Vascular continuity and auxin signals

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Plant vascular tissues form systems of interconnected cell files throughout the plant body. Vascular tissues usually differentiate at predictable positions but the wide range of functional patterns generated in response to abnormal growth conditions or wounding reveals partially self-organizing patterning mechanisms. Signals ensuring aligned cell differentiation within vascular strands are crucial in self-organized vascular patterning, and the apical–basal flow of indole acetic acid has been suspected to act as an orienting signal in this process. Several recent advances appear to converge on a more precise definition of the role of auxin flow in vascular tissue patterning.

Branched cellular systems exist in most multicellular organisms and the principles underlying their reticulate patterns have long intrigued philosophers, mathematicians and experimental biologists. In particular, the exposed and often beautiful vascular system of plants has fascinated many, yet – as for other branched systems – the patterning cues have remained elusive.

The plant vascular system is a network of interconnected cells for the transport of water and dissolved materials throughout the plant[1,2]. Vascular tissues are typically organized in bundles or strands that contain two kinds of conducting tissues, phloem and xylem, each comprising a variety of distinguishable cell types. Dissolved photoassimilates from source organs in the shoot are transported in the phloem, and the transfer of water and minerals from roots occurs in the xylem.

Vascular patterns are typically both variable in the course and arrangement of vascular strands and reproducible in their integration into the local tissue context (Fig. 1). These seemingly conflicting features suggest that genetic controls of tissue patterns in plant organs leave room for variable vascular strand arrangements and that vascular tissues have partially self-organizing capacities to ensure tissue continuity irrespective of the particular routes and arrangements of vascular strands.

At present, neither the molecular mechanisms underlying overall tissue patterning within plant organs nor the specific signals ensuring vascular continuity within variable networks are known. However, auxin and its apical–basal flow have long been implicated in promoting continuous vascular differentiation[3,4]. In this article, we briefly summarize evidence that auxin has a role in continuous vascular differentiation and discuss several recent findings that together have generated an experimental basis for exploring auxin functions in vascular development at the cellular and molecular levels. We finally discuss several newly isolated *Arabidopsis* mutants that might identify auxin-independent mechanisms in vascular strand formation. The focus is on mechanisms underlying aligned cell differentiation leading to the formation of vascular strands; further aspects of vascular development and research in a broader spectrum of plant species have been thoughtfully reviewed in Refs 4–6.

Auxin and vascular differentiation

Several lines of evidence have implicated indole acetic acid (IAA) (the predominant auxin in higher plants) in vascular development. IAA (supported by cytokinins) can induce xylem tracheary element differentiation in suspension culture cells of suitable species[7]. Auxin-overproducing transgenic plants have increased amounts of vascular tissues[8], IAA application can replace leaf primordia in inducing vascular ('leaf trace') connections in stems[9], and local auxin sources can induce the formation of new vascular strands from parenchymatic cells[3]. Vascular differentiation in response to IAA application does not occur readily in all genotypes, suggesting that further factors are often required. These factors probably include other plant hormones, and a vascular-differentiation-promoting influence of cytokinins, ethylene, gibberellins and brassinosteroids has indeed been reported[4,10–12].

The role of IAA is nevertheless unique because the position of IAA application can define the site of vascular differentiation: a new functional vascular strand will extend basally from a local IAA source[3] (Fig. 2). Remarkably, IAA does not just trigger vascular differentiation *per se* but also induces the formation of a continuous vascular strand – a cellular response with peculiar geometrical properties. First, the response is polar: local IAA application typically induces vascular strand formation towards the basal pole of the plant. Second, the responding cells differentiate in a continuous area to form a file of interconnected cells. Third, the differentiation zone is restricted in the radial and tangential dimensions, because differentiation occurs only within a narrow strip of cells rather than isotropically around the IAA source.

The capacity of a simple signal to trigger a complex and oriented cellular response suggests that the signaling mechanism co-opts directional cues already present in plant tissues. Not surprisingly, therefore, it is the polar (apical–basal) transport of the same molecule, IAA, which has been postulated to integrate cell polarity and aligned differentiation across the entire plant. In normal plant growth, IAA is predominantly produced in apical regions, such as young leaves or flowers, from which it is transported basally. Auxin transport is thought to proceed in a cell-to-cell fashion through the action of specific membrane-bound influx and efflux carriers[13–15] (Fig. 2). Although the molecular details remain hypothetical, the apical–basal transport of IAA itself is experimentally well established and its properties could account for the geometrical peculiarities of vascular strand formation (Fig. 2).

