

Vascular Patterning: More Than Just Auxin? Dispatch

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The plant hormone auxin has long been known to play a pivotal role in vascular patterning and differentiation. But auxin is not the whole story: recent genetic analyses have identified additional factors required for vascular patterning, one of them involving sterols.

Plants exhibit characteristic vascular patterns in the stem and in leaves [1,2]. A large body of evidence points to the growth regulator auxin having a central role in vascular patterning [3–5]. But a number of recently described mutants with defective vascular patterning do not exhibit obvious defects related to auxin [6,7]. Do these mutants reveal patterning mechanisms that are independent of auxin function?

During plant development, auxin induces the formation of vascular strands [3,4], along which auxin is subsequently transported in a polar fashion from the shoot towards the root [8]. These findings led Sachs to formulate the so-called ‘canalization hypothesis’ [3,4]: this states that cells that experience elevated levels of auxin are induced to absorb more auxin from adjacent tissues, and to transport it downwards more efficiently than their neighbours (Figure 1A,B). This leads to the accumulation of auxin in narrow cell files. The continuous polar flow of auxin through these cells induces them to differentiate as vascular strands. Support for the canalization hypothesis comes from the analysis of vascular development in plants in which auxin transport is defective, either because of a mutation in the putative auxin efflux carrier PINFORMED1 (PIN1), or because the plant was treated with chemical transport inhibitors (Figure 1C–F) [9–11].

A role for canalization of auxin flow in vascular patterning is plausible where vascular strands develop progressively, as in the case of the major veins. In the interstitial spaces between major veins of an expanding leaf, however, networks of minor veins often appear to be formed simultaneously. In such cases, vascular patterning might be controlled by a reaction–diffusion mechanism [12]. Such a mechanism is based on a short-range autocatalytic activator of vascular differentiation and a long-range inhibitor of the same process (released from the activated cells). The combination of short-range activation and long-range inhibition results in the amplification of small random differences from an initially unpatterned situation. Mathematical modelling of reaction–diffusion models can recreate reticulate patterns like the ones found in leaf vasculature [1].

In the case of vascular development in plants, candidate activator and inhibitor molecules that might

mediate such a patterning mechanism have not yet been identified, but formally, auxin transport might be responsible for both functions. The accumulation of auxin in cells with elevated auxin levels would lead to short-range autocatalytic activation, whereas depletion of auxin from surrounding tissues would result in long-range inhibition of vascular differentiation. Depending on the conditions, canalization and reaction–diffusion mechanisms might thus be two sides of the same coin. Indeed, mutants with defects in auxin transport or auxin response exhibit aberrant vascular patterning in minor, as well as major veins [9,13–15]. Furthermore, an auxin reporter gene (DR5-GUS) was found to be expressed in all vein classes, first in the major, and later in the minor veins (Figure 1G) [11].

Besides *PIN1*, the genes *MONOPTEROS (MP)*, *BODENLOS (BDL)* and *AUXIN-RESISTANT6 (AXR6)* – all of which play a part in the auxin response – are required for vascular patterning (reviewed in [5]). Are additional factors involved? To answer this question, several groups have initiated systematic genetic screens in *Arabidopsis*. Screening for mutants with abnormal vascular patterns in cotyledons or leaves has yielded mutants such as *lopped1 (lop1)* [16] and *scarface (scf)* [17] in which patterning defects coincide with changes in auxin transport capacity and auxin sensitivity, respectively. In some of the vascular patterning mutants, however, auxin transport and responsiveness were not affected, namely *cotyledon vascular pattern1* and *2 (cvp1* and *cvp2)* [6]. Another series of mutants, the vascular network mutants (*van1* through *van7*), have not yet been tested for their auxin transport and response capacities [18].

The recent cloning of *CVP1* brought an unexpected new player onto the stage. *CVP1* encodes sterol methyltransferase 2 (SMT2), an enzyme in the sterol biosynthetic pathway [19]. *cvp1* mutants exhibit decreased levels of sterols, and have cotyledons with reduced and poorly connected vascular systems (Figure 2A,B). This implies that sterols are required for correct vascular patterning, though their precise function remains unknown. Sterols might function as specific patterning signals or, alternatively, sterols in the membrane might fulfill structural requirements for the assembly and/or function of membrane proteins involved in patterning.

