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Connecting emerging with existing vasculature above and below ground

Noel Blanco-Touriñán and Christian S. Hardtke

Abstract

The vascular system was essential for plants to colonize land by facilitating the transport of water, nutrients, and minerals throughout the body. Our current knowledge on the molecular-genetic control of vascular tissue specification and differentiation is mostly based on studies in the Arabidopsis primary root. To what degree these regulatory mechanisms in the root meristem can be extrapolated to vascular tissue development in other organs is a question of great interest. In this review, we discuss the most recent progress on cotyledon vein formation, with a focus on polar auxin transport-dependent and -independent mechanisms. We also provide an overview of vasculature formation in postembryonic organs, namely lateral roots, which is more complex than anticipated as several tissues of the parent root must act in a spatio-temporally coordinated manner.

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Auxin canalization, Vein patterning, Lateral root primordium, Vascular connections, Developmental plasticity.

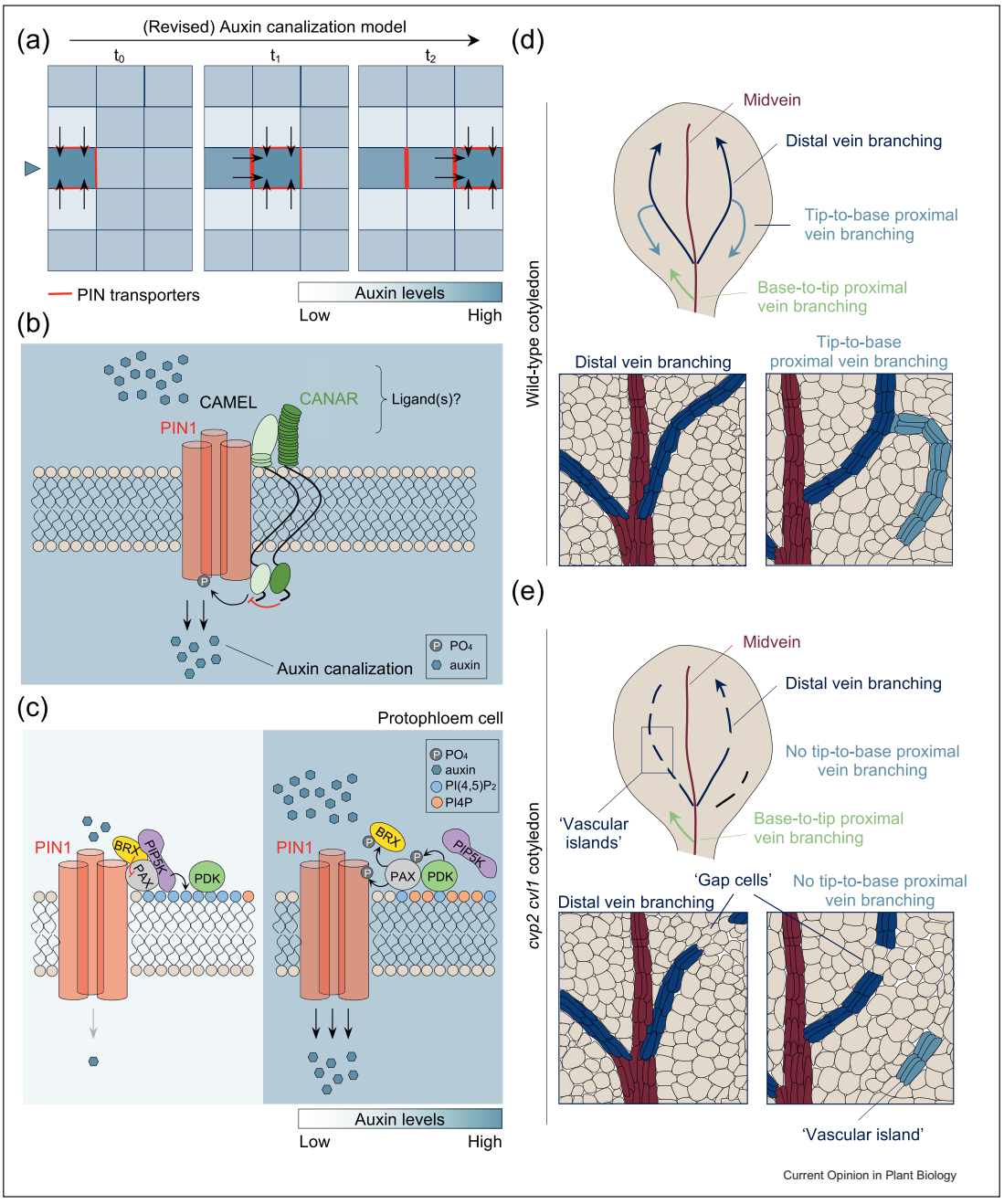
Introduction

Plant embryogenesis produces a minimal set of organs and primary meristems that provide a coordinate system for the post-embryonic elaboration of the plant body [1]. This includes the formation of a continuous vascular system that is required to exchange water and nutrients within the plant [2]. Thus, understanding the formation of vascular tissue networks in different organs is an essential question in plant developmental biology. The bulk of our knowledge on the molecular mechanisms of vascular tissue formation is derived from its study in primary roots of the dicotyledonous model plant, *Arabidopsis thaliana* (Arabidopsis) [3,4]. It remains unclear however to what degree these mechanisms operate in the ontogenesis of post-embryonic organs, and whether they are co-opted in spatially more complex situations, like the formation of vein networks in leaf (-like) organs. Recent progress in the analysis of vascular tissue formation in lateral roots and cotyledons starts to shed light on these issues.