First, the polar effect of auxin application can be explained by the integration of the applied IAA in the general apical–basal flow of IAA. Second, differentiation in response to a transported signaling molecule would be inherently continuous. Third, the restriction of vascular differentiation to a narrow zone could be because of efficient drainage of IAA through incipient provascular strands. This drainage would prevent auxin accumulation and high IAA exposure of all cells outside the narrow 'canal' region. This interpretation forms the basis of the 'auxin canalization hypothesis', which postulates a positive feedback by which IAA-conducting cells differentiate towards increased IAA conductivity[3] (Figs 2 and 3). An auxin-triggered feedback mechanism is an attractive explanation for the reproducible position of the induced vascular strand relative

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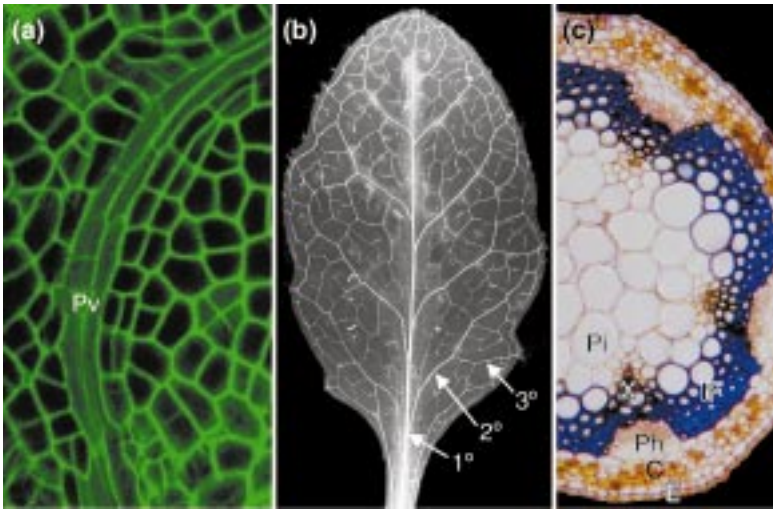

Fig. 1. Levels of vascular tissue organization. Vascular tissues connect organs across the plant body and are simultaneously integrated into the local tissue context. Their organization therefore appears to involve at least two types of control. First, directional signals involving indole acetic acid in combination with self-organizing strand-forming capacities generate continuous strands; these link to form variable strand networks. Second, genetic cues integrate vascular and non-vascular tissue patterns in plant organs and might also constrain the variability of vascular strand patterns. Self-organizing capacities are reflected in the formation of perfectly aligned strands along unpredictable routes. Integration into a larger tissue context is reflected in the organ-specific position and internal organization of vascular bundles and in the reproducible differentiation of non-vascular cell types in fixed spatial relationship to vascular bundles. (a) Alignment of cells in a provascular strand of a young leaf primordium. The strand emerges as a row of narrow cells, generated by aligned divisions and the elongation of previously isodiametric cells. The differentiation zone is always continuous but its position can vary. (b) The network of vascular strands in an *Arabidopsis* rosette leaf is highly variable (the degree of variability differs among plant species). (c) Organ-specific internal organization of vascular bundles in *Arabidopsis* stems. Abbreviations: 1°, primary vein; 2°, secondary vein; 3°, tertiary vein; C, cortex; E, epidermis; IF, interfascicular fibers; Ph, phloem; Pi, pith; Pv, provascular strand; X, xylem.

to the IAA source and is supported by a variety of vascular regeneration studies[3,12] and by the experimentally confirmed transport of auxin through differentiating vascular tissues[16].

Some aspects of naturally occurring vascular patterns can also be explained by alternative models involving prepatterns generated by local diffusion–reaction mechanisms[6]. Therefore, it is important to realize that these and other vascular patterning models are not mutually exclusive and might act in concert, possibly involving further fine-tuning cues. Of all the postulated mechanisms, present experimental and genetic tools only allow the manipulation of auxin flow and auxin perception. This has recently been performed in various ways and the associated vascular responses seem to have led to a more precise definition of the role of auxin in vascular development.