Are sterols critical for the functioning of auxin transport proteins? Interestingly, a gene related to *CVP1*, *ORC*, which encodes sterol methyltransferase 1 (SMT1), is required for the correct subcellular localization of the auxin efflux carriers PIN1 and PIN3, and for normal auxin distribution in the root [20]. *orc* mutants exhibit various auxin-related defects, revealing a requirement for sterols in auxin-dependent patterning. In contrast, *cvp1* mutants exhibit normal responsiveness to auxin in roots and normal auxin transport capacity in the stem [6], though a specific defect in auxin response or transport in their cotyledons – the

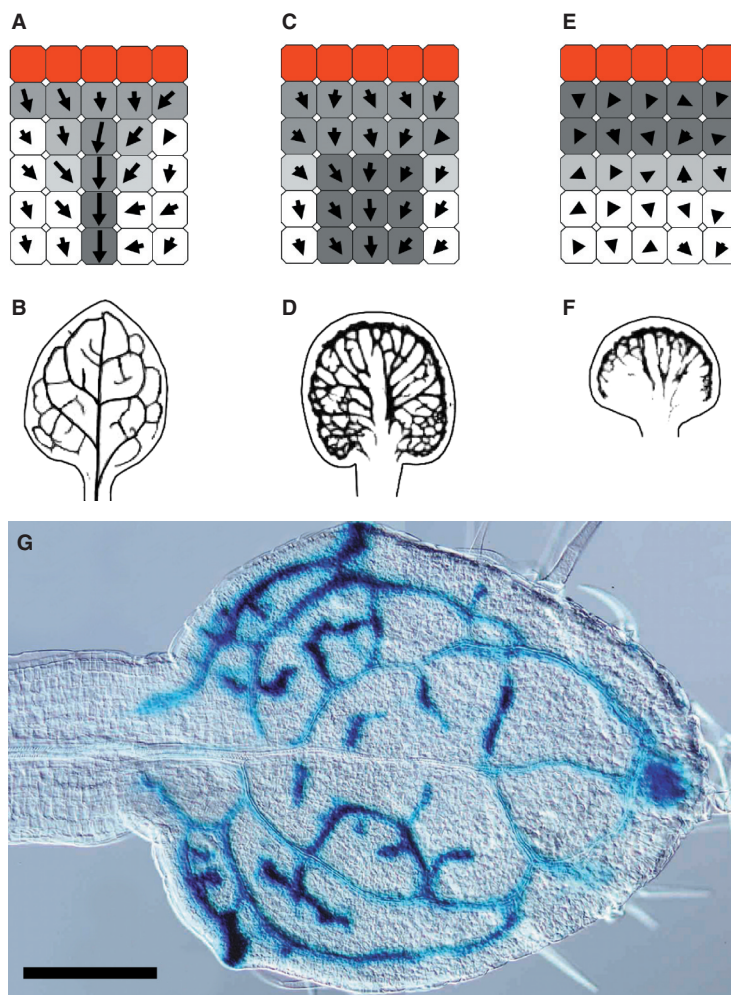


Figure 1. The role of auxin in vascular patterning of leaves.

(A) Role of auxin canalization in the determination of a vascular strand. Auxin-producing cells (red) are envisaged to reside at the leaf margin. Neighbouring cells in the second row transport auxin downwards. Cells with higher auxin levels are induced to accumulate more auxin, and to transport it downwards more efficiently (central column of cells). This positive feedback mechanism amplifies even small random differences in auxin levels. The resulting ‘canalization’ leads to the formation of distinct, narrow cell files that differentiate to vascular strands. (B) *Arabidopsis* leaf with a normal vascular pattern. (C) Weak inhibition of polar auxin transport leads to less efficient canalization, resulting in wider auxin-conducting strands. (D) *Arabidopsis* leaf treated with low concentration of an inhibitor of polar auxin transport. The vascular strands are thicker and strongly developed at the leaf margin, but less in the centre and the base of the leaf. The leaf vascular system is not connected to the stem through the petiole. (E) Strong inhibition of polar auxin transport results in accumulation of auxin in cells adjacent to the auxin-producing cells. (F) *Arabidopsis* leaf treated with a high concentration of an inhibitor of polar auxin transport. Vascular differentiation is restricted almost completely to the margin of the leaf. (G) Developing *Arabidopsis* leaf transformed with the auxin sensitive DR5–GUS reporter: GUS activity coincides with developing vascular strands. Bar = 200 μm. Parts A–F adapted with permission from [9] (©Company of Biologists); (G) adapted with permission from [11] (©American Society of Plant Biologists).

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only organ with a vascular phenotype in *cvp1* mutant plants — cannot be ruled out.