Polar auxin transport-dependent regulation of vascular patterning in cotyledons

An important concept in vascular tissue formation is the 'auxin canalization hypothesis', which was formulated over half a century ago [5]. Although it still posits a valid framework for vascular tissue formation, revisions were proposed because predictions of the original hypothesis are at odds with various experimental observations [6–8]. One of its revised formulations suggests that a (pro)vascular cell attracts slightly more auxin than its neighboring cells, which enhances its own polar auxin transport (PAT) capacity and leads to the export of auxin from the cell. The increase in auxin levels in the adjacent cell then initiates a repetition of the process, ultimately resulting in auxin canalization and the formation of narrow cell strands that differentiate into vascular tissues of the foliar organs [6,8–10] (Figure 1a). Molecular markers of auxin activity indicate that the provascular cell strands accumulate auxin and thus, counterintuitively, appear as auxin sources rather than sinks. However, much as a river that drains a landscape accumulates water, they may still constitute preferred routes for auxin evacuation, highlighting the importance of PAT control. Although the key components for polar auxin efflux, the typically asymmetrically localized PIN-FORMED (PIN) auxin efflux carriers, have been identified decades ago, it remained unclear how the auxin signal could influence PIN polarity in neighboring cells. A significant breakthrough in this area is the recent finding that downstream of the canonical auxin signaling pathway, the WRKY DNA-BINDING PROTEIN 23 (WRK23) transcription factor stimulates expression of the CANALIZATION-RELATED AUXIN-REGULATED MALECTIN-TYPE RLK (CAMEL) cell-surface transmembrane receptor. The complex of

Figure 1



Regulators of cotyledon vein formation (a) Schematic illustration of a revised view of the auxin canalization model. The blue arrowhead indicates the narrow channel with high auxin-transport capacity that will become the vascular cells. **(b)** Schematic showing phosphorylation of PIN1 by CAMEL for auxin canalization during cotyledon vascular formation. The absence of CAMEL-mediated phosphorylation in the PIN1 cytosolic loop leads to a failure in PIN1 polarization in response to auxin. CANAR, which does not exhibit kinase activity, antagonizes CAMEL-mediated phosphorylation of PIN1. **(c)** PAT regulation in developing protophloem sieve elements. At low level of auxin inside the cell, BRX inhibits PAX-mediated PIN1 activation and also recruits PIP5Ks to reinforce PAX and its own polarity. Reduced auxin efflux gradually increases intracellular auxin levels, resulting in PDK1/2-mediated PAX activation. PAX then phosphorylates BRX and its displacement from the plasma membrane allows PAX to phosphorylate PIN1 to promote PAT. The subsequent gradual decrease in auxin levels facilitates BRX association with the plasma membrane, once again inhibiting PAX activity. **(d)** Schematic representation of the branching sites of distal and proximal secondary veins in a wild-type cotyledon. Note that distal vein branching is formed from the base to the tip, whereas proximal vein branching can occur in both directions (base-tip, tip-base). **(e)** Schematic representation of secondary vein formation in *cvp2 cvl1* cotyledons. Note the absence of the proximal vein branching that emerges from the tip towards the base as well as the presence of 'gap cells' and 'vascular islands'.

CAMEL with CANALIZATION-RELATED RLK (CANAR) controls auxin-mediated PIN trafficking and polarity in individual cells through PIN phosphorylation (Figure 1b) [9,11]. Importantly, CANAR seems to act as a negative regulator of CAMEL, as it reduces autophosphorylation of CAMEL and its kinase activity towards PIN1. Mutation of the pertinent phosphosites in PIN1 impacts its polar localization and results in defects in the vein pattern of cotyledons [9,11].