Reduced auxin transport

Several classes of auxin efflux inhibitors can be used to explore the development of vascular tissues under conditions of reduced auxin transport. Two recent studies have taken this approach by applying several IAA efflux inhibitors to young *Arabidopsis* plants. Both studies report the development of similar vascular patterns in several organs[17,18]. Typically, two types of alterations were observed. First, cells within individual vascular strands were less properly aligned and, second, more vascular tissue was generally formed. These observations support roles for auxin flow in orienting cell differentiation and in restricting vascular differentiation to narrow zones, possibly by mediating efficient auxin drainage[3].

Inhibition of auxin transport also profoundly affected leaf venation patterns. *Arabidopsis* leaf venation is normally pinnate, characterized by several distinguishable vein size orders, with secondary veins branching laterally from a single prominent midvein[6] (Fig. 3). Under the influence of auxin transport inhibitors, vascular strands along the leaf margin became more prominent, associated with increased numbers of secondary veins and multiple parallel vascular strands in the center. Remarkably, this pattern shift was already visible at low inhibitor concentrations that had no detectable effects on overall leaf morphology. Thus, several alternative functional venation patterns can be generated in a given genetic background, depending on the overall auxin transport properties of a leaf primordium.

This finding implicates auxin signaling in the genetic control of leaf venation patterns in *Arabidopsis* (and three other dicot species in one of the studies[17]). Stronger inhibition of auxin transport progressively restricts vascular differentiation to the leaf margin, suggesting that this region harbors major auxin sources critical for the formation of major (primary and secondary) veins (Fig. 3). Veins of different hierarchical orders emerge at successive stages of leaf development[17–19]. When leaf primordia were exposed to auxin transport inhibitors at specific stages during this process, individual vein classes became unresponsive to the inhibition of auxin transport at stages that matched – or even slightly preceded – their anatomical emergence[17]. This finding suggests that, with the appearance of early provascular strands, local IAA accumulation is prevented even in the presence of auxin transport inhibitors. This might be because of provascular cells expressing amounts or types of auxin transport proteins that render them less sensitive to the applied inhibitors. In sum, these studies demonstrate the extent and limits of vascular pattern flexibility in *Arabidopsis*, and suggest that auxin sources at the margins of early leaf primordia are critical for the formation of *Arabidopsis* leaf venation patterns.

The recent identification of potential auxin influx and efflux carrier genes in *Arabidopsis* should provide molecular access to explore the developmental role of auxin flow in further detail[20,21]. Thus far, one of these genes, *PIN FORMED 1* (*PIN1* or *AtPIN1*), has been implicated in vascular development and related genes appear to mediate auxin transport in root gravitropic responses. Mutations in *PIN1* result in reduced auxin transport in stem segments and in pin-shaped inflorescence morphology, a feature also observed upon chemical inhibition of auxin efflux[22] (Table 1).

Molecular features of the recently cloned *PIN1* gene support its involvement in auxin efflux[21]. The deduced PIN1 protein contains presumptive membrane-spanning domains and independent evidence has implicated several other members of the same gene family in auxin transport. Most strikingly, PIN1 is conspicuously localized at the basal end of xylem parenchyma cells, matching the predictions of the chemiosmotic model. If PIN1 is essential for IAA efflux and auxin flow is involved in vascular patterning, *pin1* mutants should have aberrant vascular systems. In agreement with this expectation, excess vascular tissue is observed in mutant

Table 1. Arabidopsis genes essential for vascular strand formation

Gene	Mutant phenotype			Molecular identity	Refs
	Seedling and adult morphology	Vascular anatomy	Auxin physiology		
EMB30/GN	Defective seedling polarity, no further organized development	Disconnected, randomly oriented vascular cells	Unknown	Guanine exchange factor thought to be essential for polar localization of auxin efflux carriers	24,26
(At)PIN1	Fused cotyledons, defective phyllotaxy, pin-shaped infloresences	Excess vascularization in stems and leaves	Reduced auxin transport	Membrane protein localized to basal end of cells inxylem parenchyma, potential efflux carrier	21,22
MP	Missing hypocotyl and primary root, fused cotyledons, pin-shaped inflorescences	Reduced vascularization	Reduced auxin perception and transport	'Auxin response factor' thought to relay auxin signals in 'axial' cell, differentiation unknown	28–30
AXR6	Missing hypocotyl and primary root, fused cotyledons[a]	Reduced vascularization[a]	Reduced auxin perception because of gain-of-function mutations	Unknown	35
BDL	Reduced hypocotyl, no primary root	Reduced vascularization	Reduced auxin perception	Unknown	36
LOP1	Various organ shape defects	Reduced vascularization midvein bifurcation	Reduced auxin transport	Unknown	39
CVP1	Reduced inflorescence internode length	Reduced cotelydon vascularization, excess stem vascularization	Normal auxin content, transport and perception	Unknown	38
CVP2	Normal	Altered cotyledon and leaf vascularization	Normal auxin content transport and perception	Unknown	38
HVE	Normal	Reduced vascularization	Unknown	Unknown	37
REV/IFL1	Defects in shoot meristem, reduced apical dominance in *rev* alleles	Missing interfascicular fibers, reduced stem vasculature	Unknown	Homeodomain-leucine zipper protein	40–42

