SIGNALS THAT CONTROL PLANT VASCULAR CELL DIFFERENTIATION

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Plant vascular cells originate from procambial cells, which are vascular stem cells. Recent studies with *Zinnia elegans* cell culture and *Arabidopsis thaliana* mutants indicate that intercellular-signalling molecules such as auxin, cytokinin, brassinosteroids and xylogen regulate the maintenance or differentiation of procambial cells through distinct intracellular-signal transduction and gene-expression machineries. This intercellular- and intracellular-signalling system might be involved in determining the continuity and pattern formation of vascular tissues.

PLANT CELL BIOLOGY

VASCULAR CELLS
Cells that form the plant vascular tissues, including procambial cells, cambial cells, sieve elements, companion cells, fibre cells, xylem and phloem parenchyma cells, and tracheary elements.

APICAL MERISTEM
The meristematic tissue that is located at the tip of the shoot and root. It is composed of stem cells, which allow plants to continue to grow in height.

XYLEM
The tissue that is responsible for transporting water and minerals. It also gives strength to the stem.

PHLOEM
The tissue that is responsible for transporting the carbohydrates that are produced in leaves.

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The body plan of plants is controlled by a combination of clonal fate and positional information that is provided by local signals, as is commonly seen in multicellular organisms. Recent advances in molecular genetics and genome biology have uncovered unique mechanisms of plant-body formation. Vascular cells are formed under a well-defined plant-differentiation programme. Although vascular cells usually differentiate at predicted positions and at a predicted time to form a specific vascular pattern, the arrangement of the vascular network can be altered by local signals or in response to environmental stimuli. This indicates the involvement of a non-clonal and flexible mechanism in vascular-pattern formation.

VASCULAR CELLS are produced continuously from the APICAL MERISTEMS of shoots and roots in adult plants (FIG. 1). In this situation, local cell–cell communication between developing vascular cells and cells that are not destined to differentiate into vascular cells might have a pivotal role in determining vascular cell differentiation. On the other hand, long-distance signalling also seems to be necessary for the continuous production of vascular strands.

Vascular strands connect plant organs that are often separated by many metres, and provide a pathway for the transport of signalling molecules as well as water and nutrients. For such vascular function, their continuity is a prerequisite. Vascular strands — which are also referred to as vascular bundles — are composed of three tissues: XYLEM, PHLOEM and meristematic tissues such as PROCAMBIUM and VASCULAR CAMBIUM. But MONOCOTYLEDONOUS PLANTS and ferns, in which secondary thickening growth does not occur, lack cambium. Individual species of vascular plants form distinct radial patterns of vascular bundles in each organ, which have been categorized as collateral, bicollateral, amphivasal or amphicribral patterns (FIG. 1a, b).

Procambial cells are vascular stem cells that are derived from the apical meristem and give rise to xylem and phloem precursor cells. The final step in vascular development is the specification into distinct types of vascular cells from the precursor cells. Phloem precursor cells differentiate into various phloem cells such as SIEVE ELEMENTS (which are components of SIEVE TUBES), COMPANION CELLS, phloem PARENCHYMA cells and phloem fibres. Xylem precursor cells give rise to TRACHEARY ELEMENTS (TEs), xylem parenchyma cells and xylem fibres, which together form xylem. TEs are components of a vessel — or tracheid — and at maturity, they are emptied by the loss of all cell contents, including the nucleus, to form hollow tubes through which fluids move. Therefore, cell death of TEs is programmed developmentally. TEs possess a characteristic secondary cell wall of annular, spiral, reticulate or pitted wall thickenings, which add strength and rigidity to the wall and prevent TEs from collapse under the high pressure that is exerted on fluid uptake.

Molecular-genetic studies with *Arabidopsis thaliana* mutants and cellular studies with *Zinnia elegans* xylogenic cultures (BOX 1) have revealed the integrated

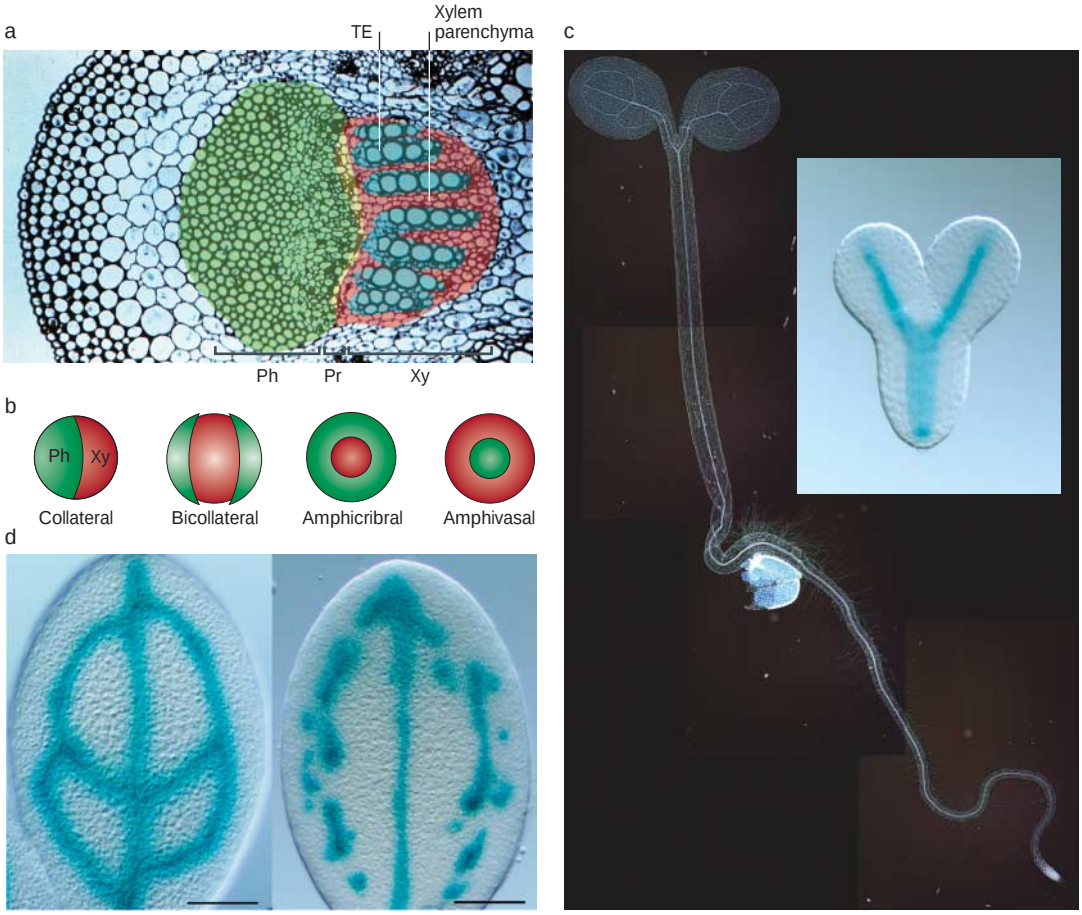


Figure 1 | **Vascular-pattern formation. a** | The vascular system is composed of phloem (green, Ph), procambium (and/or vascular cambium; yellow, Pr) and xylem (Xy) that contains tracheary elements (grey) and xylem parenchyma cells (red), which make a distinct radial pattern of vascular bundles depending on the organ and plant species. Reproduced with permission from REF. 120 © (1982) John Wiley & Sons Inc. **b** | Four distinct radial patterns of phloem and xylem (red) within vascular bundles. **c** | The procambium, stained blue to show the promoter activity of the *HD-ZIP-III* homeobox gene *AtHB8*, is formed as continuous columns of cells in embryos. In seedlings, the shoot and root meristems produce procambial cells to keep the continuity of vascular bundles. Reproduced with permission from REF. 119 © (2000) Shujyunsha Co. Ltd. **d** | *van3* mutants have a fragmented vascular network in a cotyledon (right) compared with that in a wild-type cotyledon (left)[14]. Reproduced with permission from REF. 14 © (2000) The Company of Biologists Ltd.