PAT regulation through a PIN 'phosphocode' can also affect PIN activity. For instance, root protophloem differentiation is promoted by regulation of auxin flux through a molecular rheostat [10], which may represent yet another mechanism of auxin canalization. In this rheostat model, the AGC kinase PROTEIN KINASE ASSOCIATED WITH BRX (PAX) enhances PIN activity as well as turnover, whereas the BREVIS RADIX (BRX) protein dampens this stimulation (Figure 1c) [12]. The BRX-PAX module recruits phosphatidylinositol-4-phosphate 5-kinases (PIP5Ks) to reinforce the polar localization of all three proteins, which is however independent of PIN polarity (Figure 1c) [10,13]. Genetic analysis has shown that 3-phosphoinositide-dependent protein kinases (PDK) 1 and 2 regulate PAT by phosphorylating and consequently activating PAX in an auxin-stimulated manner (Figure 1c) [14]. Interestingly, mutations in *BRX* and *PIP5K*s also affect the cotyledon vein pattern [15,16]. Likewise, *PDK1/2* loss-of-function leads to highly fragmented and complex cotyledon veins, which could be partially restored by introducing a constitutively active phosphomimic version of PAX [14]. These results suggest that the machinery controlling PAT in root protophloem formation is also deployed in cotyledon vascularization. Yet, this process may use additional factors [17,18], whose role in root protophloem development could however also be masked by redundancy. For example, VASCULATURE COMPLEXITY AND CONNECTIVITY (VCC), a plant-specific transmembrane protein, is necessary for the appropriate polarization and stability of PIN1 in (pro)vascular cells of embryonic cotyledons, and thereby, proper vascularization [17]. Mutations in *VCC* enhance the reduced cotyledon vein pattern complexity of mutations in *OCTOPUS (OPS)*, which encodes another key regulator of root protophloem development [19–22].

An often overlooked aspect of the various mutants is their phenotypic variability, which reaches from reduced complexity of vascular loops, to open ended vascular strands, to the occurrence of 'vascular islands'. An understanding of cotyledon vascularization thus cannot be achieved without appreciating the precise cellular basis of vein specification and differentiation. Recent publications propose that the emergence of distal secondary veins follows the formation of the midvein in embryonic cotyledons, and that proximal secondary veins form subsequently (Figure 1d) [17,23]. Whereas distal secondary veins branch out directly from cell files comprising the midvein (Figure 1d), the origin of proximal veins remains unclear [23]. It is possible that proximal veins initiate through the periclinal division of a distal vein cell, or by recruiting a ground meristem cell located adjacent to the vascular cell at the branching point. A re-examination of pertinent mutants may allow to genetically dissect different aspects of cotyledon vascularization and to determine the role of different genes in either distal or proximal secondary venation, or both. For instance, recent findings demonstrated that the phosphoinositide 5-phosphatases *COTYLEDON VASCULAR PATTERN 2* (*CVP2*) and its homologue *CVP2-LIKE 1* (*CVL1*) as well as *OPS* positively regulate proximal secondary vein branching but are apparently not required for distal secondary branching (Figure 1e, see vascular defects in *cvp2 cvl1* cotyledons) [22,23].

Evidence for PAT-independent regulation of cotyledon vein patterning

Discontinuities, and even more so vascular islands in the cotyledon or leaf vein network appear to contradict the auxin canalization model. They may however represent local, eventually unsuccessful canalization events. This emerges for example from detailed analysis of *PIN1* expression in discontinuous veins of *vascular network 3* (*van3; a.k.a. scarface*) leaves, which revealed that the primary defect is in the maintenance rather than the establishment of a continuous vascular pattern [24]. The VAN3 ARF-GAP might thus promote vascular continuity through *PIN* genes, as evidenced by the gradual decay of *PIN1* expression and polarity pattern that precedes the onset of fragmentation [24]. However, genetic evidence also suggests that VAN3 and other factors function at least partially in a PAT-independent manner in cotyledon vein formation. First, *van3 pin1* double mutants display an additive phenotype of *pin1* (bifurcation of the midvein) and *van3* (discontinuities) single mutants [25]. Second, the vascular defects in loss-of-function mutants of the VAN3 regulator CVP2 and its homolog CVL1 are not related to PAT alterations [23,26]. The third piece of evidence comes from genetic analysis of *gnom* mutant leaves, whose discontinuous vascular patterning has been attributed to disrupted auxin transport because *GNOM* encodes a membrane trafficking regulator that guides the correct PIN localization [27,28]. Mutual suppression of *van3* and *gnom* vein cotyledon defects suggests however that VAN3 and GNOM may have opposing functions [25,29,30]. Moreover, as the vascular defects of *gnom* mutants in both cotyledons and leaves are more severe than in sextuple mutants of vascular PINs, it appears unlikely that they are solely caused by altered PIN localization [28]. Instead, GNOM may as well function in a PIN-independent manner in leaf vein patterning, although this function appears to be still (partially) mediated by

canonical auxin signaling [28]. Finally, silencing the expression of the putative CLAVATA3/EMBRYO SURROUNDING REGION-RELATED (CLE) peptide receptor *RECEPTOR-LIKE PROTEIN KINASE 2* (*RPK2*; a.k.a. *TOADSTOOL 2*) in *cvp2 cvl1* mutants rescues secondary proximal branching defects but not the vascular islands of cotyledons, presumably in a PAT-independent manner [23] although direct interaction between CLE signaling and PAT regulation has been observed in root protophloem [31]. CLE peptides were recently implicated in the repression of xylem differentiation in cotyledons of etiolated seedlings as well [32], but it is yet to be determined whether their function in this context entails PAT regulation.