[a]In homozygous mutants.

stems at sites of leaf insertion and at the margins of mutant leaves[17,21]. These abnormalities are similar to those found in plants exposed to auxin efflux inhibitors[17]. However, even in *pin1* null mutants the defects are not severe. Thus, the vascular phenotype supports a role for PIN1 in auxin efflux, but also indicates the existence of multiple redundantly acting genes in IAA efflux.

What would a severe auxin transport mutant look like? Auxin transport has long been suspected to be essential as a polar signal in embryo development. Applying IAA efflux inhibitor to *Brassica juncea* embryos interferes with embryo axis formation, occasionally generating ball-shaped individuals[23]. Interestingly, there is a phenotypically related *Arabidopsis* mutant, *emb30*/*gn*, in which vascular cells are entirely disconnected and randomly oriented (Table 1). Again, mutant seedlings can be ball-shaped, with no detectable apical–basal polarity and a cloud of randomly oriented vessel elements in the center[24]. Although these features indicate an essential function for *EMB30*/*GN* in plant cell polarity, a possible connection to auxin transport has become apparent only recently as a result of advances in the understanding of vesicle transport in yeast and plant cells.

Genetic analysis in yeast cells has identified essential components for targeted vesicle transport, among them the Ras-like GTPase, ADP ribosylation factor. This protein is required for the assembly of coatomeric transport vesicles and its activity is regulated through the action of a specific class of guanine exchange factors (GEFs) that can be specifically inhibited by the fungal metabolite brefeldin A (BFA). Interestingly, BFA has also been found to block auxin efflux but not influx in plant cells, suggesting that ADP ribosylation factor-mediated vesicle transport is required for proper localization of auxin efflux membrane proteins[25].

EMB30/GN is similar to one of the BFA-sensitive GEFs in yeast, GEA1. The *Arabidopsis* gene *EMB30*/*GN* can complement yeast *gea1* mutations and has a BFA-sensitive GEF activity[26]. These findings suggest that *EMB30*/*GN* GEF activity is required for vesicle

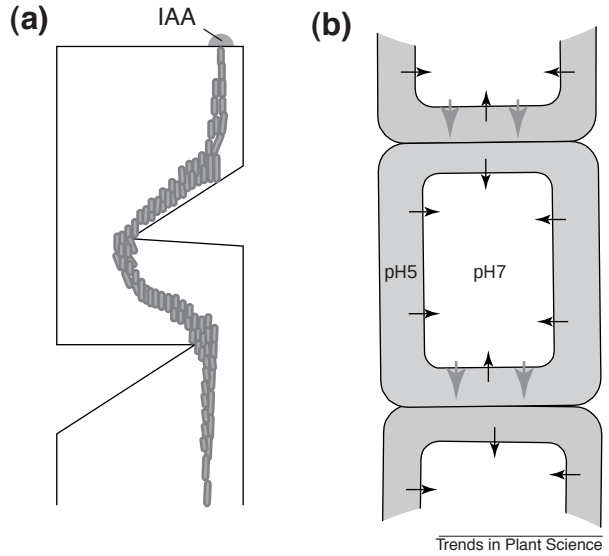

Fig. 2. Polar vascular response and auxin transport. Local application of indole acetic acid (IAA) can induce the formation of vascular strands in suitable organs, such as a bean hypocotyl[3]. The cellular response to local IAA application is non-isotropic: vascular differentiation occurs within a narrow, extremely elongated zone (i.e. a strand) that connects to the pre-existing vasculature. The route-selection process is highly flexible and generates continuous vascular strands even around lateral incisions (a). The hypothetical signaling process underlying continuous vascular differentiation is strictly dependent on polar auxin transport. (b) IAA is thought to be imported efficiently into the cell via proton symport as $IAAH + H^+$ (blue arrows). The process depends on a stable proton gradient across the plasma membrane, maintained by proton-pumping ATPases (not shown). At the higher intracellular pH, IAAH readily dissociates and efflux of IAA^- depends strictly on specialized efflux carriers (red arrows). The overall polar movement of IAA is attributed to the selective localization of efflux carriers on the basal plasma membrane.

