Plant vascular development: from early specification to differentiation

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Abstract | Vascular tissues in plants are crucial to provide physical support and to transport water, sugars and hormones and other small signalling molecules throughout the plant. Recent genetic and molecular studies have identified interconnections among some of the major signalling networks that regulate plant vascular development. Using *Arabidopsis thaliana* as a model system, these studies enable the description of vascular development from the earliest tissue specification events during embryogenesis to the differentiation of phloem and xylem tissues. Moreover, we propose a model for how oriented cell divisions give rise to a three-dimensional vascular bundle within the root meristem.

Ground tissues
One of the three major tissue types in plants, located between the outer layer (epidermis) and the inner vascular cylinder.

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The development of vascular tissues was one of the most important evolutionary adaptations that allowed plants to grow in environments other than water and to populate the land[1]. Vascular tissues provide mechanical support and facilitate the transport of water, nutrients, hormones and other signalling molecules throughout the plant. These functions have enabled land plants to grow beyond the size of mosses. Early land plants adopted a tissue organization comprising three major tissue types, which can be found in almost all organs: the outer epidermis, ground tissues and centrally localized vascular tissues. This organization proved to be evolutionarily very successful, as it is still found in leaves, stems and roots of most modern land plants (FIG. 1).

Our current understanding of the molecular pathways that regulate vascular development is mostly based on studies in *Arabidopsis thaliana*. Vascular development in *A. thaliana* occurs in four main processes: specification, during which cells obtain their specific vascular cell identity from naive precursor cells; establishment, which combines growth and patterning; maintenance; and differentiation. During specification, which occurs early in embryogenesis, four cells become provascular initial cells[2–4]. Following specification, highly regulated cell divisions with defined stereotypical orientations and simultaneous patterning events establish the vascular tissue. This results, by the end of embryogenesis, in the formation of fully specified tissues that contain an adequate number of cells with correct identities (FIG. 1). These embryonic provascular cells generate the vascular tissues of the root and the hypocotyl, whereas vascular tissues of the shoot originate from the shoot apical meristem (FIG. 1).

During post-embryonic development, the growth and maintenance of patterned vascular tissues occurs through cell divisions in zones of the plant with high mitotic activity, known as meristems. Vascular tissues comprise two functionally distinct domains — phloem and xylem — which transport solutes and water, respectively, through the plant. When cells that have acquired xylem and phloem fate exit meristematic regions, their differentiation generates specialized conducting cell types — tracheary and sieve elements, respectively — with a characteristic secondary cell wall or other cellular modifications[1] (FIG. 1).

The organization of vascular systems is very different depending on the plant organ. For example, the young root has a central xylem axis flanked by two phloem poles (diarch pattern), whereas stems contain several vascular bundles consisting of phloem on the outside and xylem on the inside (collateral pattern) (FIG. 1). It is important to note that the vascular organization found in *A. thaliana* is just one of many different vascular topologies that are found throughout the plant kingdom. Readers should refer to other recent reviews[1,5,6] for more detailed information on vascular development.

The field of plant vascular biology has seen major advances in the past few years, substantially increasing our understanding of vascular development from early embryonic development to late differentiation. Our knowledge of the molecular pathways involved in vascular development has been extended, and previously unknown links between these pathways have recently been uncovered. In this Review, we discuss the most recent progress in identifying the regulatory networks that control vascular development during *A. thaliana* embryonic root formation and its post-embryonic maintenance. We refer to excellent reviews for discussions on cambial secondary growth[1,5,6], leaf venation[7] and other topics throughout the text when relevant. We also highlight current open questions and discuss how general

concepts regarding stem cell functions in the root meristem can be extrapolated to vascular tissues.

Ontogeny and specification of root vasculature

Vascular tissues are first formed during the early globular stage of embryogenesis from four inner procambium precursor cells that each generate a ground tissue cell and a vascular tissue cell through a periclinal division[2,4] (FIG. 1). The vascular cells then undergo another round of periclinal cell divisions that generate the outer pericycle cell layer[2–4]. The resulting four inner provascular cells will produce all the cells of the xylem, phloem and procambium in the root and hypocotyl. By contrast, all vascular tissues in the above-ground tissues originate from the shoot apical meristem[1].

Although the ontogeny of these cell types in the root was first described over 20 years ago[2,8], and in 3D more recently[4], molecular markers for the earliest cellular identities remain scarce. Thus, it is at present unclear whether vascular and ground tissue lineages are both established *de novo* from an uncommitted precursor cell, or whether one of the cell lineages derives from the other. This also implies that how the actual vascular identity itself is determined or controlled is still unknown. Despite this gap in our understanding of vascular development, it is possible to speculate on when the different cell types are specified within the tissue once a generic vascular identity has been established. On the basis of studies that used cell type-specific reporter genes and hormone response markers[9,10], it was found that xylem identity is established first in two of the four initial cells around the globular stage (FIG. 2a). Although some phloem markers have been described during the later stages of embryogenesis, no early phloem markers have been identified so far[11,12]. It thus seems possible that phloem identity is established later than xylem identity, towards the end of embryogenesis (after the heart stage), when many more cell files are present.

Although final differentiation only occurs post-embryonically, all of the cell identities of the root vasculature — including xylem (which comprises protoxylem and metaxylem), phloem (which comprises sieve elements, companion cells and protophloem) and procambium — were found to be present at the end of embryogenesis, on the basis of morphology and marker analysis[11,13] (FIG. 2a). Post-embryonic primary root development is thus marked by patterning and cell specification events that are based on a previously established template (FIG. 2a,b). In most other plant organs, such as stems, leaves, flowers and lateral organs, vascular tissues are formed from non-vascular precursor cells derived from the shoot apical meristem (FIG. 1). It is very likely that the early processes of specification, growth and patterning are reiterated in these different tissue contexts.

Self-organization versus determinism

Although the stereotypic diarch vascular pattern of the *A. thaliana* root (FIG. 2) is established during embryogenesis, the regulatory mechanisms that determine this pattern are also able to generate different architectures when fewer or more cells are present. For example, mutants with half the usual number of vascular cell files can generate a monarch symmetry with opposing xylem and phloem poles[3,14], whereas in other dicot species with larger vascular bundles, the number of xylem and phloem poles is generally positively correlated with the size of the bundle[1]. Also, when new organs are formed or the vascular continuity is physically damaged, new strands will be quickly formed to restore the connectivity of this elaborate network[14–16]. This intrinsic flexibility suggests a high degree of self-organization underlying the arrangement of cell types in vascular tissues. In all cases, there is a link between the size of the vascular bundle and the number of xylem and phloem poles. Moreover, some mutants have revealed a possible correlation between the number of xylem poles and the number of cotyledons[17].