Vascular tissue development in lateral roots

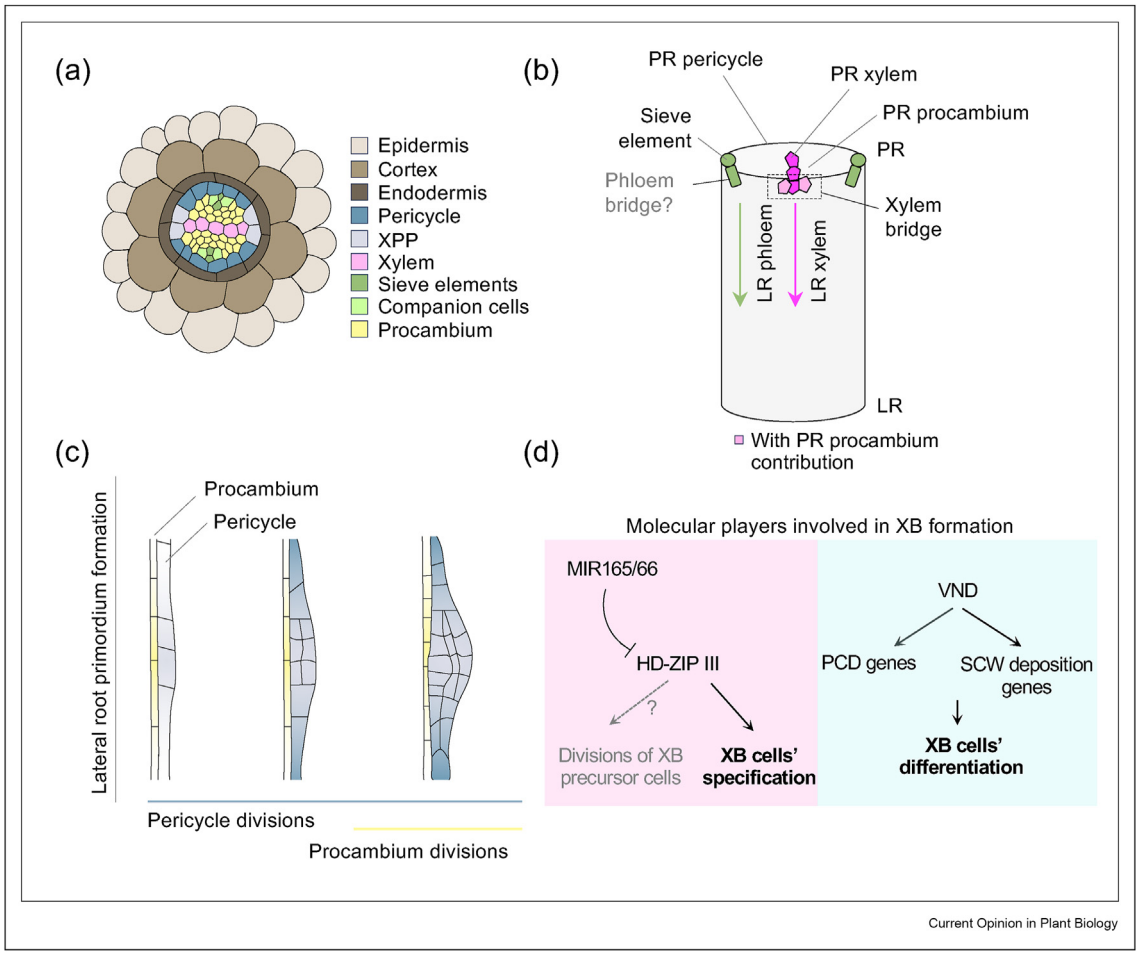
The topic of vein branching and vascular islands is also pertinent for lateral organ formation. For example, lateral roots are formed post-embryonically (with the exception of *Cucurbitaceae* and *Polygonaceae*, which also produce embryonic lateral roots) [33,34] and have to connect to the existing vasculature of their parent, primary root. In most seed plants, lateral roots arise from a specific group of founder cells (FCs) in the pericycle [35], although in some species other primary root tissues such as endodermis, cortex and vascular parenchyma contribute to its morphogenesis as well [33,36]. In Arabidopsis, pericycle FCs divide to create a meristem that resembles the primary root meristem. It is then assumed that vascular cells in lateral roots arise exclusively from their own vascular initials. The process of Arabidopsis lateral root development can be divided into eight stages (stages I-VII and emergence). Lateral root primordium (LRP) initiation starts with the specification of one xylem pericycle cell (XPP) as an FC (Figure 2a), which divides and gradually recruits neighboring pericycle cells to become FCs [35,37]. Cell type-specific reporter lines indicate that cell lineages begin to form at an early stage during LRP formation [38]. Already in stage II, two layers are differentiated, an internal layer that will develop into the vascular tissues, and an external layer that will give rise to the outer tissues [38]. Recently, single-cell mRNA sequencing through the first four stages of LRP formation revealed transitional stages that drive the acquisition of stem cell characteristics, provascular tissue identities and a flexible pluripotent state [39]. In the vascular development branch, certain cells would have transitioned to protophloem-like identity by stage IV [39]. These cells are located next to the primary root phloem and expected to ensure the continuity of phloem tissues, which is essential to support lateral root meristem growth. Although vascular tissues in lateral roots thus appear to originate from the pericycle, earlier studies suggested that the vascular parenchyma is also involved in establishing the connection between the nascent lateral root and the primary root [33]. These anatomical studies found that the vascular parenchyma starts to divide very early during lateral root development, in stages I and II in monocot and eudicot plant species, respectively [33,40–42]. However, conclusive evidence for this was only obtained from recent studies, which characterized the progression of vascular connections between nascent lateral and primary roots at spatio-temporal cellular detail [35,39,43].