transport-mediated localization of auxin efflux membrane proteins. A direct test of this prediction by immunolocalization of PIN1 revealed that PIN1 is indeed not polarly localized in either *emb30*/*gn* mutant or BFA-treated plant tissue, implicating targeted vesicle transport and EMB30/GN in the establishment of auxin transport polarity.

Defective auxin perception

Although polar localization of membrane proteins seems to be required for the directionality of auxin flow, proper auxin perception should be essential for triggering vascular differentiation. Consistent with this expectation, vascular abnormalities have been reported for auxin perception mutants[27], but it now appears that severe auxin insensitivity can be associated with embryo and/or seedling lethality and that therefore many mutants in this class might still be unidentified. Mutations in three *Arabidopsis* genes result in seedling lethality associated with defective auxin perception, vascular differentiation and embryo axis formation. This common complex phenotype suggests that there are related primary defects affecting the molecular machinery underlying the alignment of cell differentiation with the axis of auxin flow at various developmental stages.

Corresponding phenotypic and molecular evidence of a role for auxin signaling in cell axis formation has been obtained for one gene in this class, *MONOPTEROS* (*MP*) (Table 1). Development of *mp* mutant embryos is abnormal from early globular stages, and heart-stage embryos lack the central provascular cylinder[28]. Subsequently, embryo growth is restricted to cotyledons and the shoot apical meristem, and the hypocotyl and primary root meristem are not formed (Fig. 4). Seedling lethality can be bypassed by generating adventitious roots in tissue culture, enabling studies of post-embryonic stages. Throughout mutant development, vascular strands are discontinuous and incompletely differentiated. In leaves, the vascular system is reduced to its most central part, the primary vein and a few secondary veins[29]. Mutants show several additional features associated with defective auxin perception or transport, such as fused cotyledons, abnormal leaf positions and pin-shaped inflorescences.

The *MP* gene encodes a transcription factor with the domain characteristics of the growing family of auxin response factors (ARFs): a conserved DNA-binding domain near the N terminus, a central activation region and two highly conserved stretches close to the C terminus[30,31]. Most importantly, the DNA-binding domain appears to be able to bind to auxin response elements – short conserved sequences essential for the rapid auxin regulation of certain classes of auxin inducible genes[32]. The two conserved C-terminal domains are present not only in ARFs but also in the related family of short-lived, nuclear AUX/IAA proteins[33]. Unlike ARF genes, *AUX*/*IAA* genes are rapidly induced by auxin and the abundance of AUX/IAA proteins seems to reflect the strength of an auxin signal. The conserved C-terminal domains have been shown to mediate homotypic and heterotypic interactions of proteins from both families, suggesting that ARF and AUX/IAA gene products might form higher order nuclear complexes[34]. Thus, the specificity of auxin responses could be encoded in nuclear complex combinations formed by ARF and AUX/IAA products, some of which could specifically promote vascular differentiation and other cell differentiation aligned with the axis of auxin flow.

Another *Arabidopsis* gene, *AUXIN RESISTANT 6* (*AXR6*), mutates to a highly related phenotype[35] (Table 1). Alleles of *AXR6* were initially identified as dominant mutations conferring auxin insensitivity in adult plants. Heterozygous mutants are bushy, form fewer lateral roots than normal and exhibit auxin-insensitive root elongation. All these features are similar to *axr1* and other well characterized auxin response mutants, suggesting that *axr6* mutations interfere with signal transduction in a broad spectrum of auxin pathways. Most interestingly, *axr6* homozygous mutants are already defective from the earliest stages of embryogenesis, fail to produce hypocotyl and primary root and form a severely reduced vascular system (Fig. 4). The vascular system in mutant seedlings is often reduced to the cotyledon midvein and cotyledons can be fused. In short, homozygous *axr6* and *mp* mutants look similar, suggesting closely related primary defects.

