Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Plant Biology

Cell-to-cell communication in vascular morphogenesis

Satu J Lehesranta, Raffael Lichtenberger and Ykä Helariutta

The plant vascular system consists of two conductive cell types, xylem and phloem, which are both produced by procambial cells. Recently, several novel regulatory mechanisms that control the specification of vascular patterning and differentiation have been uncovered. The non-cell-autonomous TDIF/CLE signalling mediates phloem–xylem cross-talk and cambial maintenance; a flowering-related long-distance signal governs secondary development; and novel genetic players such as *LHW* regulate vascular morphogenesis. A future challenge is to conflate data on the various genetic, hormonal and other factors to understand the networks underlying vascular tissue formation.

Address
Department of Biological and Environmental Sciences/Institute of Biotechnology, University of Helsinki, P.O. Box 65, 00014 Helsinki, Finland

Corresponding author: Helariutta, Ykä (yrjo.helariutta@helsinki.fi)

Current Opinion in Plant Biology 2010, **13**:59–65

This review comes from a themed issue on
Growth and Development
Edited by Dominique C. Bergmann and Andrew J. Fleming

Available online 25th September 2009

1369-5266/$ – see front matter

DOI 10.1016/j.pbi.2009.09.004

Introduction

The plant vascular tissues form bundles that connect all parts of the plant and serve two important functions: they give mechanical support, and distribute water and nutrients as well as other substances needed for growth and defence. Individual vascular bundles consist of two differentiated, highly specialised conductive tissue types, xylem and phloem, and intervening procambial cells of pluripotent nature that can differentiate into both conductive cell types (Figure 1). Developmental decisions require precise spatial and temporal organisation to produce continuous vascular strands.

Novel genomic and molecular techniques using several model plant systems (such as *Arabidopsis*, *Populus* and *Zinnia*) have recently provided insights into the regulation of vascular development. Signals controlling aspects of vascular morphogenesis include hormones (auxin, cytokinins and brassinosteroids), other small regulatory molecules, their transporters, receptors, and various temporal and spatial regulators of gene expression. Several recent studies suggest that the control of xylem and phloem development is highly integrated. Many of the regulatory factors have been shown to act on cell autonomously; however, an emerging theme in these processes is the movement of signals controlling vascular development and cross-talk between cell types and tissues.

This review will focus on the emerging concepts of how these components and long-range and short-range signals might function to set up a regulatory network governing vascular development.

Formation of vascular strands

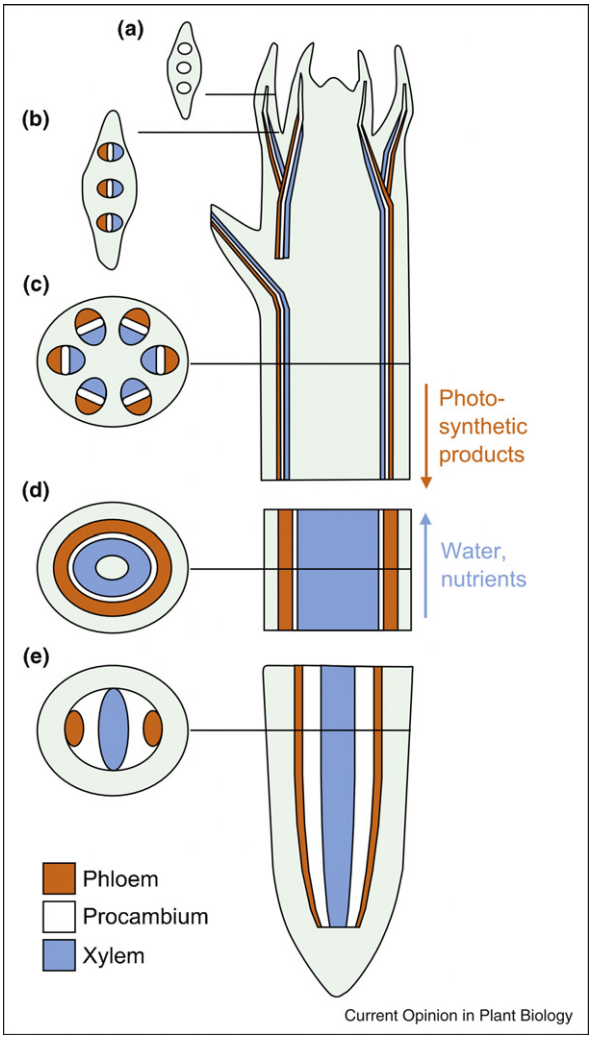
Continuous procambial strands need to be established before vasculature differentiates. This has long been thought to be caused by the accumulation of the phytohormone auxin through polar transport mechanisms [1,2]. Indeed, expression of the synthetic auxin reporter DR5 and the auxin-induced pre-procambial marker *AtHB8* precedes formation of vascular strands in leaves [3]. To achieve this, auxin is channelled to the provascular regions by the auxin transporter PIN1, which is already expressed before procambium formation (Figure 2a).