PROCAMBIUM
A primary meristem that is derived from the apical meristem and which gives rise to primary vascular tissues and vascular cambium.

VASCULAR CAMBIUM
A lateral meristem that gives rise to secondary vascular tissues — secondary xylem on the inner side and secondary phloem on the outer side in stems and roots, which results in an increase in the diameter of those organs.

MONOCOTYLEDONOUS PLANTS
A class of angiospermous plants that is characterized by the production of seeds with one cotyledon, such as rice, maize and wheat.

SIEVE ELEMENT
The components of sieve tubes that are separated from each other by sieve plates. In contrast to tracheary elements, which are depleted of all cell contents, sieve elements contain a nucleus even after maturation.

SIEVE TUBE
A series of sieve elements that form a long cellular tube that functions in the transport of photosynthetic products.

COMPANION CELLS
Cells that are associated with sieve elements and support them. An asymmetrical cell division of a precursor cell produces a sieve element and a companion cell.

PARENCHYMA
A tissue that is composed of cells that have thin cell walls and has a relatively flexible ability to differentiate.

TRACHEARY ELEMENT
The thick-walled cylindrical cell that is a component of vessels, or tracheids, which are water-conducting tissues.

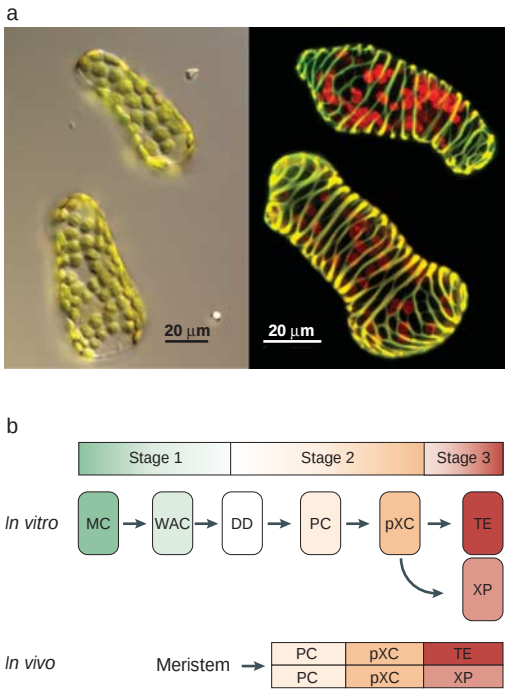
BASIPETALLY
Toward the root tip from the shoot tip.

nature of plant vascular formation, including the integrity of the vascular system, intravascular-radial-pattern formation and vascular cell specification[1,2]. Here, I review recent findings on plant vascular cell differentiation, with a special emphasis on intercellular and intracellular signalling. Because little is known about the molecular mechanism of phloem cell differentiation, vascular cell differentiation is described mainly with regard to xylem cell differentiation. In appropriate cases, *A. thaliana* and *Z. elegans* genes and proteins are distinguished by the prefixes At and Ze, respectively.

Intercellular signals and vascular continuity

A role for auxin flow. Pioneering studies by Jacobs[3] and Sachs[4,5] showed that indoleacetic acid (IAA) — a natural auxin that is produced in the apical region of shoots (including the apical meristem and expanding leaves) and transported BASIPETALLY — was the limiting and controlling factor in the regeneration of vascular strands around a wound. These studies also showed that polar auxin flow is needed for continuous vascular-pattern formation. Indeed, the prevention of polar auxin transport by specific inhibitors causes the formation of local aggregates of vascular cells and discontinuous veins in newly developed leaves[6,7]. By contrast, such inhibitors were less effective at inhibiting vascular differentiation from the procambium, patterns of which had already formed in embryonic cotyledons. So, these inhibitors seem to affect vascular development through disruptions in the development of procambial patterns. Sachs[4,5] proposed the 'auxin-flow canalization hypothesis', which suggests that auxin flow, starting initially by diffusion, induces the formation of the polar-auxin-transport-cell system.This, in turn, promotes auxin transport, leading to canalization of the auxin flow along a narrow column of cells (FIG. 2). This continuous

Box 1 | Xylem cell differentiation induced *in vitro* without cell division



Single mesophyll cells (see figure part **a**, left panel) can be isolated mechanically from expanding leaves of *Zinnia elegans*. When the isolated mesophyll cells are cultured *in vitro* in the presence of auxin and cytokinin, they transdifferentiate synchronously within 72 hours, and at a high frequency, into tracheary elements (TEs; see figure part **a**, right panel) and at a low frequency into xylem parenchyma cells (not shown)[59,106]. This transdifferentiation can occur without intervening cell division. It is interesting that differentiating TEs still have chloroplasts, as shown by red fluorescence. This xylogenic *Z. elegans* system is useful for studying the sequence of events during xylem differentiation, largely because differentiation occurs at a high frequency and because the process can be followed in single cells. For example, a recent microarray analysis with this system has uncovered an expression profile of 9,000 genes that change during xylem cell transdifferentiation[21].

The process of transdifferentiation is divided into three stages (see figure part **b**)[59]. Stage 1 immediately follows the induction of differentiation by wounding and a combination of auxin and cytokinin, and corresponds to the functional dedifferentiation process. During stage 1, isolated mesophyll cells (MC in the figure) lose their ability to carry out photosynthesis and dedifferentiate via wound-activated cells (WAC in the figure). However, it should be emphasized that this process of dedifferentiation is not accompanied by cell division. At stage 2, dedifferentiated cells (DD in the figure) differentiate into procambial cells (PC in the figure) and then into xylem cell precursors (pXC in the figure). At stage 3, the differentiation of TEs and xylem parenchyma cells (XP in the figure) occurs from xylem cell precursors. The TE-differentiation process involves patterned secondary-cell-wall deposition and programmed cell death. Brassinosteroids are synthesized actively during stage 2 and are essential for differentiation of procambial cells to xylem cell precursors and/or of xylem cell precursors to TEs and xylem parenchyma cells[77,78]. This *in vitro* transdifferentiation process mimics *in vivo* xylem cell development during which meristematic cells differentiate into TEs and xylem parenchyma cells via procambial cells and xylem cell precursors. The image in the right panel of part **a** of the figure is reproduced with permission from Macmillan Magazines Ltd.

polar transport of auxin through cells finally results in the differentiation of strands of procambial cells and, subsequently, vascular strands.

Mechanisms of auxin transport

The recent characterization of *A. thaliana* mutants that are defective in auxin transport and signalling has provided a cellular and molecular basis for the polar transport of auxin along the plant axis, thereby supporting the canalization hypothesis[5,8]. An auxin-efflux carrier, AtPIN FORMED 1 (AtPIN1), locates specifically on the plasma membrane that contacts the basal end wall in both procambial cells and xylem parenchyma cells, and contributes to basipetal transport of auxin[9,10]. Indeed, quantification of the IAA concentration in *Pinus sylvestris* trees showed the highest IAA concentration in the cambial zone and a gradual decrease in IAA from the cambial zone to the mature xylem[11]. Asymmetrically transported AtPIN1 causes a polarized auxin flow, which might lead to the formation of continuous columns of procambial cells. AtPIN1 is recycled in cells — it is transported from endomembranes to the plasma membrane by endosome-like, AtPIN1-specific vesicles (AtPINSVs; FIG. 2). A specific guanine-nucleotide-exchange factor for ADP-ribosylation factor G protein, GNOM (also known as VAN7 or EMB30), promotes the asymmetrical transport of AtPINSVs by the activation of ADP-ribosylation factor [12,13]. In a *van7* (*emb30-7*) mutant, an excess of fragmented vascular structure is induced in leaves and cotyledons[14]. A serine/threonine kinase known as PINOID might also be involved in the asymmetrical transport of AtPINSVs — possibly by phosphorylating an unknown component of AtPINSVs — because the phenotype of the *pinoid* mutant is similar to that of *Atpin1* (REF. 15). Interestingly, *PINOID* is expressed preferentially in xylem precursor cells and xylem parenchyma cells[16].

