E2. The Induction of Vascular Tissues by Auxin

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INTRODUCTION

The vascular system connects the shoot organs with the roots and enables efficient long-distance transport between them. In higher plants it is composed of two kinds of conducting tissues: the *phloem*, through which organic materials are transported and the *xylem*, which is the pathway for water and soil nutrients. In angiosperms, the functional conduits of the *phloem* are the *sieve tubes*, and the most specialized conduits of the *xylem* are the *vessels* (2). Vascular development in a plant is an open type of differentiation, continuing as long as the plant grows from apical and lateral meristems. The continuous development of new vascular tissues enables regeneration of the plant and its adaptation to changes in the environment. The differentiation of vascular tissues along the plant is induced and controlled by longitudinal streams of inductive signals (4, 52). In spite of the structural and developmental complexity of vascular tissues (47, 51), there is evidence that the differentiation of both the vessels and the sieve tubes is induced by one major hormonal signal, namely, the auxin indole-3-acetic acid (IAA), produced mainly by young leaves (11, 33, 39). Such evidence raises endless questions as to how this hormonal signal induces and controls complex patterns of xylem and phloem, and emphasizes the need to understand where and how the IAA signal is produced and transported, what are the mechanisms that control the formation of various cell types, their relationships, dimensions, differentiation and maturation patterns in the vascular system. Nevertheless, it should be emphasized that additional growth regulators may influence vascular differentiation, such as cytokinins which increase the sensitivity of the vascular cambium to auxin (3, 4), gibberellin that induces fibers (2, 47), and ethylene which reduces vessel diameter and retards fiber formation (12). These hormonal signals are beyond the scope of this article and the reader is directed to reviews on these topics (2, 36, 51, 58).

Molecular genetic approaches, using Arabidopsis as a major model system, have substantially increased interest in the subject and have boosted our understanding in the processes of vascular differentiation and vein pattern formation. However, these analytical tools, which have already yielded

impressive contributions (17, 56, 61), are in an early phase of uncovering new genes and identifying their role in vascular differentiation. They promise to provide new insights on the mechanisms that control organized vascular development. The models by which we can currently explain vascular pattern formation are limited (4, 11, 17, 23, 43, 52, 56). Hence, there is need for additional analysis at the molecular, cellular and organismic levels, which should be integrated for better understanding (4, 43, 52).

Recently, the sites of auxin production in shoot organs were discovered and their role in vein pattern formation has been demonstrated (4, 11). Additional basic unexplained vascular patterns (e.g., diameter changes in the primary vessels of roots and internal phloem formation in bicollateral bundles) require better understanding of the role of auxin transport pathways in organized vascular differentiation. Likewise, also, are possible changes which may have occurred during plant evolution in the pathways of auxin in the vascular tissues: from transport through the active cambium of trees to transport through xylem parenchyma in the advanced herbaceous species in which cambium activity is minimized, or absent (in most of the monocots). Another key issue in vascular differentiation that requires clarification is the process of xylem maturation during bundle formation, which should not be confused with the induction of vascular bundles. Xylem maturation, which is characterized by the initiation and progression of discontinuous basipetal and acropetal patterns, will also be analyzed here because of its importance for correct interpretations.

The aim of this chapter is to present general concepts on the induction and control of vascular tissues by IAA. It provides a summary of major topics in organized vascular differentiation and the recent advances made in each. I start with recent findings on the sites of auxin production and accumulation, then proceed to propose a new hypothesis for elucidating IAA transport pathways, and finally focus on the role of auxin in controlling basic patterns of xylem and phloem formation in plants.

SITES OF AUXIN PRODUCTION

IAA Production and Vein Pattern Formation in Leaves

The pioneering study of Jacobs (33) demonstrated that auxin produced in young leaves is a limiting and controlling factor in xylem regeneration around a wound. Although fifty years have elapsed since Jacobs' (33) discovery, until recently there was no physiological or molecular understanding of how and where auxin is synthesized in a young leaf and how this regulates vascular differentiation and venation pattern formation (4, 56). In an attempt to confront this challenge, auxin transport inhibitors were applied to Arabidopsis plants (40, 53) revealing that the inhibitors restricted the vascular tissues to the leaf margin, indicating that vascular tissues in a leaf depend on an inductive signal, likely IAA, from the leaf margin.

