Vascular Patterning: More Than Just Auxin?

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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 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 cycledon 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
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 cvv1 mutant stem with largely expanded phloem strands (arrows). Bars = 250 µm.

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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.

References