In addition to GNOM and PINOID, many proteins with a role in IAA efflux have been identified[17]. Muday and Murphy[17] have pointed out striking similarities between the IAA-efflux-related proteins and proteins that mediate the insulin-inducible, asymmetric vesicle cycling of mammalian glucose transporters[18,19], raising the possibility that a mammalian glucose-transporter-like mechanism might underlie polarized AtPIN1 cycling (FIG. 2). Although auxin can be transported inside the cell without a transporter,

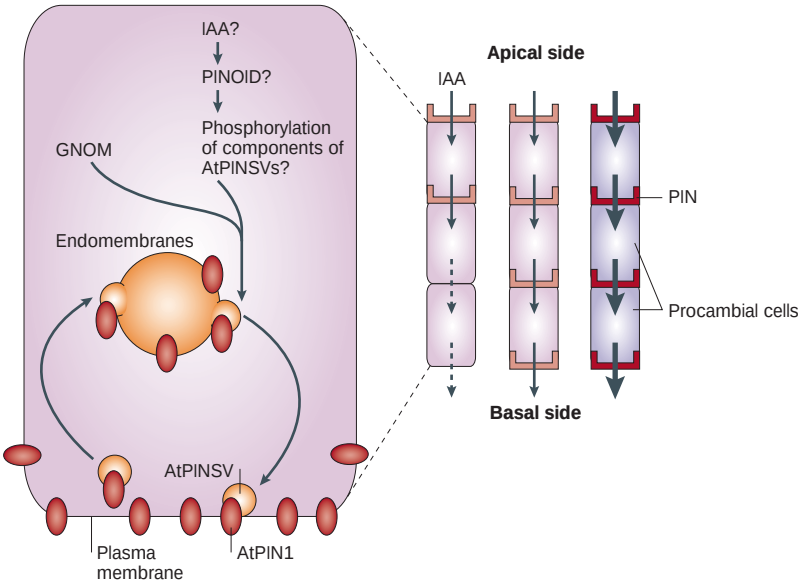


Figure 2 | **Molecular mechanism of auxin polar transport.** Asymmetrically transported auxin-efflux carriers (PIN proteins) cause a polarized flow of auxin, which leads to the formation of continuous columns of procambial cells. AtPIN1 is recycled in cells — it is transported from endomembranes to the plasma membrane by endosome-like AtPIN1-specific vesicles (AtPINSVs). A guanine-nucleotide-exchange factor for the ADP-ribosylation factor G protein, GNOM/EMB30, and the serine/threonine kinase PINOID are thought to promote the asymmetrical transport of AtPINSVs by the activation of ADP-ribosylation factor and the phosphorylation of an unknown component of AtPINSVs, respectively. This process might be initiated by the natural auxin indoleacetic acid (IAA) to create a positive-feedback loop of IAA flow, which is consistent with the IAA-flow canalization model.

auxin-influx carriers support the rapid influx of auxin into cells. AUX1, an auxin-influx carrier, is also asymmetrically localized on one side of the cell[20]. Although AUX1 locates in PROTOPHLOEM in roots[20], which is different from the localization of AtPIN1, it is still possible that a distinct member of the *AUX1*-gene family might have a role in auxin flow in procambium or xylem columns. Interestingly, exogenously supplied auxin enhances the expression of the *PINOID* gene in xylem[16] and of a *Z. elegans AUX1* homologue in the xylogenic culture[21]. Therefore, auxin-dependent transcriptional activation of these genes could function as a component of a positive-feedback loop of polar auxin transport that was predicted in the auxin-flow canalization hypothesis.

Auxin perception

Not all cells that are subjected to polar auxin flow differentiate into vascular cells. Therefore, in addition to polar auxin flow, auxin perception is needed for the continuous formation of vascular strands. Indeed, *A. thaliana* mutants, such as *auxin resistant-6* (REF. 22) and *bodenlos*[23], which are defective in perceiving auxin, show a severely reduced vascular network. The *MONOPTEROS* (*MP*) gene encodes a transcription factor that belongs to a family of 23 auxin-response factors (ARFs)[24], and MP binds to *cis*-acting auxin-response elements to activate transcription[25]. *MP* is initially expressed in a broad area of the embryo, but becomes gradually confined to the procambium[24]. *mp* mutations disturb the body organization along the apical–basal axis and cause the formation of discontinuous and reduced vascular strands[24,26]. These findings have indicated that MP might function to promote continuous vascular-pattern formation by mediating the axial formation of plant cells in response to auxin cues[27].

The auxin-induced short-lived AUX/IAA PROTEINS (of which there are at least 24 in *A. thaliana*) are thought to bind to specific ARFs and repress their transcriptional activities[28]. Auxin stimulates the degradation of AUX/IAA proteins by the activation of a specific ubiquitin ligase, which, in turn, promotes ARF-dependent gene expression. Because auxin also induces the expression of *AUX/IAA* genes, ARF-dependent gene expression is again repressed by the increased levels of AUX/IAA proteins. Therefore, it is important to find the AUX/IAA protein(s) that bind specifically to MP. Even though *IAA8*, which encodes an AUX/IAA protein, and *MP* are both expressed preferentially in procambial cells[29], there is no evidence for an interaction between IAA8 and MP in these cells. Altogether, it is clear that polar auxin transport is crucial for the continuous formation of vascular bundles; however, the precise molecular mechanisms are still mostly unknown. To gain a better understanding of this process, we need to identify more components that are involved in auxin-flow-dependent procambial cell differentiation.

Auxin-flow-independent patterning

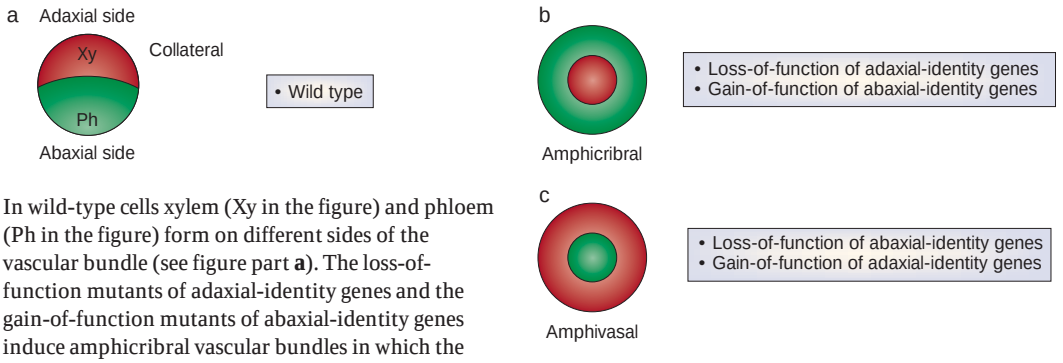
The auxin-flow canalization hypothesis assumes that polarized auxin flow establishes a canal for conducting auxin flow by itself, without any blueprints, and that this canal results in the formation of a vascular pattern. Therefore, mutations that prevent canalized auxin flow and vascular continuity are expected to have defects in the overall architecture of the vascular pattern. There are a number of *A. thaliana* mutants that have discontinuous secondary vascular strands in cotyledons and leaves, although the main vein in cotyledons and leaves, and vascular strands in other organs, are continuously formed[4,30–32]. Interestingly, in most of the mutants, although the vein networks were fragmented, the overall architecture was normal.

Detailed analysis of a mutant, *van3*, showed that vein fragmentation occurred at the time of procambium formation. This pattern of vein formation cannot be explained simply by the auxin-flow canalization hypothesis. To explain vein patterns, some other hypotheses have been proposed. These include the leaf-venation hypothesis, according to which shifts in the sites and concentrations of auxin, in association with leaf development, control venation-pattern formation[33]. And they include the diffusion–reaction prepattern hypothesis, in which local autocatalysis and long-range inhibition of the reaction by interacting substances that have different diffusion rates generate stable patterns autonomously[34,35]. The discontinuous vein formation might be explained by the diffusion–reaction prepattern hypothesis. According

PROTOPHLOEM
The primary phloem that is first formed from procambium during organ development.

AUX/IAA PROTEINS
A class of short-lived nuclear proteins that share four conserved domains and that are generally encoded by early-auxin-response genes. They repress auxin responses by dimerizing with auxin-response factor (ARF) transcriptional activators that reside on auxin-responsive promoter elements.