Procambial cells of primary root build a xylem bridge to lateral roots

Similar to primary roots, phloem tissues differentiate earlier than xylem tissue in lateral roots [39]. These first differentiated phloem cells might establish the connection to the primary root phloem (Figure 2b). Indeed, symplastic tracer experiments suggest that these cells are derived from phloem pole pericycle daughter cells [35]. Once lateral roots emerge and become able to absorb water, xylem is formed and connected to the primary root xylem. To this end, a xylem bridge (XB) is formed between primary and lateral root xylem strands [43]. In agreement with earlier studies, clonal analysis and long-term time-lapse experiments demonstrated that during LRP formation, the primary root procambium starts to divide and the XB is derived from some of these precursor cells (Figure 2c) [43]. This procambium contribution to xylem formation is spatio-temporally coordinated with the participation of the pericycle [43]. How can this be understood in the context of LRP formation? LRP initiation starts by specifying the first pericycle FC, which is typically in direct contact with the primary root protoxylem, before any procambial cell proliferation. The centrally located XB elements formed at the base of the lateral root originate from the pericycle and connect with the primary root protoxylem (Figure 2b). The recruitment of FCs then spreads laterally to the phloem poles. Procambium cells adjacent to the recruited FCs then start to divide and switch their identity to xylem precursors (Figure 2b). These procambium-derived XB cells are located lateral to the junction between the LRP and the primary root protoxylem. Finally, since procambial cells can produce either xylem or phloem, it appears possible that procambial daughter cells also contribute to the phloem connection.

While regulators of phloem connection between the primary root and nascent lateral roots are not known, regulators of the xylem connection have been described recently [43]. That is, CLASS III HOMEODOMAIN-LEUCINE ZIPPER (HD-ZIPIII) and VASCULAR-RELATED NAC-DOMAIN (VND) transcriptions factors are critical for XB specification and differentiation, respectively (Figure 2d) [43]. Therein, HD-ZIPIIIs appear to be regulated by the microRNAs miR165/6, similar to their regulation in the shoot apical meristem and primary root (Figure 2d) [44]. It is uncertain

Figure 2



Establishment of vascular connections during lateral root development. (a) Schematic of the primary tissues of an Arabidopsis root in the transverse plane. XPP, xylem pole pericycle. **(b)** Schematic showing vascular connections during LR morphogenesis. The formation of a phloem bridge is hypothesized by the authors, whereas the formation of a xylem bridge has been recently demonstrated. PR, primary root; LR, lateral root. **(c)** Schematic showing that the proliferation of parental procambium during LR morphogenesis contributes to LR formation (i.e., xylem connections). **(d)** Model summarizing the role of HD-ZIP III and VND transcription factors in XB specification and differentiation, respectively. PCD, programmed cell death; SCW, secondary cell wall.

however whether the severe inhibition of XB formation in *hd-zipIII* loss-of-function mutants is due to their role in XB cell specification directly, or rather represents an indirect consequence of vascular (pro)cambium cell proliferation defects [45]. In the primary root, the MONOPTEROS (MP) transcription factor acts downstream of HD-ZIPIIIs, whereas ARGONAUTE10 (AGO10) promotes miR165/6 degradation to stabilize HD-ZIPIIIs [44]. The lack of XB formation defects in *mp* and *ago10* mutants thus suggests that upstream and downstream elements of the miR165/6-HD-ZIPIII pathway may vary depending on context [43]. Collectively, these studies indicate that while many regulators associated with vasculature development of primary roots are involved in vascular connections, this is not true for all of them. An intriguing question is thus whether distinct regulators that are solely responsible for building vascular connections between the primary and lateral roots exist? Moreover, comparatively little is known about molecular mechanisms of vascular tissue formation in lateral roots post-emergence [15,43]. Yet, because mutants with impaired protophloem differentiation in the primary root often show similar defects in lateral roots [15], the pathways are likely similar, and phenotypic differences between primary and lateral roots may mainly reflect organ-specific unequal redundancies.