In contrast to the potential self-organizing properties underlying vascular pattern formation, recent observations have shown that the bisymmetry of the embryo, and thus also of the post-embryonic plant, is determined early after fertilization of the egg cell. Intriguingly, the orientations of the first divisions of the embryo are constrained relative to the axis of the surrounding developing seed[4] (FIG. 1). Because four-way junctions of adjoining cell walls are rare — if not actively prevented — in plant development[18], a small connection between two of the four cells in the four-cell-stage embryo is formed[9] (FIG. 2a). This connection between two cells at the centre of the embryo is maintained throughout embryogenesis and may later contribute to xylem axis formation[9]. Although there is no molecular evidence to support these observations, they suggest some degree of early determinism in plant development. It is plausible that during early stages of development, when the number of cells participating in tissue establishment is limited and seeds provide external constraints, a deterministic mode of development ensures the formation of a minimal but correct pattern. Conversely, during later, post-embryonic development, vascular development becomes plastic and acquires self-organizing properties, to allow maximal adaptability to the environment. An important future question is whether the same regulatory network can have both deterministic and plastic properties, depending on the number of cells available.

Early establishment of root vascular tissues

Early establishment of the root vascular tissue is tightly linked to growth, patterning and hormone signalling pathways. Although the plant hormones auxin and cytokinin have long been known to be crucially involved, we have only recently begun to understand how these signalling pathways interact to control vascular development on a molecular level.

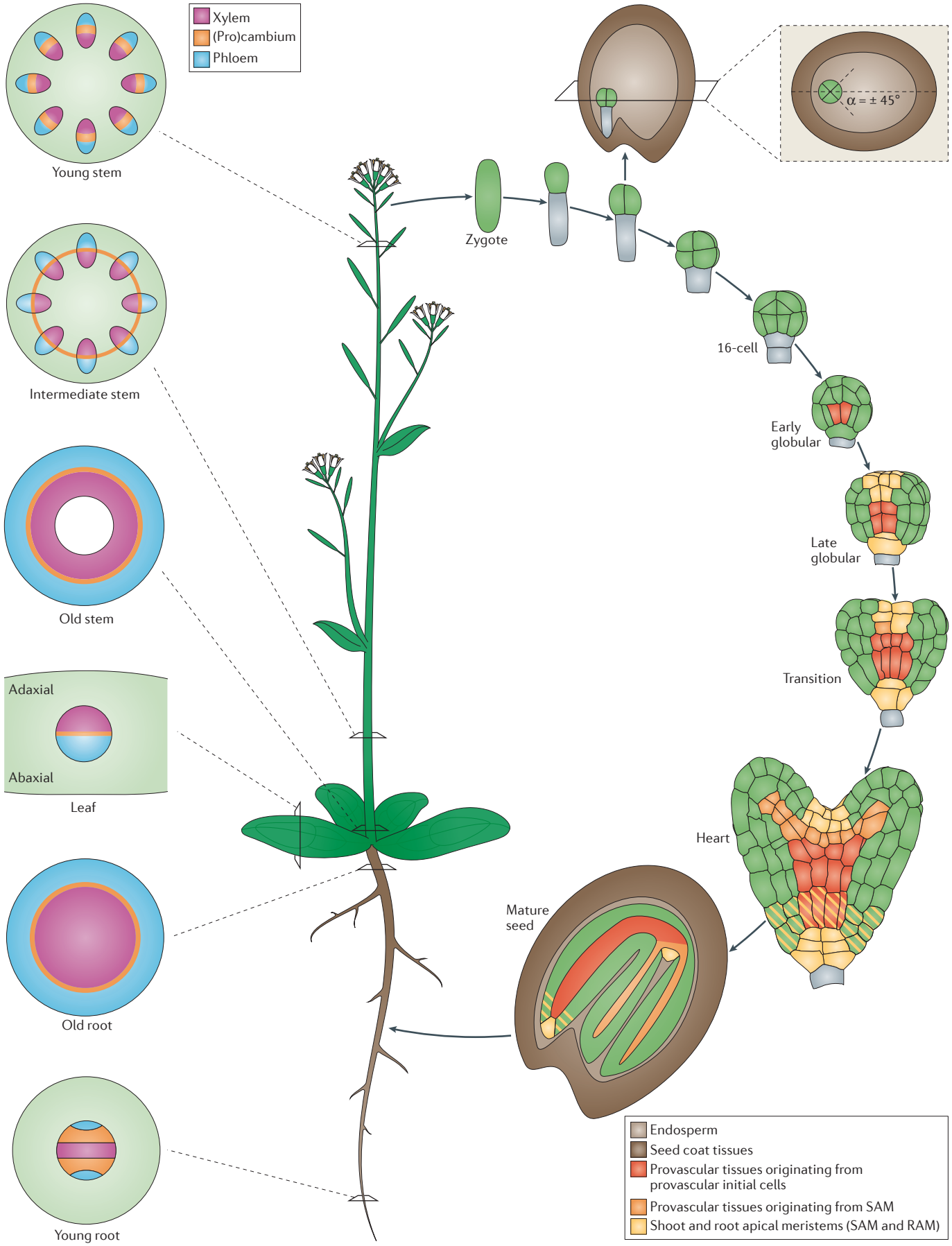
Mobile signals control vascular patterning. When the two cotyledons initiate early in embryogenesis, auxin produced at these sites is transported towards the embryonic root through auxin transporters of the PIN-FORMED (PIN) family[19,20]. Because of their positions relative to the incipient cotyledons, the two connected provascular initial cells receive more auxin than

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

Meristem
The actively dividing part of the plant, containing the stem cell niche.