Box 2 | Relationship between abaxial–adaxial identity and radial patterns of vascular tissues.



In wild-type cells xylem (Xy in the figure) and phloem (Ph in the figure) form on different sides of the vascular bundle (see figure part **a**). The loss-of-function mutants of adaxial-identity genes and the gain-of-function mutants of abaxial-identity genes induce amphicribral vascular bundles in which the phloem encircles the xylem (see figure part **b**). The loss-of-function mutants of abaxial-identity genes and the gain-of-function mutants of adaxial-identity genes induce amphivasal vascular bundles, whereby the xylem surrounds the phloem (see figure part **c**).

to this hypothesis, slight changes in the autocatalytic condition can be expected to induce a spotted pattern of vascularization as a result of fragmentation of the striped pattern without destroying the overall architecture. The *VAN3* gene, therefore, might encode components of the diffusion–reaction systems, as in the case of the *Leopard* gene of zebrafish, mutations of which change the pigmentation pattern from stripes to spots[36]. These results indicate the presence of at least two different mechanisms for the continuous formation of vascular networks — a polar-auxin-transport-controlled mechanism and a second mechanism, for example, an autonomous pattern-formation mechanism that is induced by a secreted activator(s) and inhibitor(s). The *A. thaliana cov1* mutant shows a dramatic increase in vascular-tissue development in place of the interfascicular region that normally separates the vascular bundles[37]. Analysis of the interaction of *cov1* with a known auxin-signalling mutant and direct analysis of auxin concentrations indicate that *cov1* affects vascular patterning by a mechanism that is independent of auxin.

Dorsoventral identity and radial patterning

In the vascular bundles of the leaves of many plant species, xylem, procambium and phloem show a distinct dorsoventral organization — xylem is localized on the dorsal (adaxial) side, phloem is on the ventral (abaxial) side and procambium is positioned between xylem and phloem. *Antirrhinum majus* and *A. thaliana* mutants that show defects in the identity or maintenance of the dorsoventral axis indicate the involvement of the dorsoventral axis in the radial-pattern formation of leaf vascular tissues (BOX 2). The *PHANTASTICA* (*PHAN*) gene, which encodes a MYB-LIKE TRANSCRIPTION FACTOR, is expressed in organ initials and in adaxial cells in developing leaves[38]. Loss-of-function mutations of *PHAN* show a phenotype in which tissues that are normally associated with the adaxial part of the wild-type leaf are replaced by tissues with abaxial characteristics, which indicates that PHAN is probably involved in the adaxial identity of leaves[39]. In *phan* leaves, the vein vascular system changes into a cylinder in which phloem encircles xylem — that is, it becomes an amphicribral vascular bundle (BOX 2). By contrast, gain-of-function mutations of an *A. thaliana* homeobox leucine-rich repeat (LRR) class-III gene (*HD-ZIP III*) — *PHABULOSA* (*PHB*)/*AtHB14* or *REVOLUTA* (*REV*)/*INTERFASCICULAR FIBERLESS 1*(*IFL1*) — cause a dramatic transformation of abaxial-leaf fates into adaxial fates and result in the formation of an amphivasal vascular bundle in which xylem surrounds phloem[40–42] (BOX 2). Plants that are homozygous for loss-of-function alleles of three genes — *phb phv* (*phavoluta/AtHB9*) *rev* — produce only a single, radial, abaxialized cotyledon with no bilateral symmetry in the most severe manifestation, and two radialized cotyledons with amphicribral vascular bundles in less-severe manifestations[42] (BOX 2).

On the other hand, the *YABBY* gene family of abaxial-identity genes are known to be expressed in the abaxial side of leaves and to specify abaxial cell fates in *A. thaliana*[43,44]. Ectopic expression of *FILAMENTOUS FLOWER* (*FIL*) or *YABBY3*, which are both members of this family, is sufficient to specify the development of ectopic abaxial tissues in lateral organs. Occasionally, amphicribral vascular bundles are also observed in the PETIOLES of *FIL*-overexpressing plants (S. Sawa, personal communication). Although loss-of-function of *FIL* and *YABBY3* changed abaxial cell fates to adaxial ones, the vascular system still retained a normal adaxial–abaxial polarity (xylem, adaxial; phloem, abaxial)[44]. *KANADI* is another gene family that specifies abaxial identity[45,46]. Triple loss-of-function mutants of *KANADI1 KANADI2 KANADI3* show radialized amphivasal vascular bundles in stems[42] (BOX 2).

Altogether, the collateral pattern of xylem and phloem in vascular bundles might be determined by the proper balance between adaxial- and abaxial-identity genes. The predominance of either adaxial or abaxial identity causes amphivasal and amphicribral vascular bundles, respectively. In other words, adaxial- and abaxial-identity genes might interact to specify xylem and phloem formation, respectively, in vascular bundles. In

MYB-LIKE TRANSCRIPTION FACTOR
A member of the transcription-factor family that contains the MYB motif, which consists of a helix-turn-helix structure with three regularly spaced Trp residues. This family is substantially larger in plants than in animals and functions in the regulation of a variety of events, including secondary metabolism and plant morphogenesis.

PETIOLE
The part of the leaf that connects the leaf blade and the stem.