Radial patterning and maintenance of proliferation

Besides the formation of vascular strands, auxin may also play a role in radial patterning of vascular bundles, such as the differentiation of adaxial and abaxial sides of the bundle. The radial pattern is initially set up during embryogenesis by the main agents of radial patterning, *KANADI* (*KAN*) and class III *HD-ZIP* (*PHABULOSA* [*PHB*], *PHAVOLUTA* [*PHV*], *REVOLUTA* [*REV*] and *CORONA* [*CNA*]) transcription factors [4–6]. The *KAN* signalling and auxin transport pathways appear to integrate, as PIN1 localisation is affected in *kan* mutants [7]. Conversely, auxin is likely to influence the spatial and temporal expression patterns of these transcription factors. The triple mutant *phb phv rev* has radialised, abaxialised leaves and vascular bundles [8] (Figure 2b). In contrast, gain-of-function *HD-ZIP* III mutants with faulty microRNA (miRNA) regulation and *kan1 kan2 kan3* have radialised, adaxialised leaves and bundles. In addition, the *HD-ZIP* III genes also appear to regulate vascular tissue proliferation [9].

Another important hormone, cytokinin (CK), is required for vascular patterning and the differentiation of all cell except protoxylem. Mutants with impaired CK signalling, such as *wooden leg* (*wol*) and the triple CK receptor mutant

Figure 1



A schematic outline showing the basic vascular patterning. **(a)** Cross-section of a developing leaf. Vascular bundles develop as pre-procambial strands. **(b)** Procambial cells give rise to phloem (abaxial side of the leaf) and xylem (adaxial side) that differentiate asymmetrically within the vascular strands. **(c)** Arrangement of vascular strands within the stem. **(d)** During the secondary phase of vascular development in the stem and root, cambial cells form a cylindrical meristem that produces secondary xylem and phloem. **(e)** Cross-section of a root tip shows the structure of primary vascular tissue within the stele.

ahk2 ahk3 ahk4 (for *ARABIDOPSIS HISTIDINE KINASE*), or with depleted levels of CK, such as transgenic lines overexpressing *CYTOKININ OXIDASE* (*CKX*), all lead to an increased number of protoxylem cell files and loss of other cell types in the root vasculature [10,11]. The CK signalling inhibitor *ARABIDOPSIS HISTIDINE PHOSPHOTRANSFER PROTEIN 6* (*AHP6*) restricts the domain of CK activity and thereby allows for protoxylem differentiation in a spatially specific manner. CK also appears to be important for the maintenance and proliferation of cambial cells [12,13] (Figure 2c).

Having fewer cells sometimes leads to altered cell type ratios, sometimes not; this relationship is not currently understood. Recently, the bHLH-domain-containing protein *LONESOME HIGHWAY* (*LHW*) has been identified as a key factor in establishing bilateral symmetry [14]. *LHW* is required to promote proliferation of cell files in the stele and to maintain longitudinal growth. Whilst *lhw* mutants still show distinct cell types, they have fewer cells in the centre of the root. This leads to roots with only single xylem and phloem poles. It remains to be seen whether it relates to CK signalling or other known mechanisms.

Also, the brassinosteroid (BR) class of plant hormones are involved in long-distance signalling to establish the vasculature [15]. Mutations in the BR receptor *BR-INSENSITIVE 1* (*BRI1*) cause an abnormal ratio in the differentiation of phloem to xylem cells, with a disproportionately high number of phloem cells.