The dominant auxin insensitivity of adult *axr6* heterozygous mutants has been studied in great detail and their association with embryonic defects in the respective homozygotes supports the importance of auxin signaling in embryo axis formation. One could speculate that *AXR6* encodes a positive regulator of vascular development similar to *MP*. However, *axr6*/*AXR6*/*AXR6* triploids are phenotypically similar to the heterozygous mutant, suggesting that it is the dose of the mutant gene product rather than of the residual wild-type product that determines the strength of the auxin response. Therefore, it should be interesting to see how the mutant gene product interferes with auxin signaling.

A third *Arabidopsis* mutant, *bodenlos* (*bdl*), has defects similar to but somewhat weaker than those observed in *mp* and *axr6* mutants[36] (Table 1). The vascular system is reduced and a hypocotyl of variable length ends in a basal peg rather than in a primary root meristem (Fig. 4). Similar to *mp*, cultured *bdl* mutants produce adventitious roots, but *bdl* mutants form relatively normal inflorescences with fertile flowers. With regard to

the bushy appearance of the adult plant as well as several auxin responses, *bdl* mutants resemble the auxin response mutant *auxin resistant 1* (*axr1*). Like *axr1* mutants, dark-grown *bdl* mutants do not form an apical hook, and are less sensitive to auxin with regard to hypocotyl swelling and callus formation. Intriguingly, *bdl–axr1* double mutants do not form a hypocotyl at all, and look similar to strong *mp* mutants. Thus, although *axr1* mutations are not associated with obvious embryonic defects, their impaired auxin perception enhances the embryonic defects in *bdl* mutants, suggesting that the *bdl* embryo defects reflect auxin functions in embryo development. As only a single allele of *bdl* has been recovered, it is possible that stronger alleles interfere with embryo viability.

How can auxin signal transduction genes control the alignment of a variety of cellular events with the direction of auxin flow? Two scenarios seem possible. First, genes such as *MP*, *BDL* or (indirectly) *AXR6* could act exclusively in incipient vascular tissues to control vascular differentiation in response to auxin. Vascular tissues, in turn, could then provide a scaffold system aligning numerous morphological features, such as cotyledon positions and meristem architecture relative to the plant axis. Alternatively, auxin flow could serve directly as an orienting signal in cellular events beyond the vascular system. Relay of this 'axializing' signal would depend on a specialized group of auxin signal transduction proteins that mediate oriented cell differentiation in embryos, organ primordia and, most critically, in vascular strands. The dynamic expression pattern of the *MP* gene, encompassing broad initial domains in embryos and meristems to become gradually restricted to provascular tissues supports the idea that auxin acts as an orienting signal beyond the vascular system[30], but current data are insufficient to exclude alternative interpretations.