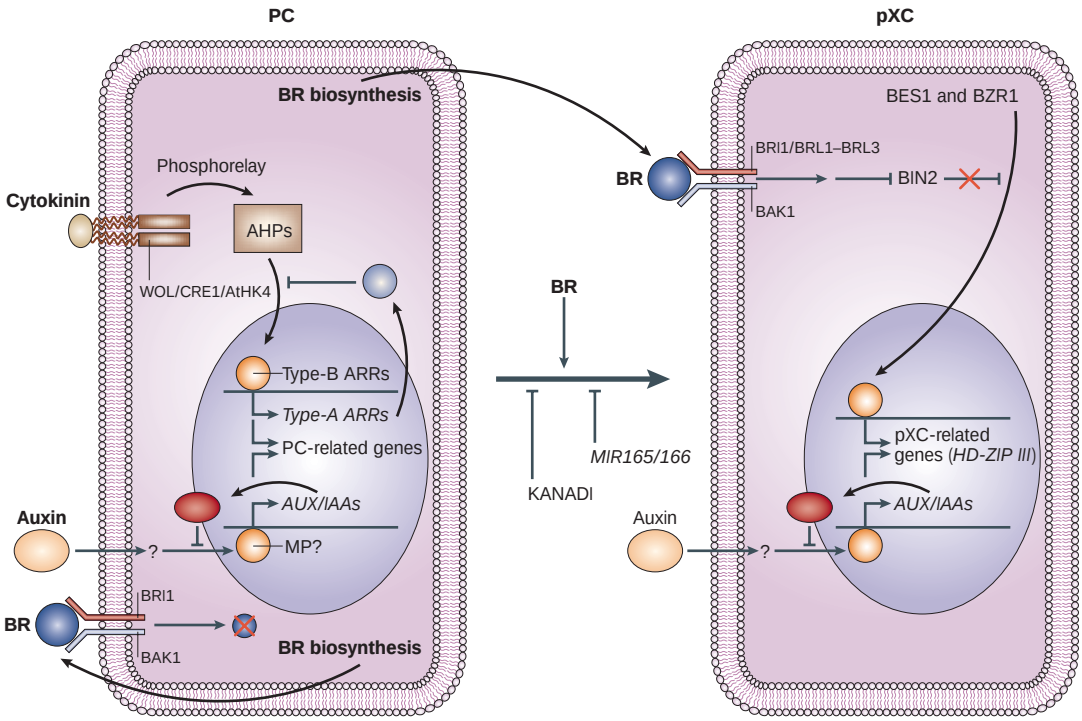


Figure 3 | **Model for signalling processes in the maintenance and/or the differentiation of procambial cells and xylem cell precursors.** In procambial cells (PCs), the coordinated signalling by cytokinin and auxin induces the expression of genes that are involved in the maintenance of procambial activities. The auxin-signalling pathway might involve gene expression of auxin-response factors, such as MONOPTEROS (MP), that also function as transcriptional activators, and their repressors, the AUX/IAA proteins. Cytokinin might be perceived by the WOL/CRE1/AtHK4 cytokinin receptor, which, in turn, transmits an intracellular signal that is mediated by a His–Asp phosphorelay mechanism to PC-related histidine-containing phosphotransfer factors (AHPs) and then to PC-related type-B response regulators (ARRs). The type-B ARRs might function as transcriptional activators of PC-related genes including the genes of their repressors, the type-A ARRs. The presence of repressors in auxin- and cytokinin-signalling pathways might allow cytokinin and auxin signalling to be temporal. Brassinosteroids (BRs in the figure) are biosynthesized actively in PCs and secreted, but brassinosteroids do not work as a signal for the maintenance of procambial activities. Instead, brassinosteroids, in the presence of auxin, might initiate differentiation of procambial cells to precursors of xylem cells (pXCs) after recognition by a receptor, which might be a heterodimer composed of either brassinosteroid-insensitive-1 (BRI1) or one of the BRI1-like proteins (BRL1–BRL3), plus BRI1-associated receptor kinase-1 (BAK1). The brassinosteroid signal inactivates the negative regulator BIN2 (brassinosteroid-insensitive-2), which allows the unphosphorylated form of *bri1*-EMS-suppressor-1 (BES1) and brassinazole-resistant-1 (BZR1) to translocate to the nucleus and to promote pXC-related gene expression. Among the most important pXC-related genes that are induced by brassinosteroids might be the *HD-ZIP-III*-homeobox gene family, which might function in further xylem cell differentiation. KANADI and the microRNAs *MIR165* and *MIR166* might suppress differentiation of PCs to pXCs. The suppression by the microRNAs might be caused by the rapid degradation of the *HD-ZIP-III* gene mRNA through RNAi machinery.

fact, the loss-of-function alleles of *KANADI* lead to an expansion of *PHB, PHV* and *REV* expression[45,47]. This indicates that abaxial-identity genes function as negative regulators of *PHB, PHV* and *REV*, leading to the suppression of xylem differentiation. The *APL* gene, which encodes a Myb-like transcriptional factor, was recently identified and found to have a dual role in promoting phloem differentiation and in repressing xylem differentiation[48]. Therefore, it will be quite interesting to uncover the interrelationship between *APL* and the *KANADI* genes, and *PHB, PHV* and *REV*.

Vascular-cell-development factors

Cytokinin. CYTOKININ has a crucial role in the formation and/or maintenance of procambial cells. In a recessive *A. thaliana* mutant known as *wooden leg* (*wol*), the number of procambial cells in embryos is reduced and the vascular system in emerging roots is composed only of xylem[49]. Mähönen and others found that *WOL* was identical to *CRE1/AtHK4*, which encodes a histidine kinase that functions as a cytokinin receptor[50]. The corresponding gene is expressed preferentially in the procambium, which indicates that cytokinin function through WOL/CRE1/AtHK4 is probably necessary for the maintenance of procambial activity (FIG. 3). By contrast, the role of WOL/CRE1/AtHK4 in phloem differentiation might be indirect and result from the regulation of cell proliferation during procambial development. In the *A. thaliana* genome, there are two other cytokinin receptor genes, *AtHK2* and *AtHK3* (REF. 51), which are also expressed in vascular cells and the products of which might have affinities for cytokinin that are different from that of AtHK4 (T. Kakimoto, personal communication). Therefore, different cytokinin affinity might be required for the maintenance of procambial cells and other vascular cells.

CYTOKININ
N^6-substituted adenine derivatives that have diverse effects on important physiological functions such as cell division, greening and differentiation in plants.

These cytokinin receptors function together with downstream components, such as histidine-containing phosphotransfer factors and response regulators. The signal is mediated by a His–Asp-phosphorelay mechanism — that is, the sequential phosphotransfer between His and Asp residues in each component. *A. thaliana* has five genes that encode histidine-containing phosphotransfer factors (*AHP1–AHP5*). AHP2 is thought to function downstream of CRE1/WOL/AtHK4 (REF. 52). There are 22 response regulators (ARR1–ARR22) in *A. thaliana*, and these can be divided into two distinct subtypes: type A, with 10 members; and type B, which has 11 members (ARR22 represents an atypical subtype). Type-B ARRs function as transcriptional activators, whereas type-A *ARR* genes are induced rapidly by cytokinin but the corresponding proteins do not have DNA-binding activity[53,54]. In *cre1* roots, cytokinin induces all the type-A *ARR* genes, except *ARR15* and *ARR16* (REF. 32). In wild-type roots, cytokinin induces *ARR15* in procambial cells, but not in xylem and phloem cells, and *ARR16* in the PERICYCLE. Recently, ARR15 was found to function as a repressor of type-B ARRs[55]. Because a loss-of-function mutant of *ARR15* shows no phenotype, other *ARR* genes might be functionally redundant. The factor(s) that activates the expression of procambium-related genes, including *ARR15*, downstream of the CRE1/WOL/AtHB4 signalling pathway, remains to be clarified.

The key step in cytokinin biosynthesis is the transfer of an isopentenyl group to the N^6 position of ADP/ATP, which is catalysed by dimethylallyl diphosphate:ATP/ADP isopentenyltransferase (IP)[56,57]. There are several *IP* genes (*AtIP1* and *AtIP3–AtIP8*) in *A. thaliana*[56]. Many *AtIP* genes are expressed in the tips or vascular bundles of *A. thaliana* roots, which indicates that the vascular bundle is an active site of cytokinin biosynthesis and might function as a source of cytokinin[58]. The demonstration of cytokinin biosynthesis in a distinct vascular cell type indicates that cytokinins are signalling molecules that induce vascular differentiation.

It should be emphasized that cytokinin function in procambial cells might require auxin (FIG. 3). For example, in *Z. elegans* xylogenic cultures, an exogenous supply of both auxin and cytokinin is a prerequisite for the differentiation of procambium-like cells from dedifferentiated cells[59]. In procambium-like cells, there is preferential expression of genes that are involved in auxin signalling, such as homologues of *A. thaliana MP, IAA8, AUX1* and a gene encoding an *A. thaliana* putative 'no apical meristem' (NAM)-like protein[21,29]. The establishment of new intracellular-auxin-signalling systems must be included in a programme for maintaining procambial activities or for further vascular development.

HD-ZIP-III-gene family and microRNAs. Differentiation of xylem cells from procambial cells is coupled with the gradual expression of *HD-ZIP-III* homeobox genes. The *A. thaliana HD-ZIP-III* gene family contains *AtHB8* and *AtHB15* in addition to *PHB, PHV* and *REV*, which were mentioned above.

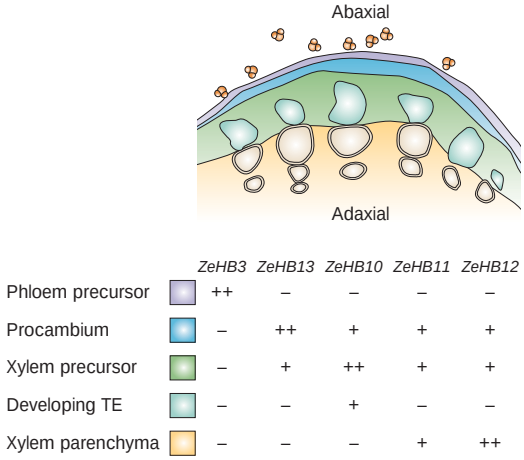


Figure 4 | **Radial-accumulation pattern of transcripts for homeobox genes in vascular tissues.** *Zinnia elegans ZeHB3, ZeHB13, ZeHB10, ZeHB11* and *ZeHB12* transcripts accumulate in vascular tissues in this order from the abaxial to the adaxial side[61]. Transcripts for *ZeHB3*, which belongs to the *HD-ZIP-I* homeobox-gene family, accumulate preferentially in phloem precursor cells on the abaxial side. Transcripts for *ZeHB13, ZeHB10* and *ZeHB11/ZeHB12*, which correspond to *AtHB15*, *AtHB8* and *REVOLUTA*, respectively, accumulate preferentially in procambial and xylem cells. Reproduced with permission from REF. 64 © (2003) The Japanese Society of Plant Physiologists. TE, tracheary element.