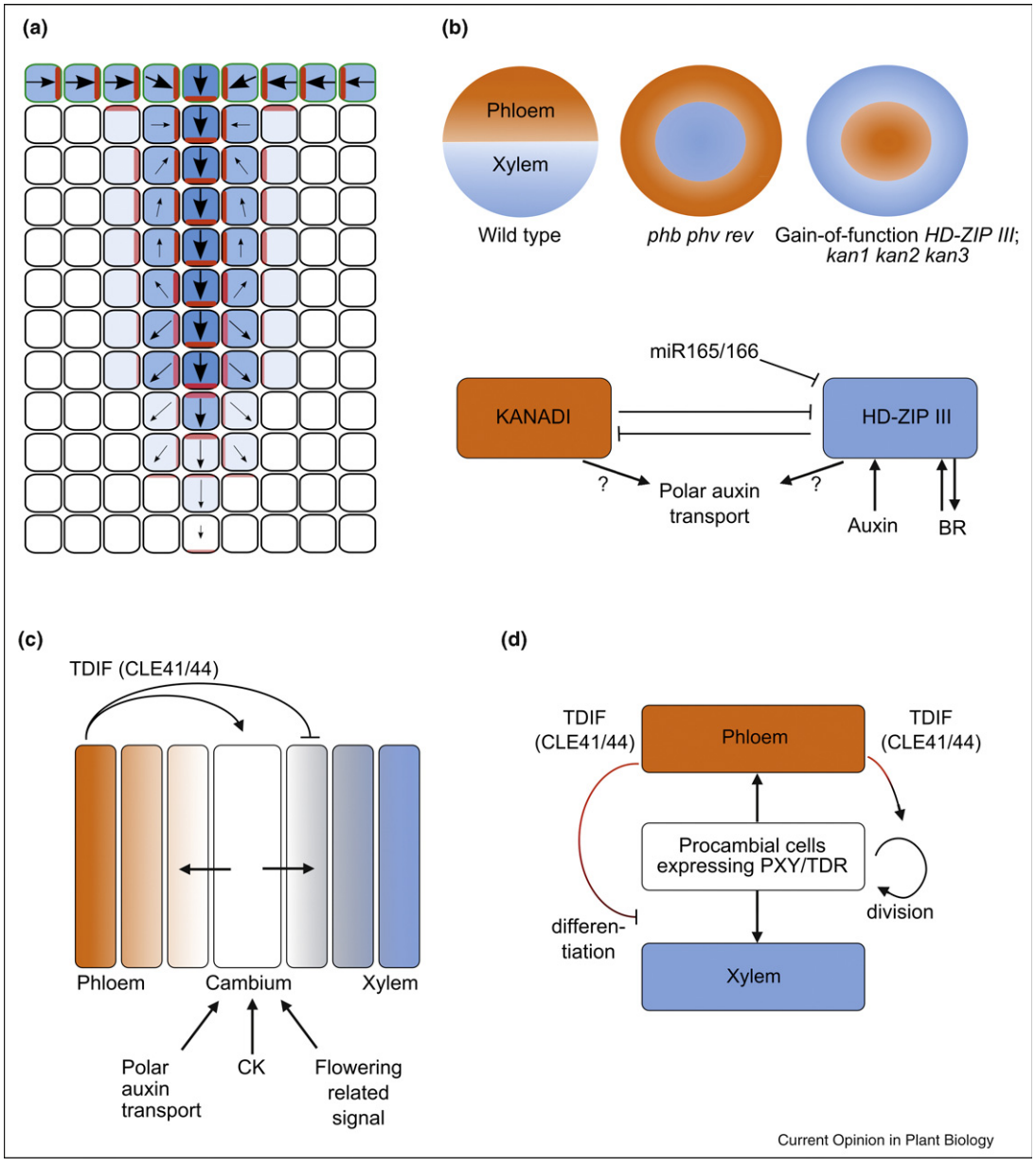
Differentiation of vascular cells

Besides *AHP6* which promotes protoxylem identity [11], several genes have been described to be specifically involved in specifying xylem or phloem identity. These genes primarily encode transcription factors, but putative signalling proteins and cell death regulators also play a role. It is currently not known what regulates the specific expression of these genes.

Fukuda [16] has previously proposed a temporal model where the balance of auxin and cytokinin is important to specify procambial cells that will adopt xylem fate. Cytokinin signalling must be counteracted early by *AHP6* to allow for protoxylem differentiation [11]. Downstream of these factors, the differentiation of xylem cells is conferred by the action of a transcriptional network, the first members of which were described as the *VASCULAR-RELATED NAC-DOMAIN* (*VND*) transcription factors *VND6* and *VND7* [17]. VND6 promotes metaxylem identity, whereas VND7 promotes protoxylem identity. Our understanding of the xylem transcriptional network has been expanded to include a cascade involving several MYB and NAC transcription factors [18•,19•]. Furthermore, the spermine biosynthesis gene *ACAULIS5* (*ACL5*) controls xylem specification. In *acl5* mutant, fibre elements and metaxylem vessel elements fail to differentiate [20•]. This is due to premature cell death, which is normally inhibited by ACL5. Vascular continuity is mediated by xylogen, a small proteoglycan-like protein that is secreted by immature tracheary elements [21]. Mutants lacking xylogen fail to form continuous xylem strands.