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

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



the other two cells[20] (FIG. 2a). Auxin is perceived by the SKP1–CUL1–F-box (SCF) ubiquitin ligase (consisting of TRANSPORT INHIBITOR RESPONSE 1 (TIR1) and AUXIN SIGNALING F-BOX (AFB) family members), which triggers the degradation of Aux/IAA transcriptional inhibitors, leading to the activation of interacting DNA-binding AUXIN RESPONSE FACTORs (ARFs) (reviewed in REF. 21). The auxin response transcription factor MONOPTEROS (also known as ARF5) is crucial for vascular tissue formation in response to auxin signalling, as mutations lead to very early division defects in the provascular initial cells[22]. A MONOPTEROS transcriptional target gene, encoding the basic helix–loop–helix (bHLH) transcription factor TARGET OF MONOPTEROS 5 (TMO5; also known as AIG1), is first expressed in the two provascular cells receiving more auxin[9,23]. TMO5 and its homologues form heterodimeric complexes *in vivo* with another bHLH subclade including LONESOME HIGHWAY (LHW) and its close homologues[3,24]. Loss of function of either TMO5 or LHW family members leads to a reduced number of periclinal cell divisions in the vasculature[3,24,25]. Thus, TMO5–LHW complexes mediate MONOPTEROS-dependent cell division activity in vascular tissues during embryogenesis.

Procambial cells undergo characteristic periclinal cell division both during and after embryogenesis, increasing the number of vascular cell files from the 4 provascular initial cell files up to 30 in a mature root[8,26] (FIG. 2b). These periclinal divisions are reduced in the *wooden leg* (*wol*) mutant, which is mutated in the gene encoding cytokinin receptor ARABIDOPSIS HISTIDINE KINASE 4 (*AHK4*; also known as *CRE1*)[26,27]. Additionally, in the *wol* mutant, all cell files within the vasculature differentiate as protoxylem and, conversely, cytokinin treatments inhibit protoxylem differentiation[8,26–28]. These results show that cytokinins have a dual role in vascular development, functioning as inhibitors of protoxylem formation and also as promoters of periclinal divisions. Protoxylem differentiation is facilitated in part by protoxylem-specific expression of cytokinin signalling inhibitor *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*)[27]; which is also a MONOPTEROS target gene[29]. *AHP6* expression is thus auxin-dependent, and protoxylem positions are therefore not only characterized by high auxin, but also by low cytokinin responses[27,29]. In the adjacent procambial cells, cytokinins promote a cell identity that is associated with the expression and/or lateral localization of PIN auxin efflux carriers, thus leading to accumulation of auxin in xylem cell files. Therefore, mutually inhibitory feedback between cytokinins and auxin establishes the bisymmetric vascular pattern[29].

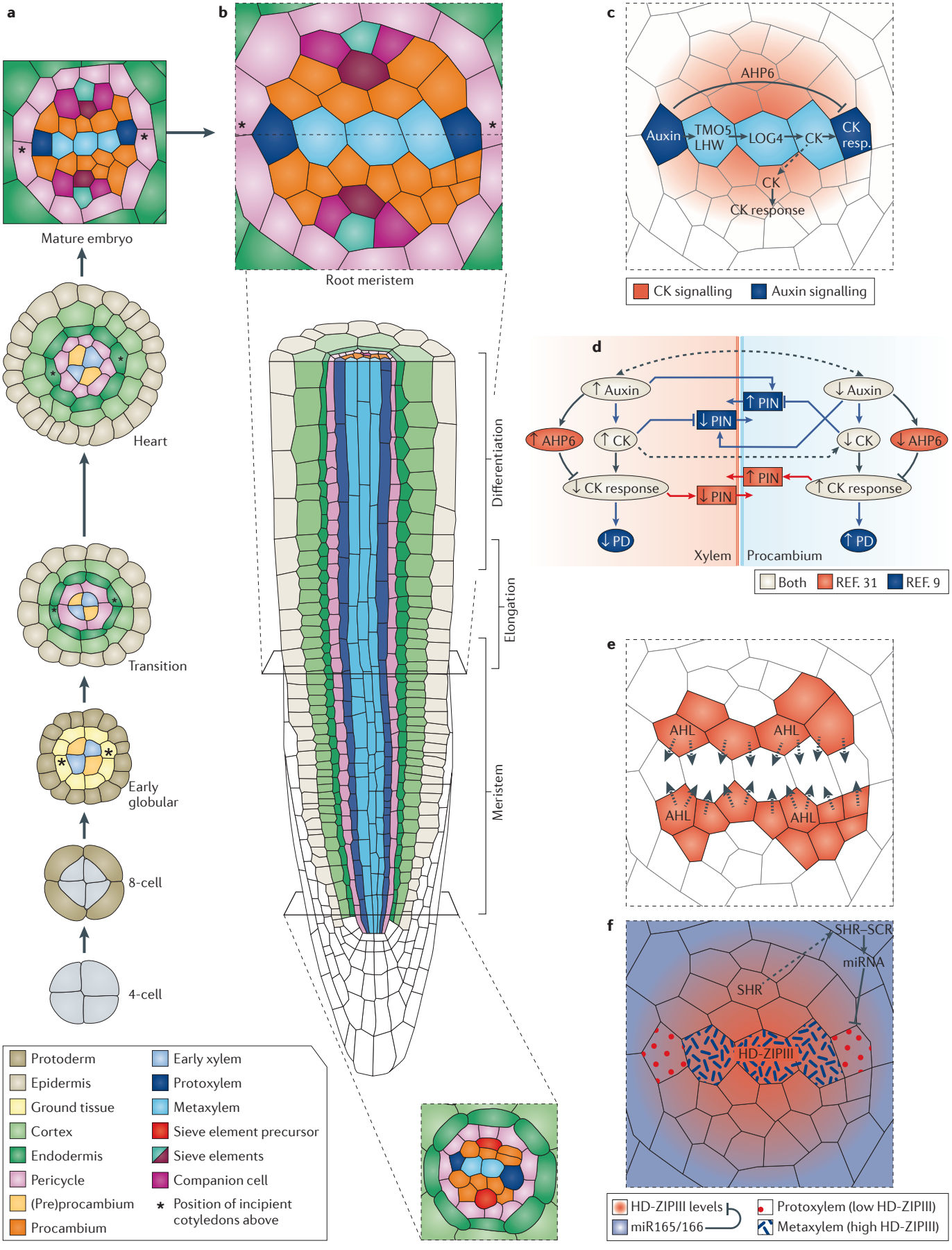
Recently, a connection between the auxin–MONOPTEROS–TMO5 and cytokinin–AHP6 pathways was identified. TMO5–LHW heterodimers were found to activate local cytokinin biosynthesis through direct transcriptional activation of the *LONELY GUY 4* (*LOG4*) gene and its closest homologue, *LOG3* (REFS 9,30) (FIG. 2c). Mathematical modelling using both growing embryonic[9] and static post-embryonic root[31] templates have shown that this regulatory network is able to create and maintain, within the growing vascular tissue, a zone of high auxin signalling along the xylem axis with flanking zones of high cytokinin signalling in procambium cells, as described above. Although the dynamic embryonic model and the static post-embryonic model used different PIN regulation dynamics (FIG. 2d), each highlights the intricate complexity in space and time of the auxin–cytokinin interactions that occur during vascular development. These modelling studies have predicted the existence of a novel inhibitor of cytokinin signalling that functions in the metaxylem[31] and have suggested that differential mobility of the key intermediates in this pathway, as well as tissue geometry, are essential for tissue patterning by this hormonal interaction network[9].