To explain how auxin induces vascular patterns in leaves, I proposed (4)

that during primordium development there are orderly shifts in the sites and concentrations of auxin production, and it is these shifts that control vein pattern formation in a leaf. Supporting evidence shows that an exogenous IAA source applied along a cut made in a very young cucumber cotyledon induced tracheary element patterns as in the hydathodes (4), thus mimicking the role of differentiating hydathodes as the primary sites of auxin production in leaves.

RT-PCR analysis of the mRNA expression of enzymes involved in auxin metabolism and transport (indole-3-glycerol synthase, nitrilase, IAA-amino acid hydrolase, chalcone synthase and PIN1) has revealed a succession of auxin production events during leaf-primordium development, starting with *de novo* build-up of a massive bound-IAA pool in the youngest primordia before vascularization (11), and from this bound-IAA reservoir the free IAA is later released by hydrolysis in a gradual basipetal pattern (Fig. 1).

Visualization of total auxin distribution (both free and conjugated auxin) in Arabidopsis leaf primordia, by immunolocalization with specific IAA antibodies and fluorescent secondary antibody conjugates, demonstrate high auxin concentrations in the chloroplasts of the mesophyll and the guard cells (11), suggesting that chloroplasts play a major role in auxin biosynthesis and metabolism.

Localization of the bioactive auxin, namely, free IAA, can be visualized in leaf primordia by auxin-response element-*GUS* expression in the DR5 Arabidopsis transformant (59). Analysis of the youngest shoot region of the DR5 Arabidopsis reveals that the promeristem and the youngest meristematic

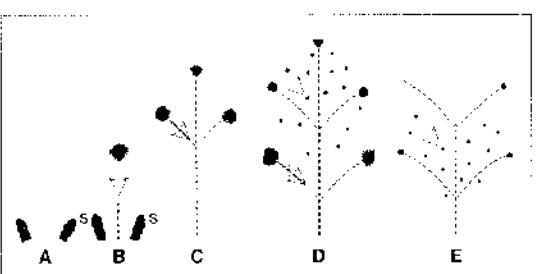

Figure 1. Schematic diagrams showing the gradual changes in sites (black spot locations) and concentrations (black symbol sizes) of free-IAA production during leaf primordium development in Arabidopsis. The arrows show the experimentally confirmed direction of the basipetal polar IAA movement, descending from the differentiating hydathodes (B-D). Recent incision experiments using the DR5 Arabidopsis transformant (R. Aloni, unpublished data), revealed that in a young leaf primordium, although the midvein matures acropetally (B), it is induced by the basipetal polar IAA flow (arrow) originating in the primordium tip. Arrowheads show the location of low free-auxin production sites in the lamina (D-E). The ontogeny of the midvein and secondary vascular bundles is illustrated by broken lines (marginal and minor veins are not shown). A. Early high auxin production occurs only in the stipules (s) of a very young leaf primordium, before auxin is detectable in the tip and prior to midvein differentiation. B. Primary auxin production in the tip of a fast growing primordium induces acropetal midvein differentiation, and illustrating leaf apical dominance. C. High primary auxin production at the differentiating hydathodes of the upper lobes inducing differentiation of the upper secondary vascular bundles. D. High primary auxin production at the differentiating hydathodes of lower lobes inducing the lower secondary bundles, and randomly distributed sites of secondary auxin production in the lamina. E. Maintenance low auxin production in hydathodes and secondary auxin production in the lamina during a late phase of leaf development.

