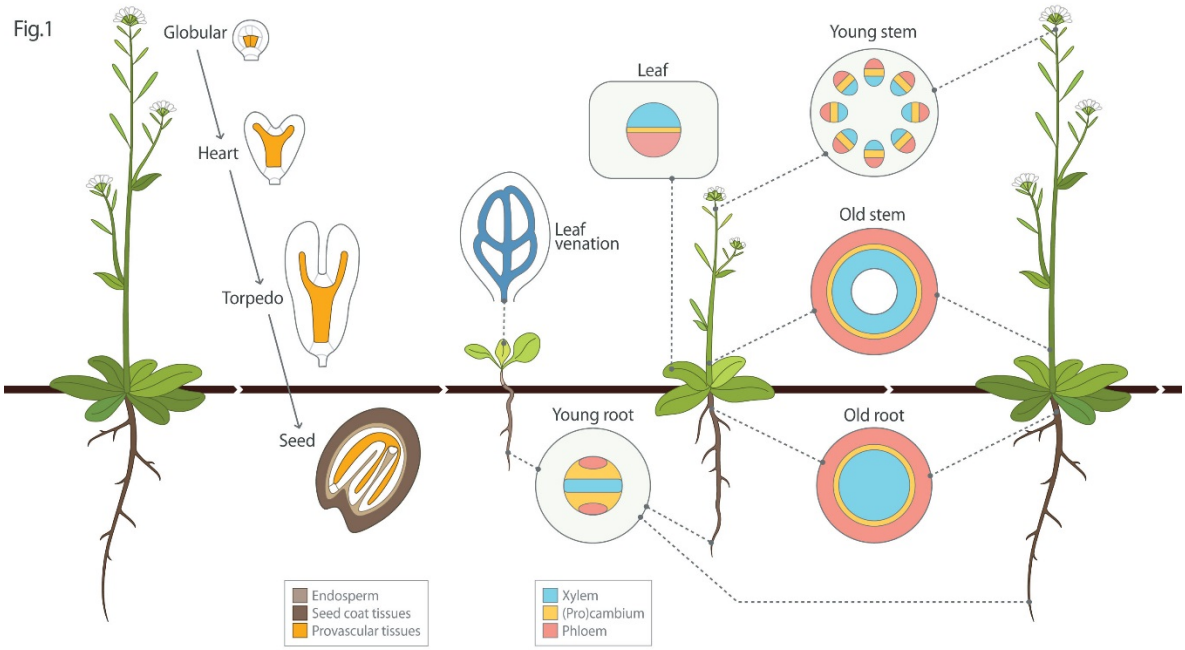

Figure 1. Vascular development in plants. Vascular development begins with the periclinal division of the lower tier of cells (marked in yellow) in globular stage embryos (top left). Controlled division of these cells forms the well-organized provascular tissue in the centre of the developing embryo throughout embryogenesis, which can be divided into 5 stages: zygote, globular, heart, torpedo and mature embryo. The vascular cylinder in newly developing post-embryonic organs originates from the shoot apical meristem or the root apical meristem. Intriguingly, despite the structural differences between vascular cylinders in different organs and growth stages, the vascular system tightly connects all of the organs together, 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 and xylem develop on the abaxial and adaxial side, respectively. A uniform structure is seen not only in the leaf vascular cylinder 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 cylinders composed of procambial cells between (inner) xylem and (outer) phloem are disconnected, but they become connected in older stems and give rise to continuous xylem tissue on the inside surrounded by cambium and then phloem tissue on the outside.