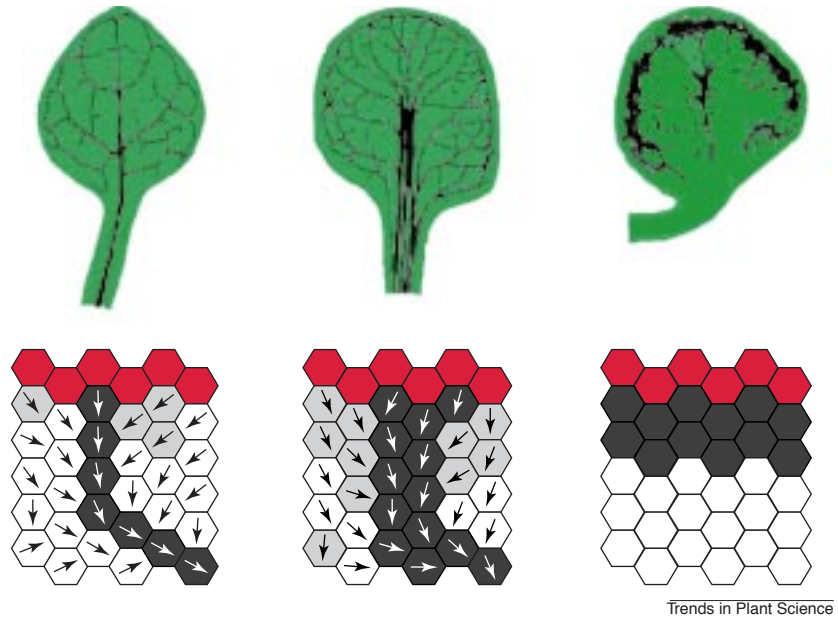

Fig. 3. Development of the *Arabidopsis* leaf venation pattern under conditions of reduced auxin transport[17]. The pinnate venation pattern in *Arabidopsis* rosette leaves (a) is transformed into an alternative pattern (b), which is characterized by strong continuous vasculature along the leaf margin, large numbers of centribasally oriented veins and multiple veins in the center. (c) Extremely strong auxin transport inhibition restricts vascular differentiation to the leaf margin. This response demonstrates that variant functional venation patterns (a,b) are generated depending on auxin transport properties of the leaf primordium. Interpretation of vascular responses: simple, highly variable venation patterns as in *Arabidopsis* could result from convergence of auxin flow emanating from auxin sources near the leaf primordium margin. Under normal conditions (a), auxin flow (arrows) from apical sources (red) would converge at subinductive concentrations of IAA (gray), and vascular differentiation (black) would therefore remain restricted to relatively few narrow zones. Under conditions of inhibited auxin transport (b), auxin would accumulate to inductive levels at more positions and, upon strong auxin transport inhibition (c), remain restricted to the presumed peripheral IAA source region. Specific features, such as the width of the central venation and vascular differentiation parallel to the margin, were attributed to features in the cellular organization of the early leaf primordium[17].

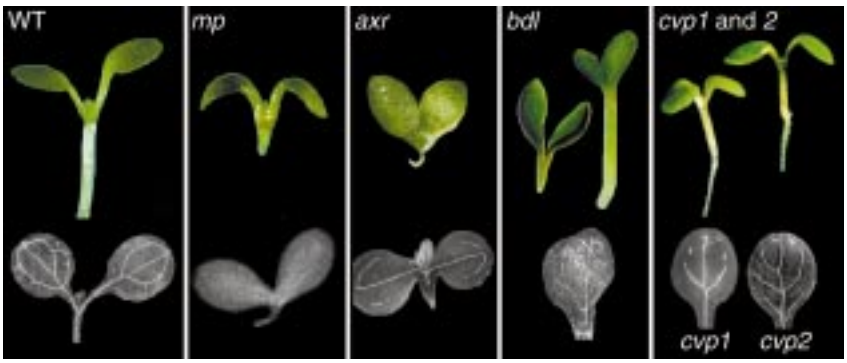

Fig. 4. General cell-axis defective mutants and mutants with impaired vascular continuity at the seedling stage. Mutations in *MP*, *AXR6* and *BDL* result in concomitant defects in the embryo axis and in the vascular system. The vascular system is strongly reduced and the hypocotyl–root axis is missing in *mp* and *axr6* mutants, and the severity of these defects is somewhat variable in *bdl* mutants. All three mutants are also compromised in certain auxin responses, indicating a common mechanism underlying oriented differentiation along the axis of auxin flow. By contrast, impaired vascular continuity in *cvp1* and *cvp2* cotyledons is not associated with embryo axis or known auxin perception defects. Mutations in *cvp1* and *cvp2* could therefore identify auxin-independent mechanisms important for vascular strand formation. *Photographs for* axr6, bdl *and* cvp *mutants reproduced, with permission, from Refs 35, 36 and 38, respectively.*

Genetic screens for vascular pattern mutants

Direct screens for vascular pattern mutants are not biased by associated embryo or auxin perception defects and might thus identify further signaling mechanisms in vascular development. However, direct inspection of vascular tissues is labor intensive and genetic analysis is thus far from saturation. Consequently, there is no reliable estimate of the number of genes involved in vascular tissue patterning in *Arabidopsis* or any other plant species. A survey of leaf venation patterns in more than 250 *Arabidopsis* ecotypes identified numerous abnormal leaf shape variants with associated aberrant venation patterns but only one ecotype with altered venation within otherwise normal leaves[37]. The reduced venation density in ecotype *Ei-5*

has been attributed to a single locus [*HEMIVENATA* (*HVE*)] and it will be interesting to explore the genetic relationship of *hve* with the auxin perception and axialization mutants discussed above (Table 1). The scarcity of specific venation mutants further raises the possibility that the genetic complexity of the *Arabidopsis* venation pattern is relatively low and that mutant screens in species with complex venation patterns could be more rewarding. Alternatively, mutations in certain patterning functions could be redundantly encoded or associated with early lethality.