leaf primordia do not show free-IAA production (do not show GUS activity) (11, 13), although these primordia are loaded with bound IAA (11). Stipules, which are outgrowths of a leaf primordium, are the earliest sites of intense free-IAA production (Fig. 1A,B). In a more developed leaf blade, hydathodes, the water secreting glands, which develop in the tip and later also in the lobes (Figs. 1B-E, 2A), are the primary sites of free-auxin production, while trichomes (Fig. 2B) and mesophyll cells (11) are the secondary sites. During early stages of primordium development, an apical dominance within the leaf is evidenced by strong *DR5::GUS* expression in the elongating tip (Fig. 1B), possibly suppressing the production of IAA in the leaf tissues below it (11). During leaf-primordium development there are gradual shifts in the sites and concentrations of free-auxin production, progressing from the elongating tip (Fig. 1B), continuing downward along the expanding blade margins (Fig. 1C-E) and ending in the central regions of the lamina (Fig. 1D,E) (11, 39). This IAA pattern was confirmed by auxin analysis by GC-MS/MS techniques (42). These successive IAA shifts are presumed to control the basipetal development of the primordia and vein pattern formation (see below; Fig. 7) in Arabidopsis leaves (11). The intense production of IAA in the differentiating hydathodes (Fig. 2A) induces the midvein and the secondary bundles (Fig. 1B-D), while low auxin levels produced by developing trichomes (Fig. 2B) and mesophyll cells induce the minor tertiary and quaternary veins as well as the freely ending veinlets (11).

IAA Production and Vascular Differentiation in Flowers

I suggest that, as in foliage leaves (4, 11, 39), free-auxin production starts at the tip of each floral organ – so that vascular tissues develop basipetally, from the site of IAA production downward. Experimental evidence obtained with the DR5 Arabidopsis transformant supports this suggestion (R. Aloni et al., unpublished data; (R.A.)). The stigma is a site of high IAA production (Fig. 3B,C), and illustrates how a major source of IAA induces immediately below it a wide fan pattern of the stylar xylem. This fanning xylem is characterized by a decreasing density of vessel elements from the auxin-producing stigma downward, as predicted from the decreasing auxin gradient down the style (see below; 9). This vascular tissue below the stigma supplies the water required for pollen hydration (Fig. 3C).

IAA Production in Decapitated Organs

Experimental analysis of *DR5::GUS* expression patterns in decapitated shoot organs (e.g., Arabidopsis stamens, gynoecia, and inflorescence internodes) shows that the distal parenchyma cells, located immediately below the cut, start to produce free auxin (R.A.). This demonstrates that the upper cells of decapitated shoot organs become active sites of free-IAA production.

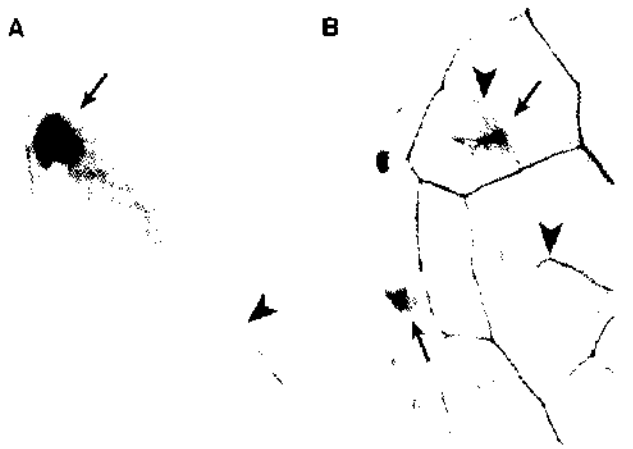

Figure 2 (Color plate page CP13). Free-IAA production detected by *DR5::GUS* gene expression (the blue GUS staining) in transformed Arabidopsis, showing histochemical localization of GUS activity during leaf morphogenesis. A. Center of strong *GUS* expression (arrow) in a developing hydathode, from which a decreasing pattern of the blue staining marks the auxin pathway towards the differentiating secondary bundle (arrowhead), x150 (x200 in color plate). B. *GUS* expression at the base of a few trichomes (arrows). Two of the trichomes are associated with freely ending veinlets (arrowheads), x30 (x40 in color plate).