Fig.2

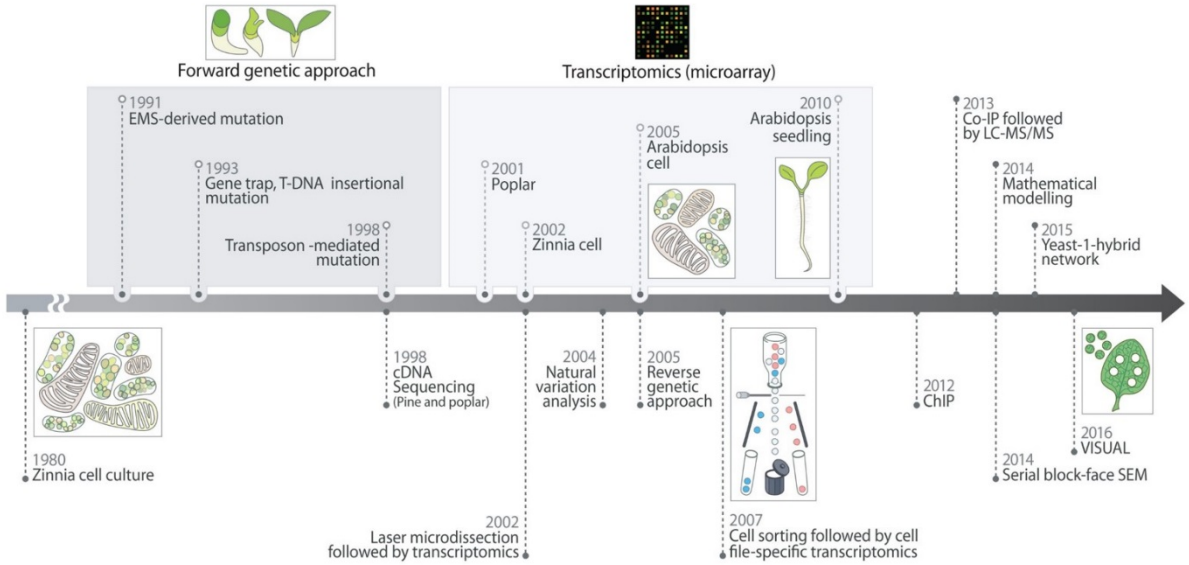

Figure 2. Major approaches used in the study of plant vascular development. Approaches are shown in consecutive order based on the time when each technique started to be used for research on vascular development.