PHB, PHV and *REV* are commonly expressed in both the vascular and adaxial region of lateral organs[40], whereas *AtHB8* and *AtHB15* are expressed strictly in vascular bundles — in particular, in the procambial or xylem precursor region[60,61]. A detailed analysis of the expression of *Z. elegans HD-ZIP-III* genes in leaf veins showed overlapping, but distinct, layer patterns of mRNA accumulation from the central (procambium) to the adaxial side (mature xylem; FIG. 4). *ZeHB13* (which corresponds to *AtHB15*) mRNA accumulates in procambial and xylem precursor cells. *ZeHB10* (which corresponds to *AtHB8*) is expressed highly in xylem precursor cells and developing TEs. mRNA for *ZeHB12* and *ZeHB11* (both corresponding to *A. thaliana REV*) is abundant in xylem precursor cells and developing xylem parenchyma cells[61,62]. This cellular localization is consistent with results from the following functional analysis. Loss-of-function mutations at the *REV* locus cause a reduction in the number of xylem cells, in particular INTERFASCICULAR fibre cells[63–65]. By contrast, overproduction of *AtHB8* under the control of a promoter that directs ubiquitous gene expression results in the formation of excess xylem, including TEs and interfascicular fibre cells[66]. So the differential expression patterns of different HD-ZIP-III proteins, together with the finding that these HD-ZIP-III proteins can form both homo- and heterodimers[62], results in various combinations of HD-ZIP-III proteins from the central to the adaxial region that might initiate or promote specific stages of xylem differentiation to produce a radial pattern of xylem tissues.

PERICYCLE
The tissue that is composed of one or a few layers of cells that are located outside of the vascular tissues in roots and stems. In roots, lateral roots originate from this tissue.

INTERFASCICULAR
'Between the bundles'; the bundles being the strands that contain the vascular tissue.

The HD-ZIP-III homeobox proteins have a predicted sterol/lipid-binding domain known as START. Many mutations in the START domain of *PHB*, *PHV* and *REV* are dominant and cause gain-of-function phenotypes[40,42]. These phenotypes are the result of an alteration of RNA sequences with no consequent alteration of amino-acid sequences in the START domain[42] — the stability of the transcripts is elevated in the gain-of-function mutants[40]. Bartel and others showed the existence of two microRNAs — *MIR165* and *MIR166* — that overlap with a region of the START domain in which mutations in *PHB*, *PHV* and *REV* occurred[67–69]. These results strongly indicate that the instability of *PHB*, *PHV* and *REV* transcripts is regulated by an RNA interference (RNAi) mechanism through *MIR165* or *MIR166*. Interestingly, the START domains of *ZeHB13* and *AtHB15*, which are expressed at the earliest stage of xylem differentiation, are complementary to *MIR166*, and those of the other *Z. elegans* and *A. thaliana HD-ZIP-III* genes were complementary to *MIR165* (REF. 61). Therefore, *MIR166* might function as a negative regulator of *ZeHB13* and *AtHB15* and restrict the distribution of its transcript within the procambium, whereas *MIR165* might limit the distribution of the other *HD-ZIP-III* transcripts in xylem tissues. Another possibility is that a combination of *MIR165* and *MIR166* suppresses the expression of all the *HD-ZIP-III* genes in tissues other than xylem, preventing them from differentiating to xylem. *A. thaliana* mutants that are defective in *ARGONAUTE1*, the gene product of which is a component of the RNAi machinery and functions in gene silencing, show pleiotropic defects in plant architecture, including reduced vascular formation[70]. A homologue of *ARGONAUTE1*, *PINHEAD/ZWILLE*, is expressed strongly in vascular tissues as well as the adaxial side of lateral organs[71]. These findings also support the idea that the RNAi machinery functions in the spatial pattern formation of vascular cells.

Brassinosteroids. BRASSINOSTEROID (BR)-deficient mutants that have defects in different enzymes of the BR biosynthetic pathway show common phenotypes including dwarf stature, altered photomorphogenesis and abnormal vascular patterning, with increased amounts of phloem and decreased amounts of xylem[72,73]. The application of brassinazole — a specific inhibitor of BR biosynthesis[74] — to cress seedlings causes excess formation of phloem and reduced formation of xylem[75]. These findings strongly indicate that endogenously biosynthesized BRs promote xylem formation and suppress phloem formation. But when are BRs synthesized and when do they function during xylem differentiation? The answers to these questions have come from experiments with xylogenic *Z. elegans* cultures (BOX 1). Iwasaki and Shibaoka showed that an inhibitor of cytochrome P450 enzymes inhibited xylem cell differentiation in cultured *Z. elegans* cells and that an active BR — brassinolide — overcame this inhibition[76]. Because such BR-biosynthesis inhibitors do not suppress gene expression during stages 1 and 2 of xylem cell differentiation, but suppress the expression of most genes that function specifically in stage 3, endogenous BRs might be necessary for the transition from stage 2 to stage 3. Indeed, a drastic increase in BR biosynthesis occurs at stage 2 when procambium-like cells are produced by differentiation[77,78]. Therefore, BRs that are biosynthesized in procambium-like cells during stage 2 might initiate the progression to stage 3 (BOX 1).

HD-ZIP-III genes might be crucial BR-regulated genes in vascular cells. In *Z. elegans* xylem cell differentiation, BR depletion severely suppresses the expression of *ZeHB10*, *ZeHB11* and *ZeHB12* in xylem precursor cells and differentiating xylem cells, but does not greatly suppress the expression of *ZeHB13* in procambial cells and xylem precursor cells[61,62]. The three genes are induced within 1 hour by brassinolide in *Z. elegans* stage-2 cells, indicating that these genes respond rapidly to BRs[61]. Therefore, BRs might regulate differentiation from procambium to xylem through the expression of specific members of the HD-ZIP-III family (FIG. 3). Interestingly, a considerable amount of BR is secreted from the cell[77], which is consistent with the fact that the perception of BR occurs on the plasma membrane by an extracellular ligand-binding domain of BR receptors, as discussed below[79].

How do BRs regulate vascular formation? BRI1 (brassinosteroid-insensitive-1) is a membrane-associated, LRR receptor serine/threonine kinase that transduces the BR signal[80]. Because BRI1 is ubiquitously expressed in plants[81] and *bri1* loss-of-function mutants show similar phenotypes to BR-deficient mutants[82], BRI1 is thought to function ubiquitously in BR-signal perception. Recently, BRI1-associated receptor kinase-1 (BAK1), an *A. thaliana* LRR receptor-like protein kinase, was shown to interact with BRI1 and modulate the BR signaling[83,84]. This protein is also expressed ubiquitously. These findings seem to imply that the BR-perception system itself is not involved in differentiation of specific tissues. However, there are other genes with high sequence homology to *BRI1* and *BAK1* in *A. thaliana*[85]. Of these, three genes — *BRL1 (BRI1-like protein-1)*, *BRL2/VH1 (vascular highway-1)* and *BRL3* — contain a sequence that encodes an LRR (which serves as a ligand-binding site) that is homologous to that of *BRI1*. Because both BRL1 and BRL3, but not BRL2, can bind brassinolide[85] and, in fact, rescue the *bri1* mutant phenotype when driven by the *BRI1* promoter (J. Li, personal communication), at least BRL1 and BRL3 seem to function as BR receptors. Interestingly, all three genes are preferentially expressed in vascular tissues[86] (J. Li, personal communication). These results strongly point to the existence of a vascular-cell-specific BR-perception system. After plasma-membrane perception of BRs, the signalling pathway begins to resemble elements of the Wingless/WNT pathway[87]. The BR signal inactivates the negative regulator BIN2 (brassinosteroid insensitive-2), a glycogen-synthase kinase-3 (GSK3)/shaggy-kinase homologue, which allows the unphosphorylated form of *bri1*-EMS-suppressor-1 (BES1) and brassinazole-resistant-1 (BZR1) to translocate to the nucleus and to promote BR-regulated gene expression (FIG. 3).