Conclusions and perspectives

In summary, whereas we have accumulated extensive knowledge on the cellular and molecular basis of vascular tissue morphogenesis in primary roots, the

formation of vascular tissues in cotyledons and lateral roots remains poorly understood. At the cellular level, key questions that require further investigation include how proximal branching is established in embryonic cotyledons and whether, similar to what happens during xylem formation, 'phloem bridges' are formed during lateral root development. At the molecular level, transcriptomic analysis by single-cell mRNA sequencing [39,46] and further genetic validation could drive further advances. Since most genes involved in vascular development have been identified through forward genetic screens, genetically redundant regulators of vein formation may have eluded detection. Chemical genetics offers a promising approach to identify small molecules that can impact leaf and cotyledon vein patterning in cases where genetic redundancy is a challenge [47]. Finally, given our limited understanding of how environmental factors affect vascular development [48,49], investigating the plasticity of this process in response to changing conditions may yield complementary critical insight.

Author contributions

N.B-T and C.S.H conceptualized and wrote the article.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

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

* of special interest
** of outstanding interest

1. Palovaara J, de Zeeuw T, Weijers D: **Tissue and organ initiation in the plant embryo: a first time for everything**. *Annu Rev Cell Dev Biol* 2016, **32**:47–75.

2. Lucas WJ, Groover A, Lichtenberger R, Furuta K, Yadav SR, Helariutta Y, He XQ, Fukuda H, Kang J, Brady SM, *et al.*: **The plant vascular system: evolution, development and functions**. *J Integr Plant Biol* 2013, **55**:294–388.

3. De Rybel B, Mahonen AP, Helariutta Y, Weijers D: **Plant vascular development: from early specification to differentiation**. *Nat Rev Mol Cell Biol* 2016, **17**:30–40.

4. Hardtke CS: **Phloem development**. *New Phytol* 2023, **239**: 852–867.

5. Sachs T: **Polarity and the induction of organized vascular tissues**. *Ann Bot* 1969, **33**:263–275.

6. Ravichandran SJ, Linh NM, Scarpella E: **The canalization hypothesis - challenges and alternatives**. *New Phytol* 2020, **227**: 1051–1059.

7. Feugier FG, Mochizuki A, Iwasa Y: **Self-organization of the vascular system in plant leaves: Inter-dependent dynamics of auxin flux and carrier proteins**. *J Theor Biol* 2005, **236**: 366–375.

8. 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.

9. Hajny J, Tan S, Friml J: **Auxin canalization: from speculative models toward molecular players**. *Curr Opin Plant Biol* 2022, **65**, 102174.

10. Aliaga Fandino AC, Hardtke CS: **Auxin transport in developing protophloem: a case study in canalization**. *J Plant Physiol* 2022, **269**, 153594.

11. ** Hajný J, Prát T, Rydza N, Rodriguez L, Tan S, Verstraeten I, Domjan D, Mazur E, Smakowska-Luzan E, Smet W, *et al.*: **Receptor kinase module targets PIN-dependent auxin transport during canalization**. *Science* 2020, **370**:550–557.
In this paper, the CANAR/CAMEL cell-surface receptor complex was identified as the first rather specific molecular player in the auxin canalization process. The phosphorylation of PIN1 by this complex is required for PIN1 trafficking and polarity.

12. Marhava P, Bassukas AEL, Zourelidou M, Kolb M, Moret B, Fastner A, Schulze WX, Cattaneo P, Hammes UZ, Schwechheimer C, *et al.*: **A molecular rheostat adjusts auxin flux to promote root protophloem differentiation**. *Nature* 2018, **558**:297–300.

13. Marhava P, Aliaga Fandino AC, Koh SWH, Jelinkova A, Kolb M, Janacek DP, Breda AS, Cattaneo P, Hammes UZ, Petrasek J, *et al.*: **Plasma membrane domain patterning and self-reinforcing polarity in Arabidopsis**. *Dev Cell* 2020, **52**:223–235 e225.

14. * Xiao Y, Offringa R: **PDK1 regulates auxin transport and Arabidopsis vascular development through AGC1 kinase PAX**. *Nat Plants* 2020, **6**:544–555.
The authors show that 3-phosphoinositide-dependent protein kinase 1 (PDK1) regulates auxin transport by phosphorylating PROTEIN KINASE ASSOCIATED WITH BRX (PAX) during vascular development.

15. Rodriguez-Villalon A, Gujas B, van Wijk R, Munnik T, Hardtke CS: **Primary root protophloem differentiation requires balanced phosphatidylinositol-4,5-biphosphate levels and systemically affects root branching**. *Development* 2015, **142**:1437–1446.

16. Tejos R, Sauer M, Vanneste S, Palacios-Gomez M, Li H, Heilmann M, van Wijk R, Vermeer JE, Heilmann I, Munnik T, *et al.*: **Bipolar plasma membrane distribution of phosphoinositides and their requirement for auxin-mediated cell polarity and patterning in Arabidopsis**. *Plant Cell* 2014, **26**:2114–2128.