The major molecular hubs in this pathway, such as MONOPTEROS, PIN1, TMO5–LHW and cytokinin signalling components, and their interactions, have been best documented in embryo and root tissues. All of these, however, are expressed in vascular tissues throughout the plant[19,24,27]. Therefore, it is plausible that the activity of this interaction network mediates vascular development in other plant tissues. However, not all vascular periclinal cell divisions (for example, those at the phloem poles) can be explained by this regulatory network. Thus, other parallel pathways may function in the phloem (see below) and elsewhere.

A second pathway involving mobile signals is next required to maintain a sharp boundary between the xylem axis and the neighbouring procambial cells. *AT-HOOK MOTIF NUCLEAR LOCALIZED 3* (*AHL3*) and the gene encoding its interacting homologue (*AHL4*) are expressed in procambium cells neighbouring the xylem axis (FIG. 2e). The AHL3 and AHL4 proteins move towards the xylem, where they are required to regulate tissue boundaries between xylem and procambium[32]. Consistent with their expression in the zone with high cytokinin signalling, they were shown to be cytokinin-inducible, suggesting that these AT-HOOK factors could function downstream of the TMO5–LHW pathway.

Patterning of the xylem axis into protoxylem and metaxylem cells is controlled by a third pathway, which

◀ Figure 1 | **Vascular development in the *Arabidopsis thaliana* lifecycle.** After fertilization of the egg cell, provascular tissues are established around the early globular stage of embryogenesis. Intriguingly, the first division of the apical cells occurs with a specific orientation relative to the surrounding seed (top right). Highly controlled oriented divisions then generate the entire vascular system throughout the plant. The vascular tissue in the post-embryonic root, hypocotyl and cotyledons derives from the embryonic vasculature (red and orange cells) from the early globular stage onwards (right), whereas the vascular tissue in all newly initiated post-embryonic organs and tissues (for example, leaf, stem and lateral roots) is established *de novo* from the shoot and root apical meristems (SAM and RAM respectively; yellow cells). Red cells with yellow stripes indicate provascular cells in the RAM. Note that the exact architecture of vascular tissues differs among the individual organs of the plant (left). For example, in young roots, a central xylem axis is separated from the phloem poles by procambium cells. In older roots that have undergone secondary growth, concentric rings of xylem (inner), cambium and phloem (outer) are formed. Leaves show xylem on the adaxial side and phloem on the abaxial side. In young stems, vascular tissues are first organized in bundles with xylem on the inside and phloem on the outside. Later in development, the procambium cells of the different bundles connect, forming a ring. Finally, in the old stem, a structure similar to that in the mature root is formed, with concentric rings of xylem (inner), procambium and phloem (outer).



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

so far has not been mechanistically linked to the two pathways described above. The SHORTROOT (SHR) transcription factor, which is involved in ground tissue specification[33,34], also has an important role in vascular tissue patterning; this is exemplified by metaxylem formation at the protoxylem position in the *shr* mutant[35]. *SHR* is expressed in the stele, and the SHR protein moves towards the endodermis[36], where it is sequestered into the nucleus by SCARECROW (SCR) and induces expression of microRNAs (miRNAs) miR165 and miR166 (REFS 35,37) (FIG. 2f). These mobile miRNAs diffuse to create a gradient, with the highest levels at the periphery of the vascular bundle and the lowest levels in the inner domain of the stele. The class III HOMEODOMAIN LEU-ZIPPER (HD-ZIPIII) family proteins PHABULOSA (PHB; also known as ATHB14), PHAVOLUTA (PHV; also known as ATHB9), REVOLUTA (REV), ATHB8 and ATHB15 (also known as CNA) are all present in the stele[38–42], require auxin biosynthesis for their proper expression[43] and are targeted by miR165 and miR166 (REFS 35,44). Therefore, high miRNA and resulting low HD-ZIPIII levels control protoxylem identity, whereas metaxylem is specified by the inverse gradient. This is supported by the observation that only protoxylem identity is found in quadruple loss-of-function *athb8 phb phv rev* mutants, and that dominant *phb7* mutants that are insensitive to the miRNAs show ectopic metaxylem at the protoxylem position, similar to *shr* mutants[35]. Moreover, a modelling study has indicated that interactions between miRNAs and *PHB* mRNA probably contribute to the formation of sharp boundaries of gene expression in the vascular bundle[31].

Although this pathway has only been shown to function post-embryonically in the root, it is probably also active during embryogenesis to establish protoxylem and metaxylem identity. Early embryonic expression has been shown for least five miR165 or miR166 members[45] in the lower tier of the embryo. These miRNAs restrict PHB expression to the upper tier during this stage of development. Nevertheless, this very early expression is most likely to be linked to its function in determining abaxial and adaxial polarity, because other HD-ZIPIII members that are involved, such as *PHB*, *PHV* and *REV*, are only expressed in around late heart to torpedo stage embryos[45–48].

From these examples, it is clear that diverse mobile signals play a crucial part in controlling xylem patterning. These signals move between the cells by various mechanisms, depending on their nature. Polar auxin transport is based on the PIN protein efflux transport[49]. The SHR and AHL proteins, as well as the miR165 and miR166 species, move through the plasmodesmata[32,50]. The role of protein and cellular factors of SHR movement has been further investigated[51,52]. The movement mechanism for cytokinins is, however, less clear. It appears possible that they might move through the plasmodesmata, but various transporters have also been implicated in cytokinin transport in contexts other than xylem development[53,54].