In addition to BRs, other sterols might function in vascular development. A discontinuous venation pattern

microRNA
The approximately 21–22-ribonucleotide RNA that arises from the action of the Dicer double-stranded ribonucleases on short stem-loop precursors. It initiates blocking of the targeted mRNAs, which have nucleotide sequences that are complementary to the microRNA.

BRASSINOSTEROID
A group of naturally occurring plant polyhydroxysteroids with wide-ranging biological activity.

is observed in several biosynthetic mutants that affect both sterols and BRs, including *orc* (which encodes sterol methyltransferase-1)[88], *fackel* (which encodes C-14 sterol reductase)[89] and *cvp1* (which encodes sterol methyltransferase-2)[90]. In contrast to mutants that are defective in the BR-specific biosynthetic pathway, the wild-type phenotype cannot be restored by supplementing these mutants with BRs. In *orc*, the membrane localization of the auxin-efflux carrier proteins AtPIN1 and AtPIN3 is disturbed, whereas polar positioning of the influx carrier AUX1 is normal[88]. These results imply that balanced sterol composition or the shortage or overproduction of an unknown specific sterol(s) might have a role in vascular continuity through the establishment of auxin efflux.

Xylogen. Motose *et al.*[91] first showed the involvement of local intercellular communication during TE differentiation using gel-embedding culture methods, in which *Z. elegans* MESOPHYLL cells embedded in a thin sheet of agarose gel were cultured on solid medium (thin-sheet culture), or those in microbeads of agarose gel were cultured in liquid medium (microbead culture). Statistical analysis of the two-dimensional distribution of TEs in thin-sheet culture indicated that TEs were not randomly distributed but, rather, were aggregated. This result indicates that local intercellular communication has a positive regulatory role in TE differentiation from *Z. elegans* mesophyll cells. The characterization of a putative factor that mediates local intercellular communication, using a bioassay system based on microbead culture, showed that the mediator is a secretory non-classical type of ARABINOGALACTAN PROTEIN, which was called xylogen[92]. The depletion of arabinogalactan proteins from the culture medium with β-GLUCOSYL YARIV REAGENT specifically inhibits TE differentiation without affecting cell division, which is consistent with the conclusion that xylogen is an arabinogalactan protein. Xylogen activity is induced in stage 2 of *Z. elegans* cell differentiation, probably in procambial and/or xylem precursor cells, by a combination of auxin and cytokinin. The specific localization of *xylogen* mRNA in procambial and xylem precursor cells has recently been revealed (H. Motose and H. Fukuda, unpublished observations). From these findings, a positive-feedback loop is assumed in which cells are drawn into the pathway towards TE differentiation by the presence of xylogen and such cells come to produce more xylogen. Therefore, xylogen might be responsible for the continuous formation of TE strands.

Tracheary-element differentiation

The final step in vascular cell development is the specification of a vascular cell with a particular task. One of the most distinctive specification processes is the TE-differentiation process, which involves two main morphological events: patterned secondary-wall formation and programmed cell death.

Secondary-wall formation and transcriptional activation. The most striking feature of TE formation is the development of patterned secondary walls. The secondary wall is composed of cellulose microfibrils and cementing substances that contain lignin, hemicellulose, pectin and other proteins, which add strength and rigidity to the wall. The coordinated and transient synthesis of these substances has provided hints regarding the regulatory mechanism of TE differentiation. Secondary-wall formation in developing TEs requires cytoskeleton-oriented cellulose microfibril deposition, deposition of other secondary-cell-wall components along the cellulose microfibrils, degradation and modification of primary cell walls, and LIGNIFICATION[59]. The formation of patterned cellulose microfibrils is a coordinated process in which actin filaments initiate the reorganization of microtubules, which, in turn, determine the spatial disposition of cellulose microfibrils by controlling the movement of the cellulose biosynthetic complex on the plasma membrane[93]. *CESA* genes encode catalytic subunits of the plant cellulose synthase[94]. Ten CESA isoforms exist in *A. thaliana* (see The Cellulose Synthase Superfamily in the online links box). Distinct CESA isoforms participate specifically in secondary-cell-wall formation in developing TEs[95]. In *A. thaliana*, IRX1/CESA8, IRX3/CESA7 and IRX5/CESA4 interact as subunits within a cellulose complex in secondary cell walls[95,96], whereas CESA1, CESA3 and CESA6 form the complex in primary walls[97]. This difference might reflect the distinct properties of cellulose in secondary walls, which have more β-glucan chains per microfibril and a higher overall cellulose content than primary walls. During maturation of TEs, IRX1/CESA8, IRX3/CESA7 and IRX5/CESA4 move from the cytoplasm to the plasma membrane to colocalize with bands of cortical microtubules[96]. Arrays of cortical microtubules, but not those of actin filaments, are required continually to maintain normal CESA protein localization, supporting the direct involvement of cortical microtubules in patterned cellulose deposition[96].

Recent systematic gene-expression analysis with the *Z. elegans* xylogenic culture[21,98], poplar trees[99] and pine trees[100] has revealed a number of genes that are newly and coordinately expressed in association with secondary-wall formation. These include genes for specific cell-wall structural proteins, enzymes that degrade cell walls, enzymes that modify the cell-wall structure, enzymes that catalyse lignin-precursor biosynthesis, and lignin-polymerizing enzymes, as well as specific *CESA* genes. Although specific MYB and LIM TRANSCRIPTION FACTORS are known to coordinately upregulate several enzymes that are related to lignin precursor biosynthesis[101,102], the transcription mechanism that upregulates genes encoding other proteins that have various tasks in secondary-wall formation remains to be elucidated. Because almost all TE-specific genes are suppressed by depletion of endogenous BRs in the *Z. elegans* culture system, profiling of genes that are induced immediately by BR treatment in developing xylem cells might provide an important insight into the coordinate induction of TE-specific genes.

Secondary-wall formation in TEs is tightly coupled with the degradation of primary walls, which is

MESOPHYLL
The photosynthetic tissue between the upper and lower epidermis of the leaf. Mesophyll cells contain chloroplasts.

ARABINOGALACTAN PROTEIN
A class of hydroxyproline-rich proteoglycans to which branched 3,6-β-D-galactans containing arabinose are attached by *O*-glycosidic bonds. They are widely distributed throughout the plant kingdom.

β-GLUCOSYL YARIV REAGENT
A synthetic phenyl glycoside that interacts selectively with arabinogalactan proteins and that is used for the purification and quantification of arabinogalactan proteins and for disturbing arabinogalactan protein function.

LIGNIFICATION
The deposition of lignin on secondary cell walls of tracheary elements and fibre cells to reinforce them mechanically and chemically.

LIM TRANSCRIPTION FACTOR
A member of the transcription-factor family that contains the LIM domain, which is a cysteine-rich polypeptide composed of two special zinc fingers separated by a two-amino-acid spacer.