17. * Yanagisawa M, Poitout A, Otegui MS: **Arabidopsis vascular complexity and connectivity controls PIN-FORMED1 dynamics and lateral vein patterning during embryogenesis**. *Development* 2021:148.
In this manuscript, the authors show that the plant-specific transmembrane protein VASCULATURE COMPLEXITY AND CONNECTIVITY (VCC) is required for the PIN1-mediated control of cotyledon vein complexity.

18. Samakovli D, Roka L, Dimopoulou A, Plitsi PK, Zukauskait A, Georgopoulou P, Novak O, Milioni D, Hatzopoulos P: **HSP90 affects root growth in Arabidopsis by regulating the polar distribution of PIN1**. *New Phytol* 2021, **231**:1814–1831.

19. Roschzttardtz H, Paez-Valencia J, Dittakavi T, Jali S, Reyes FC, Baisa G, Anne P, Gissot L, Palauqui JC, Masson PH, Bednarek SY, Otegui MS: **The VASCULATURE COMPLEXITY AND CONNECTIVITY gene encodes a plant-specific protein required for embryo provasculature development**. *Plant Phys* 2014, **166**:889–902.

20. Breda AS, Hazak O, Hardtke CS: **Phosphosite charge rather than shootward localization determines OCTOPUS activity in root protophloem**. *Proc Natl Acad Sci U S A* 2017, **114**: E5721–E5730.

21. Breda AS, Hazak O, Schultz P, Anne P, Graeff M, Simon R, Hardtke CS: **A cellular insulator against CLE45 peptide signaling**. *Curr Biol* 2019, **29**:2501–2508 e2503.

22. Truernit E, Bauby H, Belcram K, Barthelemy J, Palauqui JC: **OCTOPUS, a polarly localised membrane-associated protein, regulates phloem differentiation entry in Arabidopsis thaliana**. *Development* 2012, **139**:1306–1315.

23. * Kastanaki E, Blanco-Tourinan N, Sarazin A, Sturchler A, Gujas B, Vera-Sirera F, Agusti J, Rodriguez-Villalon A: **A genetic framework for proximal secondary vein branching in the Arabidopsis thaliana embryo**. *Development* 2022:149.

By comparing wild-type and *cotyledon vascular pattern 2 (cvp2) cvp2-like 1 (cvl1)* mutant embryonic cotyledons, the authors propose the existence of two distinct branching mechanisms contributing to cotyledon vein formation. A PIN1-independent genetic framework for proximal secondary vein branching is defined and involves the activity of CVP2 and CVL1 phosphatidylinositol 4,5-bisphosphate 5-phosphatases.

24. Scarpella E, Marcos D, Friml J, Berleth T: **Control of leaf vascular patterning by polar auxin transport**. *Genes Dev* 2006, **20**:1015–1027.

25. Koizumi K, Naramoto S, Sawa S, Yahara N, Ueda T, Nakano A, Sugiyama M, Fukuda H: **VAN3 ARF-GAP-mediated vesicle transport is involved in leaf vascular network formation**. *Development* 2005, **132**:1699–1711.

26. Carland F, Nelson T: **CVP2- and CVL1-mediated phosphoinositide signaling as a regulator of the ARF GAP SFC/VAN3 in establishment of foliar vein patterns**. *Plant J* 2009, **59**: 895–907.

27. Geldner N, Anders N, Wolters H, Keicher J, Kornberger W, Muller P, Delbarre A, Ueda T, Nakano A, Jürgens G: **The Arabidopsis GNOM ARF-GEF mediates endosomal recycling, auxin transport, and auxin-dependent plant growth**. *Cell* 2003, **112**:219–230.

28. ** Verna C, Ravichandran SJ, Sawchuk MG, Linh NM, Scarpella E: **Coordination of tissue cell polarity by auxin transport and signaling**. *Elife* 2019, **8**.

In this paper, the authors conducted a detailed genetic analysis to discover that PIN transporters are not essential during vein formation and that the GNOM ARF-GEF acts upstream of auxin response.

29. Naramoto S, Kyozuka J: **ARF GTPase machinery at the plasma membrane regulates auxin transport-mediated plant growth**. *Plant Biotechnol* 2018, **35**:155–159.

30. Sieburth LE, Muday GK, King EJ, Benton G, Kim S, Metcalf KE, Meyers L, Seamen E, Van Norman JM: **SCARFACE encodes an ARF-GAP that is required for normal auxin efflux and vein patterning in Arabidopsis**. *Plant Cell* 2006, **18**:1396–1411.

31. Wang Q, Aliaga Fandino AC, Graeff M, DeFalco TA, Zipfel C, Hardtke CS: **A phosphoinositide hub connects CLE peptide signaling and polar auxin efflux regulation**. *Nat Commun* 2023, **14**:423.