◀ Figure 2 | **Regulatory networks controlling early vascular development.** **a** | The schematic shows, from bottom to top, the ontogeny of the xylem tissues during embryogenesis. Two provascular initial cells (already at the four-cell stage) share a cellular connection with each other and receive more auxin than the other two through the incipient cotyledons forming above (indicated by an asterisk next to the early xylem (blue) in the early globular stage embryo). These cells will form the xylem axis of the root and are marked by high levels of auxin signalling. The other provascular cells will form the procambium and phloem cell lineages (including sieve elements and companion cells), with the procambium marked by high cytokinin (CK) signalling. **b** | Schematic radial (upper panel) and longitudinal (lower panel) cross-sections through the vascular bundle of the root apical meristem are shown. Different colours indicate the various cell types. Note that all distinct cell identities are present in the mature embryo. Longitudinal zones in the root are not drawn to scale. **c** | The auxin–TARGET OF MONOPTEROS 5 (TMO5)–LONESOME HIGHWAY (LHW)–LONELY GUY 4 (LOG4)–CK pathway controls growth and patterning of the vascular bundle through local production of CKs (shown as a red gradient) along the xylem axis (protoxylem in dark blue; metaxylem in light blue), which triggers periclinal divisions (PD) in the neighbouring procambium cells. ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6 (AHP6) functions as an auxin-dependent negative regulator of the CK response in the xylem cells. **d** | A summary of regulatory connections included in two computational models describing vascular patterning in the root meristem, as described in part **c**. Connections based on one model[31] are shown in red, connections based on another model[9] are shown in blue, and those common to both models are shown in grey. Although different templates and PIN-FORMED (PIN) dynamics were used; similar outputs were generated. **e** | AT-HOOK MOTIF NUCLEAR LOCALIZED (AHL) proteins are expressed in procambium cells and migrate towards the xylem axis, thereby controlling strict boundaries between xylem and procambium through an unknown mechanism. **f** | Control of metaxylem versus protoxylem identity by the SHORTROOT (SHR)–miR165/166–class III HOMEODOMAIN LEU-ZIPPER (HD-ZIPIII) pathway. SHR expressed in the stele moves to the endodermis, where it is sequestered in the nucleus by SCARECROW (SCR). There, microRNAs (miRNAs) miR165 and miR166 are induced and move back inwards, inhibiting members of the HD-ZIPIII family of transcription factors. Levels of these HD-ZIPIII proteins determine whether cells have metaxylem or protoxylem identity.

Unravelling early phloem development. The first factor controlling phloem development to be identified was the MYB-type transcription factor ALTERED PHLOEM DEVELOPMENT (APL)[11]. The *apl* mutant has xylem-like cells at the phloem positions, while ectopic expression represses xylem development. Moreover, because the *apl* mutants lack sieve elements and companion cells, APL is most likely to be both a negative regulator of xylem differentiation and a positive regulator of phloem differentiation. In addition to APL functioning as a master regulator of phloem identity, several counteracting pathways specify the individual cell types within the phloem lineage (protophloem, metaphloem and companion cells; FIG. 3).

The membrane protein OCTOPUS (OPS) was identified through screening gene-trap lines[13] for phloem-specific genes. In *ops* mutants, individual protophloem cells fail to differentiate and thus interrupt the phloem strand integrity[12]. Very similar phloem defects have been described in another of these gene-trap lines, previously identified as *BREVIS RADIX* (*BRX*), which is itself a MONOPTEROS target gene[55,56] and shows low penetrance *MONOPTEROS*-like embryo phenotypes[57]. Both OPS and BRX are polar membrane-associated proteins, although BRX also seems to be nuclear[12,56]. These factors promote the transition to sieve element identity and help to maintain it; whereas CLAVATA 3/EMBRYO SURROUNDING REGION 45 (CLE45) peptide treatments suppress protophloem differentiation[58]. The CLE45 response requires the BARELY ANY MERISTEM 3 (BAM3) receptor-like kinase[58]. All of these factors are