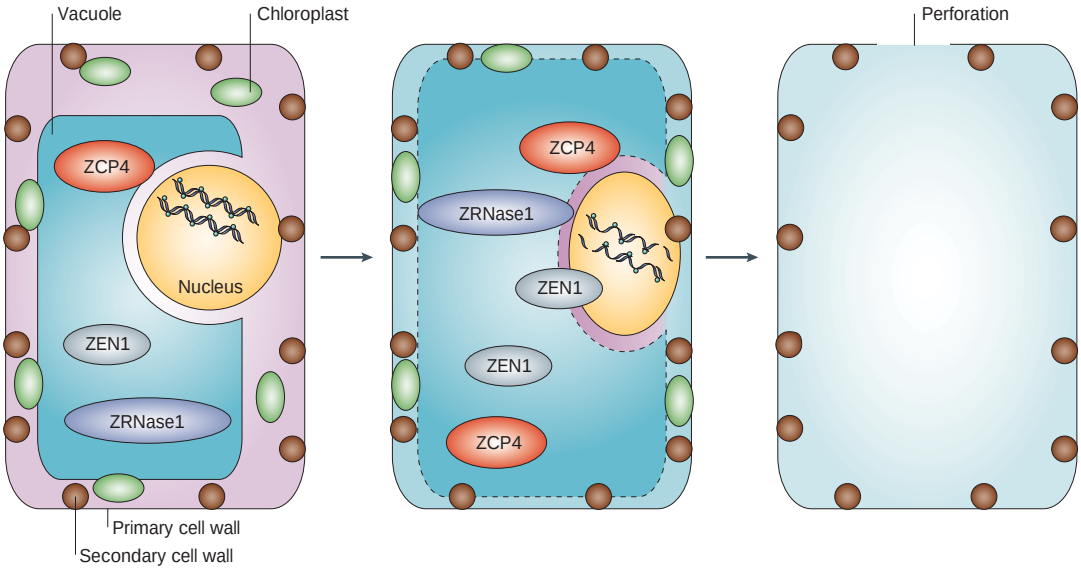


Figure 5 | **Programmed cell death during tracheary-element differentiation.** Brassinosteroids (BRs) induce tracheary element (TE) differentiation through the expression of genes that are related to secondary-cell-wall formation and programmed cell death (PCD). In developing TEs, PCD-specific hydrolytic enzymes — such as an S1-nuclease (ZEN1), RNases (ZRNase1) and cysteine proteases (ZCP4) — are newly synthesized and accumulate in the vacuole. The vacuole enlarges and the enlarged vacuole then bursts, shrinks and fragments. Vacuole collapse causes the insulated hydrolytic enzymes to be released into the cytoplasm and to attack various organelles, resulting in autolysis of the cell contents and part of the cell walls. Finally, perforation of the wall leads to the loss of all cell contents from TEs and the formation of mature hollow tubes that are reinforced by secondary walls. It takes about 6 hours to lose all the cell contents after the formation of visible secondary-wall thickenings and only 15 minutes to lose nuclear DNA after vacuole collapse.

accompanied by new and specific expression of many cell-wall-degrading enzymes in developing TEs[21,103]. Moreover, localized degradation and modification of walls in TEs require intracellular positional information. The MIDDLE LAMELLA is resistant to autolysis when it is between neighbouring cells and when only one cell will differentiate into a TE, although the middle lamella between two neighbouring TEs is digested completely[104]. In a continuously formed vessel strand, a differentiating TE opens pores at one longitudinal end adjacent to a mature dead TE but not to an immature TE. Even in single TEs that are formed *in vitro*, the perforation is restricted to one longitudinal end[105]. These findings indicate the existence of anisotrophy (polarity), which might occur autonomously, without cell–cell interaction, on cell walls of developing TEs. Because monoclonal antibodies against TE-specific cell-wall components recognize cell walls only at the tip of TE-precursor cells[106], cell polarization of TEs must occur at an early stage of TE differentiation. Transcripts for a Rac-like small G protein are restricted to the site that faces developing TEs in TE precursor cells and xylem parenchyma cells *in situ*[107]. Similarly polarized intracellular mRNA localization in procambial cells has been reported for genes that encode expansins — cell-wall proteins that promote cell expansion[108]. Therefore, it is likely that polarized intracellular organization directing polarized cell-wall patterning occurs in the early development of xylem cells.

MIDDLE LAMELLA
The thin layer that connects two plant cells and is rich in pectin.

Programmed cell death. TE differentiation culminates with the loss of cell contents, leaving behind a functional cellular corpse. This cell-death process has been studied extensively in the *Z. elegans* culture system and described in detail in recent reviews[109,110]. Here, the essence of the TE cell-death programme will be described by focusing on the plant-specific idiosyncrasies of programmed cell death (PCD).

FIGURE 5 illustrates the process of TE PCD. As TE PCD occurs autonomously and does not require input from other cells, TEs themselves must therefore provide all the components that are necessary for the execution of cell death. The cell-death programme in TEs is tightly coupled with secondary-wall formation. So far, no agent or mutation has been found that prevents the cell-death programme without affecting secondary-wall formation, or vice versa. A crucial step in TE PCD is the transcriptional regulation of TE-PCD-specific genes and, indeed, a number of PCD-specific genes are expressed at the same time[21]. BRs are some of the earliest signalling molecules that direct the cell-death programme, as well as secondary-wall formation, by upregulating the expression of specific genes[78]. Proteins that are encoded by PCD-specific genes include hydrolytic enzymes that function optimally at acidic pHs, or are predicted to do so, such as cysteine proteases, serine proteases, RNases, S1-type nucleases, acid phosphatases and lipases[21,110]. Most of these newly synthesized enzymes are transported to the vacuole where they are activated[111]. This means that in developing TEs, the vacuole changes into

TONOPLAST
The membrane that encompasses the central vacuole.

ENDOCARP CELL
The cell that composes the innermost fruit wall. Some endocarp cells are destined to die during maturation of the fruit.

ALEURONE CELL
The layered cell that surrounds the starchy endosperm of cereal grains and is instrumental in digesting the materials that are stored in the endosperm.

a highly lytic vacuole that is similar to animal lysosomes. Indeed, cell extracts from developing TEs, but not from non-TE cells, can degrade nuclear DNA[112].

The autolysis of TEs starts with the rupture of the vacuole[113–115]. The degradation of nuclear and chloroplast DNA is triggered by vacuole rupture and is completed within only 15 minutes of vacuole collapse, although chlorophyll is degraded much more slowly[115]. For nuclear-DNA degradation, the S1-type Zn^{2+}-dependent nuclease ZEN1 accumulates in an active form in the vacuole, where it has a pivotal role[112]. Although Kuriyama[114] proposed that a change in the organic-anion permeability of the TONOPLAST initiates vacuole collapse in TEs *in vivo*, the actual molecular mechanism for vacuole collapse is not yet known. Vacuole-executed PCD might be of widespread occurrence in plants and is seen, for example, in the death of ENDOCARP CELLS during ovary senescence of pea plants[116] and the ALEURONE-CELL-death process[117]. Although similar hydrolytic enzymes are commonly expressed during vacuole-induced cell death, some genes, such as *ZCP4* (which encodes a cysteine protease) and *ZEN1*, seem to be expressed specifically during TE PCD[115]. Therefore, there must be some minor variations in the vacuole-mediated cell-death programme in plants. At the final stage of TE maturation, the digested cell contents — containing proteases and nucleases — are released into the extracellular space, usually into a neighbouring hollow TE.

Conclusions and perspectives

Recent advances using *A. thaliana* mutants that have defects in vascular development and cellular studies with *Z. elegans* xylogenic cultures have provided insights into the formation of the three-dimensional plant vascular system at the cellular level. A picture has now emerged of the molecular organization of plant vascular cell development. Crosstalk among plant hormones, which function as intercellular signals, and their endogenous biosynthesis in distinct vascular cells are involved in the activity of procambial cells and the differentiation from procambial cells into various vascular cells with distinct functions. Downstream of such intercellular signals, new regulatory factors such as HD-ZIP-III homeobox proteins and microRNAs function during the differentiation of procambial cells to xylem cells. The detailed analysis of the process of vascular cell differentiation has also uncovered some unique features of plant cell differentiation, including the cell-death programme.

It is important to note, however, that we still know only a little about procambial cells. For example, in *A. thaliana* root tips, xylem cell poles start from procambial cells next to the quiescent centre. However, there are a few dozen procambial cells between the quiescent centre and developing TEs along the pole. We do not know what makes these procambial cells different in nature, although it has been reported that a homeobox gene, *Oshox1*, might be a molecular marker for distinguishing developmental stages of procambial cells in rice roots[118]. Therefore, our next target should be to dissect the procambial and xylem precursor stages into smaller steps on the basis of their cellular and molecular functions, which will allow us to identify new inter- and intracellular signals that initiate each step. Another important issue for the future is the establishment and maintenance of the polarity of vascular cells, which controls the formation of continuous columns of vascular cells. To understand the molecular mechanism of vascular cell polarity, we have to identify the asymmetrical intracellular-signalling pathways that establish this polarity.

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The author declares that he has no competing financial interests.

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