The only currently known factor for phloem specification is *ALTERED PHLOEM DEVELOPMENT* (*APL*) [22]. This MYB-coiled-coil transcription factor is essential for the proper differentiation of companion cells and

Figure 2



Signalling and genetic components during different stages of vascular tissue development. **(a)** Model for polar auxin transport visualises vascular bundle formation in the leaf midvein (adapted from [28•]). First, polar localisation of PIN1 (red) directs auxin flow (arrows; auxin concentration shown in blue) in the surface layer of the meristem (green cells) to form an auxin maximum. Subsequently, auxin flux is directed from this maximum towards inner tissue. A procambial strand forms at the site of the auxin flux. **(b)** The *HD-ZIP III* and *KANADI* genes are important regulators of vascular patterning and have an antagonistic relationship. In shoot vascular bundles, they determine the adaxial and abaxial localisation of xylem and phloem, respectively. In *phb phv rev* triple mutants, phloem surrounds xylem, and in gain-of-function *HD-ZIP III* mutants or *kan1 kan2 kan3* triple mutants, xylem surrounds phloem. *HD-ZIP III* genes are regulated by miR165/166 and apparently interact with polar auxin transport and brassinosteroids. **(c)** Cambium, the vascular meristem, produces both xylem and phloem cells. Polar auxin transport and cytokinins have been suggested to regulate cambial activity. A currently unknown flowering-related signal from the shoot is required for secondary cambial activity. **(d)** A model according to Hirakawa *et al*. [37••] suggests that a CLE receptor/ligand system maintains cambial cell activity in a non-cell-autonomous manner. The TDIF/CLE41/44 peptide is secreted from the phloem and perceived by the receptor PXY/TDR in procambial cells. This signal promotes proliferation of cambium and inhibits its differentiation into xylem.

sieve elements, and contributes to spatially limiting the differentiation of xylem.

Long-range and short-range signalling molecules are involved in vascular development

Results from several recent studies suggest that the control of differentiation of xylem and phloem requires short-range and long-range intercellular transport of signalling molecules.

Auxin has been shown to regulate a number of key developmental events such as organisation of meristems. This is possible because short-range morphogenic auxin gradients are regulated by intricate mechanisms to provide positional information; for an in-depth update on polar auxin transport, we refer to recent reviews [23,24]. The asymmetric localisation of PIN proteins directs auxin within tissues [2,25,26]. This polarity and the fact that auxin can redirect PIN1 transporter in pre-procambial cells explain how auxin flow can channel and amplify itself in a self-organising process to establish a new auxin maximum [3,27] (Figure 2a). Computer simulations give further support to this canalisation hypothesis during leaf midvein formation [28•]. There is also evidence for the long-range transport of auxin in the phloem from source to sink tissues [29–31]. Mechanisms for short-range or long-range transport of other hormones have not yet been described. Cytokinin probably acts both as a local and a long-distance signal and may be transported in the vasculature — by both phloem and xylem — but the mechanisms involved are so far poorly characterised [32].

Short-range signalling by TDIF/CLE41/44 and PXY mediates phloem–xylem cross-talk

The control of stem cell maintenance in the shoot apical meristem of Arabidopsis involves an interaction between the CLV3 peptide and receptor components CLV1 and CLV2. The CLV pathway induces downstream components that limit the expression domain of the WOX family homeodomain transcription factor WUSCHEL; WUS acts non-cell-autonomously to maintain stem cell identity and promote CLV3 expression [33]. Another feedback regulon maintains the root apical meristem and involves the peptide CLE40 and the WUS-related WOX5 [34•,35].

A similar cross-talk model between phloem and xylem has been suggested as the regulator for vascular patterning. The CLE peptide TDIF was originally identified in the *Zinnia* cell culture system and shown to be a dodecapeptide that could suppress the differentiation of cultured cells to tracheary elements at very low levels but did not inhibit meristem function in plants [36]. TDIF inhibits xylem differentiation and increases proliferation of procambial cells in leaves *in planta* [37••]. These results suggested that a CLV-like signalling mechanism operates in the vasculature. More evidence for this kind of regulon came from the identification of PXY, a CLV-like receptor kinase which is required for normal cambial cell divisions and spatial organisation of xylem and phloem in *Arabidopsis* [38••]. In *pxy* mutants (for *phloem intercalated with xylem*), the organisation of vascular bundles in stems is severely disrupted in both transverse and longitudinal directions: cell divisions occur irregularly, and xylem and phloem are no longer neatly separated. The model for the functions of these two genes came together when TDIF/CLE41/44/42 was shown to act as ligand for PXY, also named TDR for putative TDIF receptor [37••]. The direct binding of TDIF was demonstrated by a biochemical approach, where photoaffinity labelling was used on recombinant TDR produced in tobacco cells. *CLE41/44* encoded peptides are secreted from the phloem and bind their receptor in the cambium (Figure 2d). Combined, these data strongly suggest that the CLE receptor/ligand system maintains cambial stem cell activity and regulates its differentiation into xylem. Whether there is a downstream factor similar to WUS that would constitute a complete regulatory loop remains to be seen.

miRNAs on the move?