Figure 3 (Color plate page CP13). Free-IAA production detected by *DR5::GUS* expression in transformed Arabidopsis, showing localization of GUS activity during flower morphogenesis. All photographs x 65. A. Young flower bud, prior to detectable IAA production and vascular development (arrow) in the gynoecium. At this developmental stage, the stamens produce high auxin concentrations (stained dark blue). B. In a young gynoecium, free-IAA production (detected by blue *GUS* expression) in the stigma induces the central bundle and an early stage of xylem fan formation (arrow) below the stigma. C. Mature gynoecium with germinating pollen grains (stained dark blue). A typical well developed wide fan xylem pattern (arrow) descending into two central bundles was induced by the IAA-producing stigma (stained blue). The discontinuous short veinlets (arrowheads) induced by the ovules do not connect with the gynoecium's central bundles, which are well supplied with auxin produced by the stigma.

SITES OF AUXIN ACCUMULATION

When either a young leaf or a young inflorescence of Arabidopsis is removed from the plant and kept in humid conditions, free IAA starts to accumulate at the petiole or the basal internode. This auxin accumulation was detected by *DR5::GUS* expression (R.A.). Similarly, after removing a distal part of the main root from a young Arabidopsis plant, free IAA starts to accumulate at the root base, above the cut (R.A.). These observations are in accordance with normal auxin accumulation observed at the tip of the main root, or the tips of emerging lateral roots (11, 49). Recently, a model involving an active AtPIN4-dependent sink for auxin in the columella region was suggested, possibly implying that auxin gradients are sink driven (26, 27).

THREE PATHWAYS FOR AUXIN TRANSPORT ALONG THE PLANT

The molecular mechanisms that control IAA transport are discussed in chapter E1. Here the focus is on the routes of IAA transport which are complex and poorly understood. A detailed analysis of IAA routes is required for understanding the mechanisms controlling basic patterns in vascular systems, which will be discussed below. Auxin produced in young leaves moves polarly in the vascular tissues (4, 47, 51), specifically along their cambium (39, 54, 57), and through the xylem parenchyma (18, 28, 44), starch sheath and the root pericycle (27). In addition, there is evidence for rapid non-polar movement of the hormone through sieve tubes. This non-polar auxin flow in the phloem conduits originates in mature leaves (41). Furthermore, there are indications that auxin flows in the epidermis (16, 27, 55). Surprisingly, these fragments of important information have never been incorporated into a general concept for explaining where IAA moves in the plant body. This confusing situation hinders proper analysis of new findings, which could consequently result in incorrect interpretations. I propose that free auxin flows along three main pathways in the entire plant. These three pathways, and how they lead to differences in the control of development, differentiation and IAA activity along these routes, will be described bellow.

All living cells in the plant body are capable of transporting auxin, but only those through which IAA is canalized become specialized to transport the hormone rapidly, resulting in canalized files of cells (51). During plant development, initial auxin flows are canalized into three main routes of IAA transport. This canalization starts during embryogenesis. The first two pathways induce and control the vascular and protective tissues, while the third pathway controls the activity of the phloem conduits.

(1) The *internal route* – which is the most complex pathway, and can be subdivided into the following longitudinal components (Fig. 4): primary shoot (I), primary root (III), and secondary body (II) which might be produced between the primary parts in woody dicotyledonous and

gymnosperm species. Each of these components has its unique anatomy and physiology as follows:

In the primary shoot (I), IAA moves polarly through the vascular bundles in three distinct streams: (i) via the vascular meristem (M), namely, the procambium or early stages of cambium, (ii) in the surrounding (B) bundle sheath at the phloem pole, and (iii) through parenchyma cells at the (X) xylem pole.

In the primary root (III), IAA moves polarly in the vascular cylinder through: (i) the vascular meristem (M), and (ii) the pericycle (Pe).

In the secondary body (II), the polar IAA streams descending from the primary shoot (I) merge into one pathway, which occurs in the cambium (C) and differentiating vascular elements.