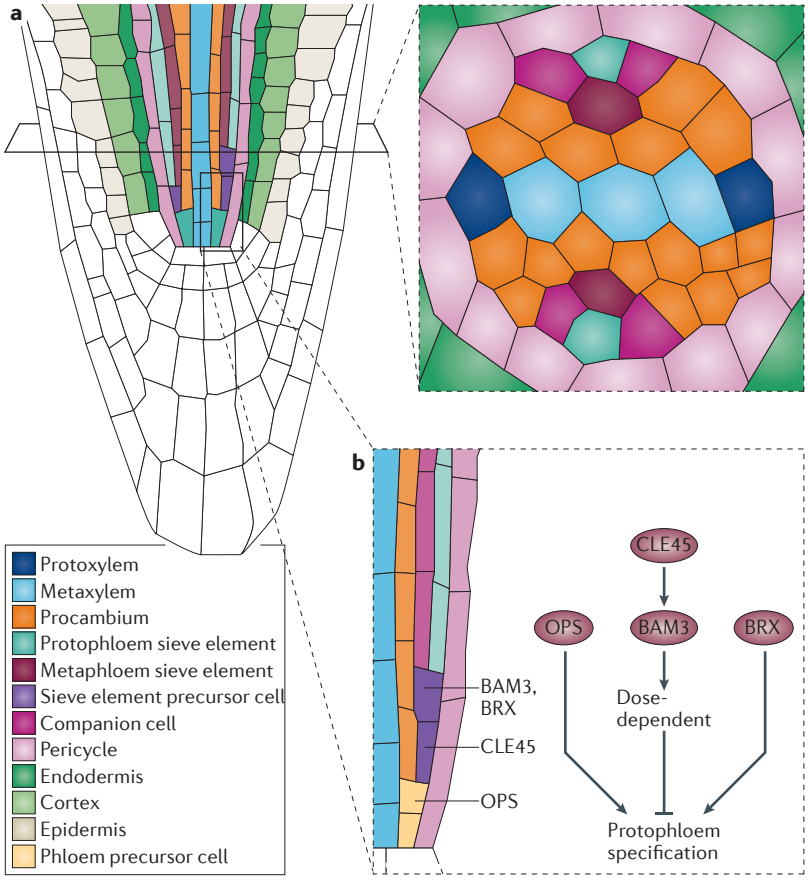


Figure 3 | **Key factors regulating early phloem development. a** | A schematic representation of phloem development in the root meristem, showing the longitudinal ontogeny and organization of phloem cell types. **b** | After one or more anticlinal cell divisions, one procambium cell next to the pericycle on each side of the xylem axis undergoes a periclinal cell division, generating another procambium cell and a sieve element precursor cell. This cell undergoes another periclinal cell division, generating protophloem and metaphloem sieve elements. The companion cells (CC) are formed through yet another periclinal division from two flanking procambium cells. Protophloem specification is controlled in parallel by the dose-dependent CLAVATA 3/EMBRYO SURROUNDING REGION 45 (CLE45)–BARELY ANY MERISTEM 3 (BAM3) factors and by the opposing activity of OCTOPUS (OPS) and BREVIS RADIX (BRX) proteins. Cells marked with OPS, CLE45, BRX and BAM3 highlight the first cells expressing the indicated marker in the protophloem sieve element lineage.

expressed in the precursor cells of the protophloem sieve element, and it seems that the balanced interplay between this CLE45–BAM3 pathway on one hand, and the BRX and OPS regulators on the other hand, regulates the timing of protophloem specification. Furthermore, auxin seems to have a role in regulating the timing of the asymmetric periclinal division, resulting in the specification of the protophloem and metaphloem cell lineages[59].

Xylem and phloem differentiation

Once specification, growth and patterning events are completed, all cell types are present in the vascular bundle. In order to create functional conductive tissues, cells with xylem and phloem identities differentiate into tracheary and sieve elements, respectively (FIG. 4). These processes involve drastic cytological changes in these cells and result in the formation of tissue-specific secondary cell walls. Because the differentiation of xylem and phloem cell types has been recently reviewed[60–62], we only briefly discuss the molecular mechanisms that control these processes in this section.

Tracheary element formation. Transcript profiling of *in vitro* xylem vessel differentiation events using *Zinnia* cell cultures led to the identification of the NAC transcription factors VASCULAR-RELATED NAC-DOMAIN 6 (VND6; also known as NAC101) and VND7 (also known as NAC030) as transcriptional switches controlling differentiation into metaxylem and protoxylem cells, respectively[63]. However, it remains unclear whether, besides their role in differentiation, they also control cell identity determination of these xylem cell types. Although fusions to a dominant transcriptional repressor domain inhibited differentiation into the respective vessel elements, loss-of-function mutants did not show phenotypes, indicating that there is functional redundancy within the VND family[63]. Both VND6 and VND7 directly upregulate genes that are involved in programmed cell death and in cell wall thickening, leading to tracheary element differentiation[64,65]. In this pathway (FIG. 4a), VND INTERACTING 2 (VNI2; also known as NAC083) was found to interact with VND7 and negatively regulate xylem differentiation[66]. A systems biology approach was recently applied to determine the intricate transcriptional networks that function during xylem differentiation and showed that a multitude of feed-forward loops in this network ensures the robust regulation of the process[67]. Intriguingly, orthologues of the same NAC-type transcription factors in the moss *Physcomitrella patens* control the differentiation of its water-conducting hydroid cells. The functional conservation in moss and vascular plants thus suggests that these transcription factors played a major part in the evolutionary adaptations of plants to life on land[68].

Differentiation of xylem, conversely, is repressed by two members of the CLE gene family, *CLE41* and *CLE44* (also known as *TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR* (*TDIF*)), which are expressed in the phloem[69,70]. The peptides that they encode move to the procambium, where they are perceived by the Leu-rich repeat receptor-like kinase PHLOEM INTERCALATED WITH XYLEM (PXY; also known as TDIF RECEPTOR (TDR))[69–72]. This peptide–receptor complex activates proteins of the GLYCOGEN SYNTHASE KINASE 3 (GSK3) family, leading to repression of the BRI1–EMS SUPPRESSOR 1 (BES1; also known as BZR2) transcription factor and thereby preventing xylem differentiation[73].

First parts of the phloem differentiation puzzle. In contrast to tracheary elements that undergo programmed cell death, phloem cells interconnect through sieve plates, generate secondary cell walls and lose most of their organelles and their nucleus. They manage to stay alive by establishing numerous cytoplasmic connections through plasmodesmata with the neighbouring companion cells. Recent work has shown that sieve plate biogenesis requires CHOLINE TRANSPORTER-LIKE 1