Small RNAs can influence many aspects of gene regulation. Whilst the well-characterised classes of small interfering RNAs (siRNAs) act mainly in defence, miRNAs and trans-acting RNAs have important functions in growth and development by regulating endogenous target genes. For instance, miR165/166 has been shown to regulate *HD-ZIP III* patterning [39,40] (Figure 2b). Is it possible that these miRNAs would provide positional information by moving short distances? There is plenty of evidence of short-range and systemic movement of siRNAs [41] but miRNAs have been considered to function cell autonomously [42]. Various miRNAs, including miR165/166, are present in phloem [43•], although it is debatable whether they could act as long-range developmental signals [44]. It has been suggested that short-range miRNA movement could have important developmental consequences [45•] but direct functional evidence for miRNA movement remains to be demonstrated.

Flowering triggers secondary development

An interesting concept for the long-range control of vascular patterning comes from work on natural variation. There is a transition of secondary growth in Arabidopsis hypocotyl and roots that leads to expansion of xylem and fibre differentiation, reminiscent of trunk formation in trees [46••]. Analyses of quantitative trait loci analyses revealed *FLOWERING LOCUS C* as the major locus controlling this transition, suggesting that flowering

induction is required for the onset of xylem expansion (Figure 2c). This implicates a flowering-related signal from the shoot but it is not currently known what this signal might be.

How do molecules move between cells?

Xylem and phloem are the primary conduits of long-distance transport within the plant. Furthermore, short-range non-cell-autonomous developmental signals such as transcription factor movement are well established in plants. Plasmodesmata are probably the main mediators for symplastic transport of non-cell-autonomous transcription factors and small RNAs [47] and especially important for phloem as they regulate the loading of molecules into the phloem stream (reviewed in [48]). Phloem sieve elements are connected to each other by sieve plate pores, plasmodesmatal complexes, the functions of which are currently poorly known. Furthermore, plasmodesmata can fine-tune cell-to-cell trafficking by setting size exclusion limits [49]. Because of this complexity and subtle regulation, novel mutants with defects in plasmodesmata may shed light on their specific roles in phloem continuity, differentiation and/or transport function. Few have so far been described, but recently, a family of novel plasmodesmata-located proteins that affect cell-to-cell communication has been identified [50•]. Another mutant with increased callose deposition and decreased plasmodesmal transport has severe defects in development and phloem unloading [51•].

Protein movement may in some cases also involve targeted regulated transport. SHORT-ROOT (SHR), an important transcription factor for ground tissue patterning, is also implicated in vascular development [52]. SHR is expressed in vascular tissues in the root but moves into the endodermis, where it promotes endodermal cell identity. SHR requires both cytoplasmic and nuclear localisation in order to be transported in cell-specific manner, and therefore this is unlikely to occur simply through diffusion [53•]. The TDIF/CLE41/42/44 peptide is likely to be secreted and distributed in the apoplast [37••].

Conclusions

Many different factors involved in vascular development have been described, but so far there has been relatively little knowledge on how they interact and regulate each other and how positional information is provided. Morphogenesis requires the integration of a multitude of signals and gene functions coming from outside and within the vascular system. Recently, novel concepts of signalling between cells and tissues such as the TDIF/CLE regulon mediating phloem–xylem cross-talk and miRNA movement have been proposed. In the future, it will be of great interest to see how these signalling networks fit in with other known players and how cell-to-cell communication occurs to produce this complex system.