The recent study by Parker *et al.* [7] specifically addresses vascular patterning in the stem of *Arabidopsis*. Mutant screening by visual inspection of stem cross sections led to the identification of the continuous vascular ring (*cov1*) mutant. Instead of discrete vascular strands, this mutant exhibits wide strands that frequently extend over most of the circumference of the stem (Figure 2C,D) [7]; vascular patterning in the leaves remains normal, however. In the *cov1* mutants, ectopic differentiation of vascular strands is already evident in young tissues at the shoot apex, where vascular pattern is laid down. The mutant phenotype is thus due to a defect in primary vascular patterning, and not secondary overproliferation of vascular strands.

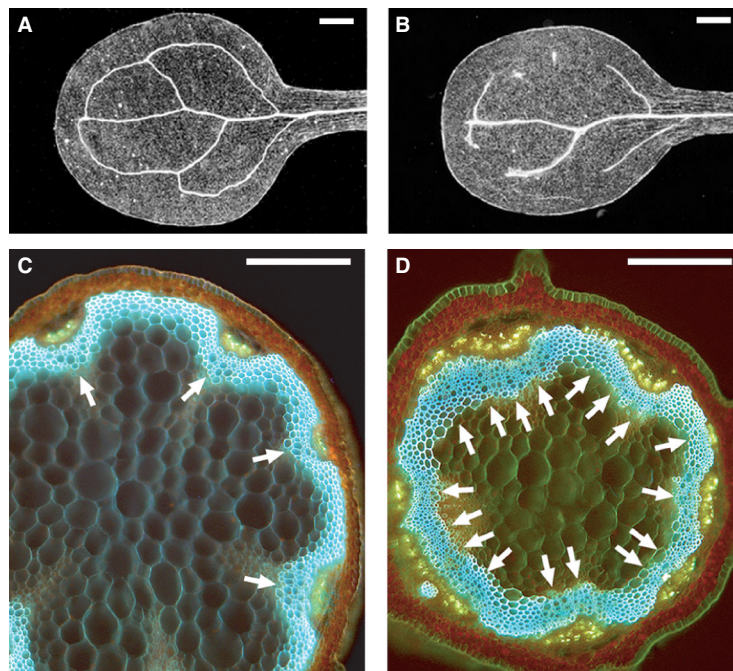
COV1 encodes a predicted membrane protein with three membrane-spanning domains. Although there is a related gene in rice, *COV1* exhibits no homology to any gene of known function. *COV1* homologues can also be found in bacteria, indicating involvement in a process conserved between plants and bacteria. Given the loss-of-function phenotype of *cov1* mutant plants — ectopic vascular differentiation — the *COV1* protein

might be involved in the generation, transport or perception of a signal molecule that negatively regulates vascular differentiation in the stem. Overproliferation of vascular tissues also occurs in plants in which polar auxin transport is inhibited either chemically, or by mutation of the PIN1 efflux carrier [9]. Unfortunately, it is not known whether the *cov1* mutation affects polar auxin transport in the stem. If the *cov1* mutant phenotype were associated with a defect in auxin transport, then *cov1* stems should exhibit decreased auxin transport, despite the existence of excess vascular tissues.

An interesting question is whether the organ-specific phenotypes of mutants such as *cvp1* and *cov1* reflect fundamental differences in vascular patterning of the various plant parts. Alternatively, we may reach a unified picture in which a general patterning mechanism is controlled by gene families with members that have at least partially redundant functions but exhibit organ-specific expression so that their mutations cause organ-specific phenotypes. To distinguish between these two possibilities, the analysis of multiple mutants in the small gene families of *SMT2* and *SMT3*, as well as *COV1* and the related *LCV1*–*LCV3* genes will provide important information.

Figure 2. *Arabidopsis* mutants with altered vascular patterning.

(A) Wild-type *Arabidopsis* cotyledon. (B) Cotyledon of the *cvp1-1* mutant. (C) Cross-section of a wild-type *Arabidopsis* stem. Light blue fluorescence indicates xylem and interfascicular fibers, yellow fluorescence indicates phloem strands (arrows). (D) Cross-section of a *cov1* mutant stem with largely expanded phloem strands (arrows). Bars = 250 μ m. A and B adapted with permission from [19] (©American Society of Plant Biologists); C and D adapted with permission from [7] (©Company of Biologists).



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Auxin is thus a major determinant of vascular patterning in all plant parts. But although auxin is at the centre of vascular patterning, it is clearly not the sole player on the stage. Additional factors are required for vascular patterning, among them sterols. The challenge now is to understand how factors such as sterols and the membrane protein COV1 are integrated in auxin-mediated vascular patterning. Further factors are likely to emerge from the cloning of genes such as SCARFACE, CVP2 and the VAN genes.

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