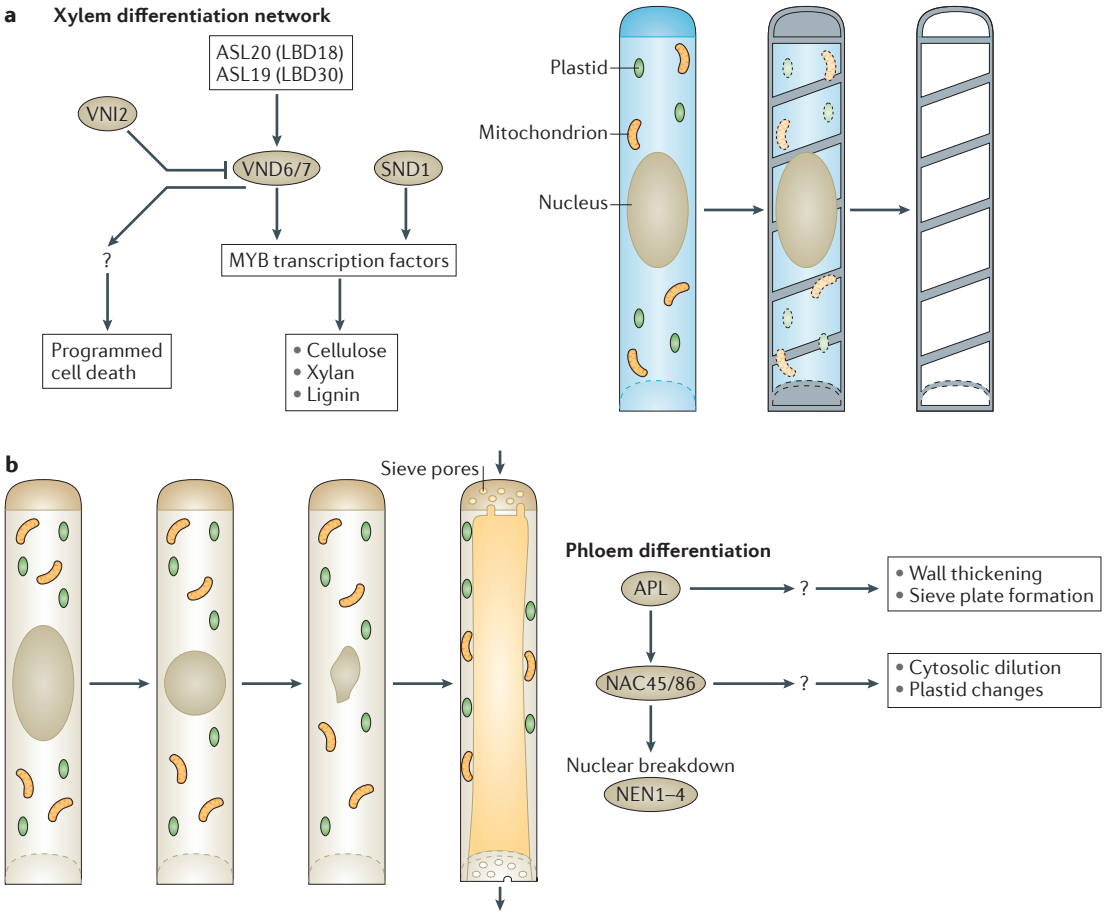


Figure 4 | **Differentiation events during xylem and phloem development. a** | A network of transcription factors, including VASCULAR-RELATED NAC DOMAIN proteins (VNDs) under the control of LOB DOMAIN-CONTAINING (LBD; also known as ASL) genes, VND INTERACTING 2 (VNI2) and SECONDARY WALL-ASSOCIATED NAC DOMAIN PROTEIN 1 (SND1), activate MYB transcription factors that regulate the expression of genes required for secondary cell wall synthesis and programmed cell death during xylem differentiation (left panel). In the first step, the secondary cell wall pattern is established and hydrolytic enzymes accumulate. Next the vacuoles rupture and programmed cell death occurs, together with perforation of the cell wall, generating a hollow tracheary tube (right panel). **b** | Phloem differentiation involves a number of distinct cellular modifications, including nuclear breakdown and sieve plate formation (left panel). These cellular events are controlled as different outputs of a regulatory network (right panel). So far, it has been shown that ALTERED PHLOEM DEVELOPMENT (APL) induces nuclear breakdown by the NAC45/86 DEPENDENT EXONUCLEASE-DOMAIN PROTEIN 1 (NEN1) to NEN4 nucleases through NAC45 and NAC86 transcription factors.

(CHER1; also known as ATCTL1), indicating that the regulation of choline levels is crucial for phloem development and long-range transport in plants[74]. Sieve element formation is further controlled by two redundant APL-targeted genes, *NAC45* and *NAC86* (REF. 75). Among the target genes of the NAC transcription factors encoded by these genes, a family of nuclease domain-containing proteins (NAC45/86-DEPENDENT EXONUCLEASE-DOMAIN PROTEIN 1 (NEN1) to NEN4) control the enucleation process[75] (FIG. 4b). Despite these recent advances, most of the molecular mechanisms that regulate the vast array of cellular changes that occur during phloem differentiation remain elusive to date.

A meristem within the root meristem

To generate a growing three-dimensional structure, the root meristem undergoes several rounds of ordered cell divisions. Those divisions underlying the longitudinal growth of the root are called anticlinal divisions, whereas radial growth is controlled by periclinal divisions (FIG. 5). More than 60 years ago, Clowes[76,77] described a group of cells at the centre of the meristem with very low division rates, which we now call the quiescent centre. These cells are also characterized by specifically high auxin signalling, as shown by auxin responsive reporters[78–80]. Although quiescent centre cells hardly divide, the cells immediately surrounding the quiescent centre are actively dividing anticlinally and are commonly called the stem cells. Elegant laser ablation studies and genetic experiments[81–85] have shown that stem cells continuously undergo asymmetric, anticlinal divisions, each generating a new stem cell and another daughter cell that is no longer in contact with the quiescent centre and that undergoes several more rounds of anticlinal divisions to finally differentiate when exiting the meristem. The PLETHORA (PLT) transcription factors are crucial

Anticlinal divisions
Cell divisions that occur perpendicular to the surface of the plant body, resulting in longitudinal growth.

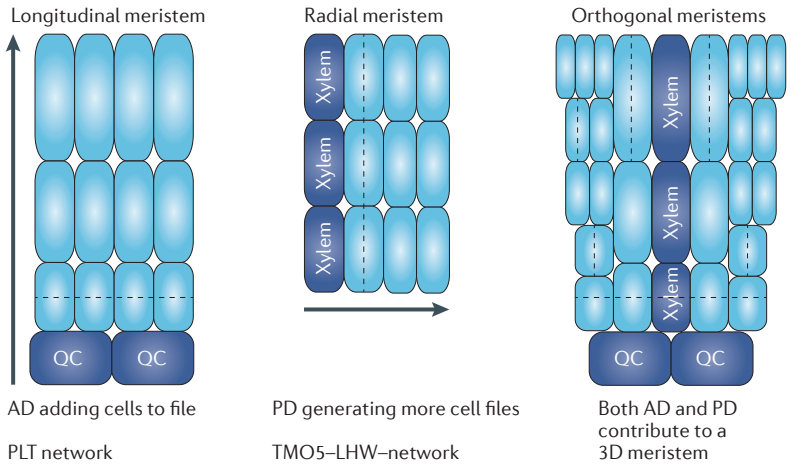


Figure 5 | **Orthogonal meristems control three-dimensional vascular growth in the root meristem.** A model for the control of vascular tissue growth in the post-embryonic root. Two orthogonal meristems operate to control extension of the tissue either in the longitudinal dimension (left panel) or in the radial dimension (middle panel). Arrows indicate the axis of growth. These two meristems are differentially regulated, as the longitudinal meristem depends on the PLETHORA (PLT) pathway, whereas the radial meristem is under control of the auxin–TARGET OF MONOPTEROS 5 (TMO5)–LONESOME HIGHWAY (LHW)–cytokinin pathway. Note that these two meristems rely on distinctly oriented cell division planes (shown as dotted lines): anticlinal divisions (AD) for the longitudinal meristem and periclinal divisions (PD) for the radial meristem. In both cases, dividing cells (light blue) are neighbouring cells that do not divide along the axis of the respective meristem (quiescent centre (QC) or xylem in dark blue). The combination of these two orthogonal meristems can explain the three-dimensional growth of the vascular tissue within the root meristem.

regulators of the stem cell niche[86–88]. The stem cell model has been well established for the distal stem cell niche (the root cap) and paved the way for our understanding of stem cell niches in plants.