Acknowledgements

We would like to thank Dr Anthony Bishopp for a critical reading of the manuscript. Financial support was provided the Academy of Finland, Tekes and the European Science Foundation.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Sachs T: **The control of the patterned differentiation of vascular tissues**. *Adv Bot Res* 1981, **9**:151-262.
2. Friml J, Yang X: **A PINOID-dependent binary switch in apical–basal PIN polar targeting directs auxin efflux**. *Science* 2004, **306**:862-865.
3. Scarpella E, Marcos D, Friml J, Berleth T: **Control of leaf vascular patterning by polar auxin transport**. *Genes Dev* 2006, **20**:1015-1027.
4. McConnell JR, Barton MK: **Leaf polarity and meristem formation in Arabidopsis**. *Development* 1998, **125**:2935-2942.
5. McConnell JR, Emery J, Eshed Y, Bao N, Bowman J, Barton MK: **Role of *PHABULOSA* and *PHAVOLUTA* in determining radial patterning in shoots**. *Nature* 2001, **411**:709-713.
6. Kerstetter RA, Bollman K, Taylor RA, Bomblies K, Poethig RS: ***KANADI* regulates organ polarity in Arabidopsis**. *Nature* 2001, **411**:706-709.
7. Izhaki A, Bowman JL: **KANADI and class III HD-Zip gene families regulate embryo patterning and modulate auxin flow during embryogenesis in *Arabidopsis***. *Plant Cell* 2007, **19**:495-508.
8. Emery JF, Floyd SK, Alvarez J, Eshed Y, Hawker NP, Izhaki A, Baum SF, Bowman JL: **Radial patterning of Arabidopsis shoots by class III HD-ZIP and KANADI genes**. *Curr Biol* 2003, **13**:1768-1774.
9. Prigge MJ, Otsuga D, Alonso JM, Ecker JR, Drews GN, Clark SE: **Class III homeodomain-leucine zipper gene family members have overlapping, antagonistic, and distinct roles in Arabidopsis development**. *Plant Cell* 2005, **17**:61-76.
10. Mähönen AP, Bonke M, Kauppinen L, Riikonen M, Benfey N, Helariutta Y: **A novel two-component hybrid molecule regulates vascular morphogenesis of the *Arabidopsis* root**. *Genes Dev* 2000, **14**:2938-2943.
11. Mähönen AP, Bishopp A, Higuchi M, Nieminen KM, Kinoshita K, Törmäkangas K, Ikeda Y, Oka A, Kakimoto T, Helariutta Y: **Cytokinin signaling and its inhibitor AHP6 regulate cell fate during vascular development**. *Science* 2006, **311**:94-98.
12. Matsumoto-Kitano M, Kusumoto T, Tarkowski P, Kinoshita-Tsujimura K, Václavíková K, Miyawaki K, Kakimoto T: **Cytokinins are central regulators of cambial activity**. *Proc Natl Acad Sci U S A* 2008, **105**:20027-20031.
13. Nieminen K, Immanen J, Laxell M, Kauppinen L, Tarkowski P, Dolezal K, Tähtiharju S, Elo A, Decourteix M, Ljung K *et al.*: **Cytokinin signaling regulates cambial development in poplar**. *Proc Natl Acad Sci U S A* 2008, **105**:20032-20037.
14. Ohashi-Ito K, Bergmann DC: **Regulation of the *Arabidopsis* root vascular initial population by *LONESOME HIGHWAY***. *Development* 2007, **134**:2959-2968.
15. Caño-Delgado A, Yin Y, Yu C, Vafeados D, Mora-Garcia S, Cheng J-C, Nam KH, Li J, Chory J: **BRL1 and BRL3 are novel brassinosteroid receptors that function in vascular differentiation in *Arabidopsis***. *Development* 2004, **131**:5341-5351.
16. Fukuda H: **Signals that control plant vascular cell differentiation**. *Nat Rev Mol Cell Biol* 2004, **5**:379-391.
17. Kubo M, Udagawa M, Nishikubo N, Horiguchi G, Yamaguchi M, Ito J, Mimura T, Fukuda H, Demura T: **Transcription switches for**

protoxylem and metaxylem vessel formation. *Genes Dev* 2005, **19**:1855-1860.

18. Zhong R, Lee C, Zhou J, McCarthy RL, Ye ZH: **A battery of**
• **transcription factors involved in the regulation of secondary cell wall biosynthesis in Arabidopsis**. *Plant Cell* 2008, **20**:2763-2782.

19. Zhou J, Lee C, Zhong R, Ye ZH: **MYB58 and MYB63 are**
• **transcriptional activators of the lignin biosynthetic pathway during secondary cell wall formation in Arabidopsis**. *Plant Cell* 2009, **21**:248-266.
These two papers describe a network of MYB, NAC and other transcription factors that are involved in secondary cell wall formation important for xylem cell development.

20. Muñiz L, Minguet EG, Singh SK, Pesquet E, Vera-Sirera F, Moreau-
• Courtois CL, Carbonell J, Blázquez MA, Tuominen H: **ACAULIS5 controls *Arabidopsis* xylem specification through the prevention of premature cell death**. *Development* 2008, **135**:2573-2582.
The putative spermine biosynthetic gene ACAULIS5 is required for proper specification of late-developing pitted xylem vessel elements. This may be due to the gene preventing premature death of the developing vessel elements.

21. Motose H, Sugiyama M, Fukuda H: **A proteoglycan mediates inductive interaction during plant vascular development**. *Nature* 2004, **429**:873-878.

22. Bonke M, Thitamadee S, Mähönen AP, Hauser MT, Helariutta Y: **APL regulates vascular tissue identity in Arabidopsis**. *Nature* 2003, **426**:181-186.