Cotyledon vascular patterns, being simple, reproducible and readily visible after germination, are well suited to large-scale mutant screening for leaf vascular mutants. Mutations in two genes, *COTYLEDON VASCULAR PATTERN 1* and *2* (*CVP1* and *CVP2*, respectively), also affects vascular patterns and anatomy in other organs but not the overall morphology of mutant leaves[38] (Table 1). Both mutations appear to interfere with vascular development from early stages and both affect xylem and phloem. Auxin content, transport and perception did not appear to be compromised in *cvp* mutants, suggesting that these defects are not further manifestations of impaired auxin flow.

The vascular defects in the two mutants are clearly distinguishable. Although no abnormalities at the level of individual vascular cells are observed in *cvp2* mutants, cell elongation and alignment are affected in vascular strands of *cvp1* mutants. Xylem strands in *cvp1* cotyledons are wider and contain more tracheary cells. By contrast, *cvp2* mutations are associated with reduced numbers of vascular cells along the length of a vascular strand. This suggests that the main function of *CVP1* is to integrate oriented differentiation and cell elongation, and that *CVP2* promotes vascular differentiation and thereby prevents premature vein termination. Distorted vascular strands in rosette leaves of an earlier identified mutant, *lop1*, are associated with reduced auxin transport and gross morphological defects[39] (Table 1). For all three genes, identification of the gene products and ensuing cell biology will probably be the most direct way to understand their potential function in vascular tissue patterning.

Another type of mutant screen, cross-sections of inflorescence stems, can identify mutants with abnormal tissue composition and vascular bundle organization[40,41]. The most noticeable defect in *interfascicular fiberless 1* (*ifl1*) mutants is the absence of fibers between stem vascular bundles (Table 1). Although this feature suggests a function for the gene in fiber differentiation, other results indicate that *IFL1* is also expressed and required in the vascular bundles[42]. In mutant vascular bundles, secondary xylem is reduced or absent. Defects in vascular bundles are more pronounced towards the base of the stem, which could indicate the involvement of auxin flow. Because of the pleiotropic effects of both meristem and auxin signaling defects, the primary defect in *ifl1* mutants is not clear. Furthermore, *IFL1* has been found to be allelic with *REVOLUTA* (*REV*), a gene implicated in apical meristem development[43]. The issue might be resolved at the molecular level, because *REV*/*IFL1* encodes a group III HD-ZIP transcription factor, which should eventually allow its function to be assigned to a particular signaling pathway.

Interestingly, another group III HD-ZIP gene, the *Arabidopsis homeobox gene 8* (*AtHB8*) appears to have an important role in vascular development. *AtHB8* is expressed early in provascular differentiation and is inducible by externally applied IAA (Ref. 44). AtHB8 could therefore act as a positive regulator of provascular differentiation in response to localized auxin signals. In the absence of loss-of-function mutations in *AtHB8*, this view is supported by the fact that ectopic overexpression of the gene leads to the formation of excess xylem tissue[45]. Interestingly, *AtHB8* expression also marks the path of provascular differentiation during regenerative vascular development. When IAA is applied in experiments similar to the one shown in Fig. 2, *AtHB8* expression is turned on in narrow domains beneath the IAA source. The expression of the auxin inducible *AtHB8* gene in all instances of provascular differentiation supports the notion that auxin signaling is essential not only in regenerative but also in normal vascular development.

Prospects

A pivotal role has long been suspected for auxin in plant cell axis formation and vascular development[3], and the cellular details are now becoming experimentally tractable owing to simultaneous advances in various fields. The molecular identity of recently discovered genes, their correlated mutant phenotypes and new experimental approaches have together generated a conceptual framework for auxin action in vascular patterning, in which model predictions can now be tested at the molecular level. The emerging picture implicates genes involved in vesicle trafficking, auxin transport and auxin regulated gene expression in the establishment of plant cell polarity and oriented differentiation, which are in turn needed for embryo axis formation and aligned vascular differentiation.

The next step will almost certainly be the identification of further components in this interplay and of genes contributing to vascular patterning through auxin-independent mechanisms. It will be particularly intriguing to see how different types of controls are integrated. Recent findings in *Arabidopsis* might provide a precedent for the interaction of auxin-independent and auxin-dependent mechanisms. The pattern of *Arabidopsis* root meristem appears to be controlled not only by numerous specific cellular interactions[46] but also by the position of a distal, auxin-dependent organizer[47]. In an optimistic scenario, molecular genetics could soon provide a detailed concept of how local auxin signals can induce continuous vascular differentiation, which could later become integrated into a molecular understanding of tissue patterning in each plant organ.

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