This concept of stem cell function in the proximal meristem, however, does not seem to seamlessly apply to the high number of periclinal divisions that occur in the vascular tissues[26], giving the root tip its typical conical shape (FIG. 2a) by increasing its width. Radial growth through periclinal division has recently been shown to depend on cytokinin and the TMO5 and LHW transcription factors (see above). The TMO5–LHW dimer is sufficient to specifically trigger periclinal division in any cell type of the root when ectopically expressed, whereas the number of anticlinal divisions is not significantly altered. Also, in *tmo5 tmo5like1* and *lhw* loss-of-function mutants, longitudinal growth is only moderately reduced compared to wild type, whereas radial growth in the vasculature is strongly reduced[3], suggesting that control of periclinal division and anticlinal division can be genetically separated. Moreover, the TMO5–LHW dimer controls periclinal division in the neighbouring procambium cells through local cytokinin production[9]. Although cytokinin induces radial growth through periclinal division in root meristem and also in vascular cambium[26,27,89–92] (BOX 1), it has a negative effect on meristem length by repressing anticlinal division[93,94]. Thus both genetic and hormone signalling networks suggest that radial and longitudinal growth are controlled by distinct networks.

Intriguingly, cells with xylem identity expressing the TMO5–LHW dimer only very rarely divide along the radial axis and contain high levels of auxin signalling, two characteristics that are shared with the quiescent centre. Indeed, root vascular bundles containing only xylem cell identities, such as in the *log* heptuple or *wol* mutants[9,26,91], show hardly any periclinal division. Radial growth thus appears to be controlled by a 'radial meristem', in which the xylem axis functions as an organizing centre driving radial growth through periclinal division in the neighbouring procambium cells. However, it seems unlikely that the TMO5–LHW-dependent network located in cells with xylem identity would be sufficient to explain all vascular formative divisions (for example, those that generate the distinct phloem cell types: companion cell, protophloem and metaphloem), suggesting the existence of as-yet-unknown factors that control, for example, phloem cell fates.

In conclusion, a growing root tip contains a longitudinal meristem that generates more cells in the existing cell files through anticlinal division, under the control of the well-described auxin–PLT signalling network[86–88]. Furthermore, we propose that a radial meristem produces more cell files within the vascular bundle through periclinal division, at least partly through the independently acting TMO5–LHW-dependent network. A combination of both meristem activities would thus generate the ordered three-dimensional structure of the root apical meristem.

Box 1 | **Vascular cambium and its similarity to the radial meristem**

Whereas a proportion of procambial cells differentiate into various xylem and phloem cell types, the remaining cells persist undifferentiated as the tissue matures. Later during development, these intervening undifferentiated procambial cells form the vascular cambium, which is a secondary meristem that undergoes periclinal cell divisions to produce new cells on each side of the meristem — xylem cells towards the inside of the stele and phloem towards the outside — thus resulting in radial (secondary) growth in a number of plant organs. Cambial activity therefore resembles the activity of the radial meristem in the root tip. Similar to the radial meristem, cytokinins are important for the periclinal divisions in cambium[26,27,89,92]. However, there are differences between the cambium and the radial root meristem: the key cambial regulators WUSCHEL-RELATED HOMEOBOX4 (WOX4), TRACHEARY ELEMENT DIFFERENTIATION INHIBITORY FACTOR (TDIF) and PHLOEM INTERCALATED WITH XYLEM (PXY; also known as TDR) are present in the cambium but are absent from the root tip, including the procambium[69–72,95]. The TDIF peptide is produced in secondary phloem and diffuses into the cambium, where it binds to its receptor PXY to regulate the rate and orientation of cell divisions, in part through promoting expression of the transcription factor WOX4 (REFS 70,71,96). Another striking dissimilarity is that, in contrast to the vascular tissue in the primary root meristem, phloem-related cells in stem secondary growth show a marked increase in auxin signalling[97]. Cambial regulators and their signalling pathways have been reviewed recently[5,6].

Stem cell niche
A group of cells and the associated organizing centre, which contribute to plant growth through local cell divisions.

Perspectives

In recent years, several important studies have increased our understanding of the development and regulation of vascular tissues. Early steps in tissue formation can now be linked to differentiation through connections identified between individual components of regulatory networks. Thus, vascular tissue initiation, formation, patterning, growth and differentiation can now be seen as parts of a continuum. However, it has also become clear where knowledge is still lacking. For example, although many genes are activated concurrently at the time of vascular tissue initiation, it is entirely unknown how the tissue identity is first specified. Secondly, as several regulatory networks have been identified in specific developmental contexts, it remains to be seen whether these are universal principles that also mediate patterning in other organs. For example, the specific vascular architecture of the root apical meristem drastically changes during root secondary growth, and vascular organizations similar to those of the root apical meristem can be obtained, starting from a cellular template that is different from the stereotypic early *A. thaliana* embryo. This raises the question of whether the same pathways are reused throughout development, or whether independent parallel pathways exist. It will also be crucial to evaluate whether the well-studied pathways in *A. thaliana* can be transferred to other plant species, and whether these mechanisms are conserved throughout evolution. Finally, it seems that oriented cell division is an important factor in vascular tissue development, and a key question is how division orientation is controlled in space and time to create and maintain the three-dimensional shape of vascular tissue. We propose a model in which two distinctly regulated orthogonal meristems each control a separate axis of growth to sustain tissue development as a whole. It will be interesting to see whether the downstream networks overlap, or whether distinct regulatory modules control radial and longitudinal growth separately.

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Competing interests statement
The authors declare no competing interests.