23. Vanneste S, Friml J: **Auxin: a trigger for change in plant development**. *Cell* 2009, **136**:1005-1016.

24. Robert HS, Friml J: **Auxin and other signals on the move in plants**. *Nat Chem Biol* 2009, **5**:325-332.

25. Wiśniewska J, Xu J, Seifertová D, Brewer PB, Růžička K, Blilou I, Rouquié D, Benková E, Scheres B, Friml J: **Polar PIN localization directs auxin flow in plants**. *Science* 2006, **312**:883.

26. Michniewicz M, Zago MK, Abas L, Weijers D, Schweighofer A, Meskiene I, Heisler MG, Ohno C, Zhang J, Huang F *et al.*: **Antagonistic regulation of PIN phosphorylation by PP2A and PINOID directs auxin flux**. *Cell* 2007, **130**:1044-1056.

27. Sauer M, Balla J, Luschnig C, Wisniewska J, Reinöhl V, Friml J, Benková E: **Canalization of auxin flow by Aux/IAA-ARF-dependent feedback regulation of PIN polarity**. *Genes Dev* 2006, **20**:2902-2911.

28. Bayer EM, Smith RS, Mandel T, Nakayama N, Sauer M,
• Prusinkiewicz P, Kuhlemeier C: **Integration of transport-based models for phyllotaxis and midvein formation**. *Genes Dev* 2009, **23**:373-384.
The authors provide further support to the canalisation hypothesis. Computer simulations of auxin fluxes during leaf midvein initiation are consistent with the idea that auxin can redirect PIN1 and amplify small auxin maxima to trigger *de novo* vascular strand formation.

29. Cambridge AP, Morris DA: **Transfer of exogenous auxin from the phloem to the polar auxin transport pathway in pea (*Pisum sativum* L)**. *Planta* 1996, **199**:583-588.

30. Marchant A, Bhalerao R, Casimiro I, Eklöf J, Casero PJ, Bennett M, Sandberg G: **AUX1 promotes lateral root formation by facilitating indole-3-acetic acid distribution between sink and source tissues in the Arabidopsis seedling**. *Plant Cell* 2002, **14**:589-597.

31. Ljung K, Hull AK, Celenza J, Yamada M, Estelle M, Normanly J, Sandberg G: **Sites and regulation of auxin biosynthesis in Arabidopsis roots**. *Plant Cell* 2005, **17**:1090-1104.

32. Hirose N, Takei K, Kuroha T, Kamada-Nobusada T, Hayashi H: **Regulation of cytokinin biosynthesis, compartmentalization and translocation**. *J Exp Bot* 2008, **59**:75-83.

33. Schoof H, Lenhard M, Haecker A, Mayer KF, Jürgens G, Laux T: **The stem cell population of Arabidopsis shoot meristems is maintained by a regulatory loop between the CLAVATA and WUSCHEL genes**. *Cell* 2000, **100**:635-644.

34. Stahl Y, Wink RH, Ingram GC, Simon R: **A signalling module**
• **controlling the stem cell niche in Arabidopsis root meristems**. *Curr Biol* 2009, **19**:909-914.
A CLE40/WOX5 signalling module similar to the TDIF/CLE41/44 system controls the root stem cell niche maintenance. This spatiotemporal communication appears to be a recurring theme in the maintenance of meristematic cells.

35. Sarkar AK, Luijten M, Miyashima S, Lenhard M, Hashimoto T, Nakajima K, Scheres B, Heidstra R, Laux T: **Conserved factors regulate signalling in *Arabidopsis thaliana* shoot and root stem cell organizers**. *Nature* 2007, **446**:811-814.

36. Ito Y, Nakanomyo I, Motose H, Iwamoto K, Sawa S, Dohmae N, Fukuda H: **Dodeca-CLE peptides as suppressors of plant stem cell differentiation**. *Science* 2006, **313**:842-845.

37. Hirakawa Y, Shinohara H, Kondo Y, Inoue A, Nakanomyo I,
•• Ogawa M, Sawa S, Ohashi-Ito K, Matsubayashi Y, Fukuda H: **Non-cell-autonomous control of vascular stem cell fate by a CLE peptide/receptor system**. *Proc Natl Acad Sci U S A* 2008, **105**:15208-15213.
A signal peptide, TDIF (CLE41/CLE44), is produced and secreted by phloem. The authors propose a role for TDIF/CLE41/44 signalling as a mobile mediator for phloem–xylem cross-talk and show that TDIF binds to its receptor PXY/TDR.

38. Fisher K, Turner S: **PXY, a receptor-like kinase essential for**
•• **maintaining polarity during plant vascular-tissue development**. *Curr Biol* 2007, **17**:1061-1066.
Procambial cells perceive the TDIF signal through the PXY (TDR) receptor. A role for *PXY/TDR* is supported by data on its expression in the cambium and the identification of the *pxy* mutant with severely impaired vascular development.