32. * Ghosh S, Nelson JF, Cobb GMC, Etchells JP, de Lucas M: **Light regulates xylem cell differentiation via PIF in Arabidopsis**. *Cell Rep* 2022, **40**, 111075.

The authors demonstrate a crucial link between light and xylem differentiation in cotyledons. A molecular mechanism involving PHYTOCHROME INTERACTING FACTORs (PIFs) and CLE peptides is proposed.

33. Torres-Martinez HH, Rodriguez-Alonso G, Shishkova S, Dubrovsky JG: **Lateral root primordium morphogenesis in angiosperms**. *Front Plant Sci* 2019, **10**:206.

34. Singh H, Singh Z, Kashyap R, Yadav SR: **Lateral root branching: evolutionary innovations and mechanistic divergence in land plants**. *New Phytol* 2023, **238**:1379–1385.

35. * Torres-Martinez HH, Hernandez-Herrera P, Corkidi G, Dubrovsky JG: **From one cell to many: Morphogenetic field of lateral root founder cells in Arabidopsis thaliana is built by gradual recruitment**. *Proc Natl Acad Sci U S A* 2020, **117**: 20943–20949.

The authors elegantly demonstrated that phloem pole pericycle cells are recruited as founder cells, presumably to aid in the establishment of the phloem connection between the parent and the nascent lateral root.

36. Xiao TT, van Velzen R, Kulikova O, Franken C, Bisseling T: **Lateral root formation involving cell division in both pericycle, cortex and endodermis is a common and ancestral trait in seed plants**. *Development* 2019:146.

37. Torres-Martinez HH, Napsucialy-Mendivil S, Dubrovsky JG: **Cellular and molecular bases of lateral root initiation and morphogenesis**. *Curr Opin Plant Biol* 2022, **65**, 102115.

38. Malamy J, Benfey PN: **Organization and cell differentiation in lateral roots of Arabidopsis thaliana**. *Development* 1997, **124**: 33–44.

39. * Serrano-Ron L, Perez-Garcia P, Sanchez-Corrionero A, Gude I, Cabrera J, Ip PL, Birnbaum KD, Moreno-Risueno MA: **Reconstruction of lateral root formation through single-cell RNA sequencing reveals order of tissue initiation**. *Mol Plant* 2021, **14**:1362–1378.

In this study, a single-cell RNA sequencing through the first four stages of lateral root primordium formation revealed that certain cells in the vascular branch exhibit protophloem-like characteristics, indicating that this vascular tissue is likely the first to be formed and connected to the primary root during lateral root development.

40. Bell JK, McCully ME: **A histological study of lateral root initiation and development in Zea mays**. *Protoplasma* 1970, **70**: 179–205.

41. Byrne JM, Pesacreta TC, Fox JA: **Development and structure of the vascular connection between the primary and secondary root of Glycine max (L.) merr**. *Am J Bot* 1977, **64**:946–959.

42. Seago JL: **Developmental anatomy in roots of Ipomoea purpurea. II. Initiation and development of secondary roots**. *Am J Bot* 1973, **60**:607–618.

43. ** Blanco-Touriñán N, Torres-Martínez HH, Augstein F, Champeyroux C, von der Mark C, Carlsbecker A, Dubrovsky JG, Rodriguez-Villalón A: **The primary root procambium contributes to lateral root formation through its impact on xylem connection**. *Curr Biol* 2023, **33**:1716–1727.

In this study, the authors propose the formation of a xylem bridge that connects the xylem of the lateral root with that of the primary root. They find that both the pericycle and parental procambium contribute to the formation of this bridge, and further investigate the role of several transcription factors during this developmental program.

44. Ramachandran P, Carlsbecker A, Etchells JP: **Class III HD-ZIPs govern vascular cell fate: an HD view on patterning and differentiation**. *J Exp Bot* 2017, **68**:55–69.

45. Smetana O, Makila R, Lyu M, Amiryousefi A, Sanchez Rodriguez F, Wu MF, Sole-Gil A, Leal Gavarron M, Siligato R, Miyashima S, *et al.*: **High levels of auxin signalling define the stem-cell organizer of the vascular cambium**. *Nature* 2019, **565**:485–489.

46. Liu Z, Wang J, Zhou Y, Zhang Y, Qin A, Yu X, Zhao Z, Wu R, Guo C, Bawa G, *et al.*: **Identification of novel regulators required for early development of vein pattern in the cotyledons by single-cell RNA-sequencing**. *Plant J* 2022, **110**:7–22.

47. Carland F, Defries A, Cutler S, Nelson T: **Novel vein patterns in Arabidopsis induced by small molecules**. *Plant Physiol* 2016, **170**:338–353.

48. Agusti J, Blazquez MA: **Plant vascular development: mechanisms and environmental regulation**. *Cell Mol Life Sci* 2020, **77**:3711–3728.

49. Lopez-Salmeron V, Cho H, Tonn N, Greb T: **The phloem as a mediator of plant growth plasticity**. *Curr Biol* 2019, **29**: R173–R181.