Fig.3

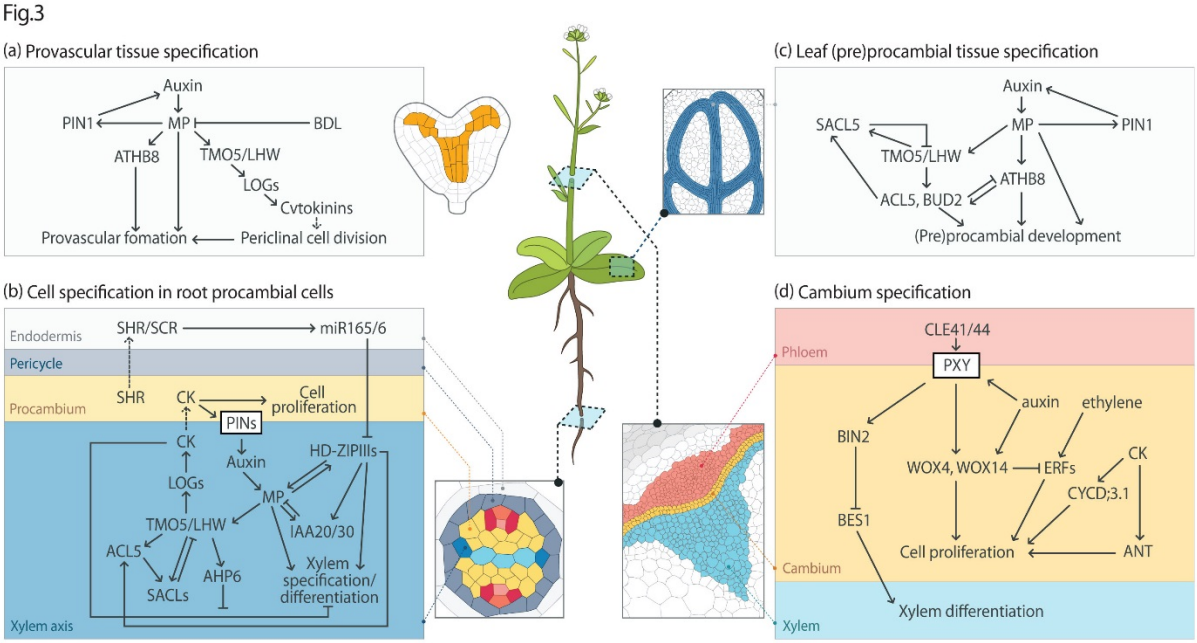

Figure 3. Genetic networks underlying vascular tissue specification. Vascular patterning in plants is controlled by complex genetic networks involving multiple transcription factors and phytohormones. (a) Provascular tissue specification. Auxin-inducible *MP* positively regulates *PIN1* expression, which, 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 HD-ZIP IIIs, including *ATHB8*, which play crucial roles 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 that stimulate periclinal cell division. The expression pattern of *MP* in heart stage embryo is marked in yellow. (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*/6 in the endodermis, which diffuse toward the centre of stele to form a gradient. *miRNA165/6* degrade the HD-ZIP IIIs, resulting in an HD-ZIP III gradient toward the centre of the xylem which specifies metaxylem versus protoxylem. In addition, the HD-ZIP III *PHABULOSA* upregulates *MP* and *IAA20/30* to stabilize the auxin response in the xylem axis of the root. *TMO5* induces expression of *ACL5*, a thermospermine synthase, as well as the SACL genes. Thermospermine generated by *ACL5* induces translation of the SACLs. Furthermore, *ATHB8* directly upregulates the expression of the SACLs, which inhibit TMO5 and LHW heterodimerization by binding to LHW. *ACL5* is likely to inhibit HD-ZIP III expression in roots based on the enhanced protoxylem cell differentiation in *acl5* roots, but this has not been experimentally demonstrated in roots. (c) Leaf (pre)procambial tissue specification. The auxin-MP pathway also plays 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 repress expression of *ATHB8*, forming a negative feedback loop. (d) Cambial specification. *PXY*, a leucine-rich repeat receptor-like kinase, is activated by its ligands, *CLE41/44*, and upregulates the expression of *WOX4/14*, 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.

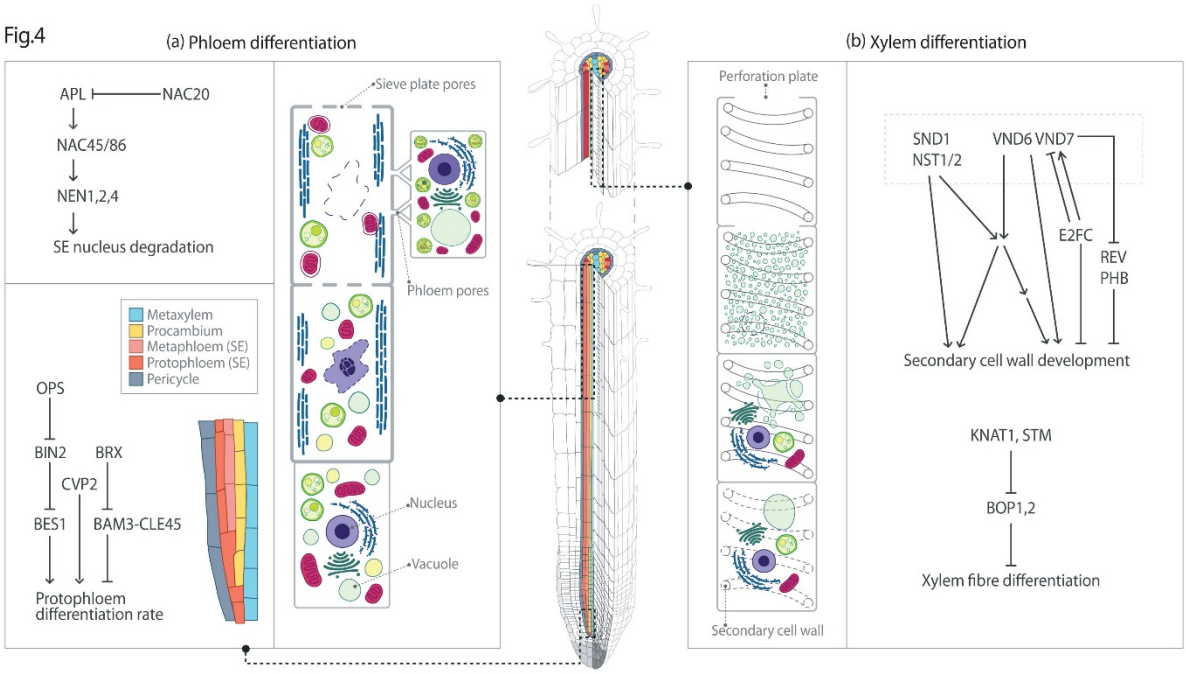

Figure 4. Morphogenetic events and transcriptional networks involved in vascular tissue differentiation. (a) Over the course of phloem differentiation from protophloem to mature sieve elements (SE), cell walls thicken and the cells undergo cellular re-arrangements during which organelles are modified and re-aligned. 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 signalling 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 *NAC45/86* expression and is downregulated by *NAC20*. *NEN1*,*2*, and *4* are key regulators of SE nuclear degradation that act downstream of *NAC45/86*. (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 feed-forward loops that are important in this process. The NAC transcription factors *VND6*, *VND7*, *SND1*, *NST1* and *NST2* are sufficient to regulate xylem formation and are therefore considered master regulators. Two recently identified novel pathways are also indicated; upstream of *VND7*, *E2Fc* may have either a positive or negative effect, while 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 *2*, which supress xylem fibre differentiation.