39. McHale NA, Koning RE: **MicroRNA-directed cleavage of *Nicotiana sylvestris PHAVOLUTA* mRNA regulates the vascular cambium and structure of apical meristems**. *Plant Cell* 2004, **16**:1730-1740.

40. Juarez MT, Kul JS, Thomas J, Heller BA, Timmermans MC: **MicroRNA-mediated repression of *rolled leaf1* specifies maize leaf polarity**. *Nature* 2004, **428**:84-88.

41. Kalantidis K, Schumacher HT, Alexiadis T, Helm JM: **RNA silencing movement in plants**. *Biol Cell* 2008, **100**:13-26.

42. Voinnet O: **Origin, biogenesis and activity of plant microRNAs**. *Cell* 2009, **136**:669-687.

43. Buhtz A, Springer F, Chappell L, Baulcombe DC, Kehr J:
• **Identification and characterization of small RNAs from the phloem of *Brassica napus***. *Plant J* 2008, **53**:739-749.
Besides transporting photosynthetic products, phloem carries various molecules with signalling functions. Analysis of small RNAs in phloem of *Brassica napus* demonstrates that many miRNAs and other small RNA species are present in the sap and may convey long-distance information.

44. Tretter EM, Alvarez JP, Eshed Y, Bowman JL: **Activity range of Arabidopsis small RNAs derived from different biogenesis pathways**. *Plant Physiol* 2008, **147**:58-62.

45. Chitwood DH, Nogueira FTS, Howell MD, Montgomery TA,
• Carrington JC, Timmermans MCP: **Pattern formation via small RNA mobility**. *Genes Dev* 2009, **23**:549-554.
The authors demonstrate incongruities between expression patterns of precursor and mature small RNAs in a developmental context and provide evidence for the movement of trans-acting siRNAs. This suggests the intriguing idea that small RNA movement combined with the activity of spatially restricting effectors could form morphogenic patterns.

46. Sibout R, Plantegenet S, Hardtke CS: **Flowering as a condition**
•• **for xylem expansion in Arabidopsis hypocotyl and root**. *Curr Biol* 2008, **18**:458-463.
The authors propose the concept that flowering is a condition required to trigger the transition to secondary vascular development, suggesting a currently unknown long-distance signal from the shoot.

47. Kobayashi K, Zambryski P: **RNA silencing and its cell-to-cell spread during Arabidopsis embryogenesis**. *Plant J* 2007, **50**:597-604.

48. Turgeon R, Wolf S: **Phloem transport: cellular pathways and molecular trafficking**. *Annu Rev Plant Biol* 2009, **60**:207-221.

49. Kim I, Cho E, Crawford K, Hempel FD, Zambryski PC: **Cell-to-cell movement of GFP during embryogenesis and early seedling development in Arabidopsis**. *Proc Natl Acad Sci U S A* 2005, **102**:2227-2231.

50. • Thomas CL, Bayer EM, Ritzenthaler C, Fernandez-Calvino L, Maule AJ: **Specific targeting of a plasmodesmal protein affecting cell-to-cell communication**. *PLoS Biol* 2008, **6**:e7.
Few mutants with defects in plasmodesmal trafficking have been described. This paper describes a family of novel plasmodesmata-located proteins that have a role in cell-to-cell communication.

51. • Benitez-Alfonso Y, Cilia M, San Roman A, Thomas C, Maule A, Hearn S, Jackson D: **Control of Arabidopsis meristem development by thioredoxin-dependent regulation of intercellular transport**. *Proc Natl Acad Sci U S A* 2009, **106**:3615-3620.
This paper describes a mutant with increased callose deposition and decreased plasmodesmal transport, resulting in severe defects in development and phloem unloading.

52. Levesque MP, Vernoux T, Busch W, Cui H, Wang JY, Blilou I, Hassan H, Nakajima K, Matsumoto N, Lohmann JU *et al.*: **Whole-genome analysis of the SHORT-ROOT developmental pathway in Arabidopsis**. *PLoS Biol* 2006, **4**:e143.

53. • Gallagher KL, Benfey PN: **Both the conserved GRAS domain and nuclear localization are required for SHORT-ROOT movement**. *Plant J* 2009, **57**:785-797.
SHORT-ROOT is a master regulator of root development and also implied in vascular morphogenesis. The movement of the SHR transcription factor from the vascular cylinder to surrounding tissues is used as a tool to study cell-to-cell trafficking. The movement of the protein is required for proper root patterning, and both nuclear and cytoplasmic localisation signals appear to be important for this phenomenon.