(2) The *peripheral route* – which courses through the protective and adjunct tissues. The auxin in this pathway originates in the epidermal cells, like the auxin-producing trichomes (Fig. 2B) and stomata, and moves polarly towards the root tips through the epidermis (E) and subepidermal cell layers, and the phellogen (Ph) that produces the cork.

(3) The *non-polar route* – which courses in the phloem conduits, where IAA moves rapidly up and down via the sieve tubes (S). This fast auxin flow should be considered as a house-keeping signal that controls callose levels in the sieve tubes (3, 10).

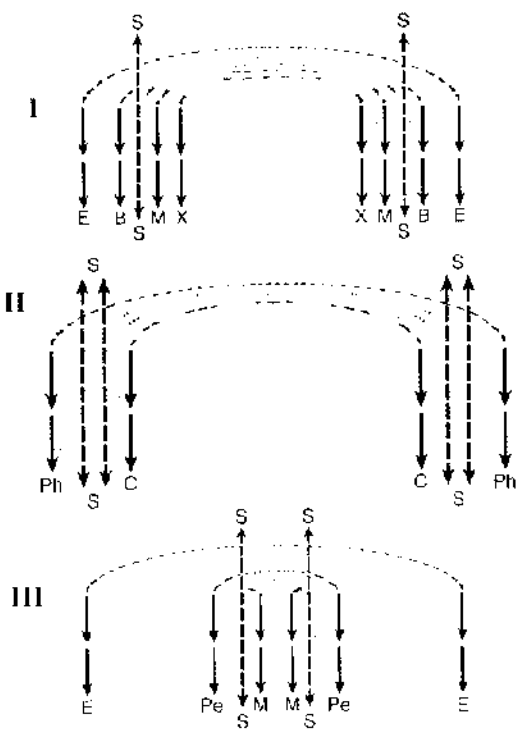

Figure 4. Schematic diagrams showing free-IAA transport pathways in the primary shoot (I), the secondary body (II), and the primary root (III). In the **internal route**, IAA moves polarly through the (M) vascular meristem (in the primary body), which is the (C) cambium in secondary body, the (X) xylem parenchyma cells, the (B) bundle sheath (in primary shoot) and the (Pe) pericycle (in primary root). In the **peripheral route**, IAA moves polarly through the (E) epidermis and the (Ph) phellogen. In the **non-polar route**, IAA moves up and down in the (S) sieve tubes.

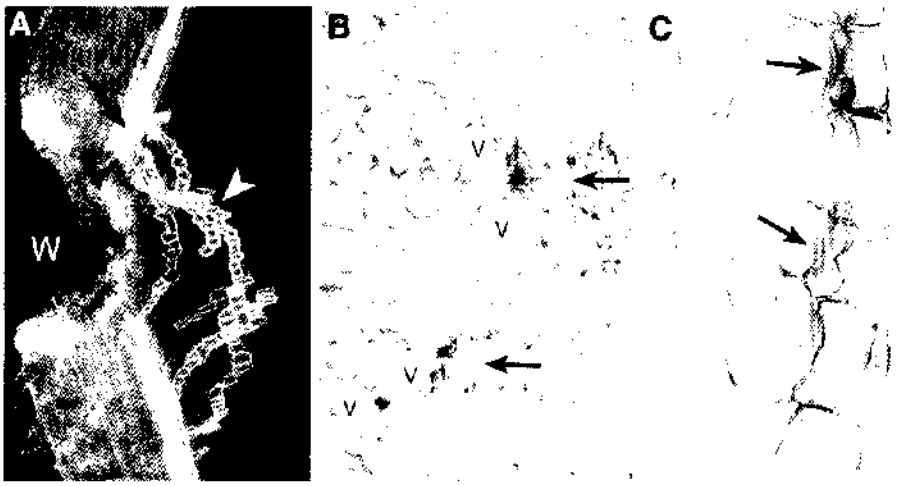

Figure 5 (Color plate page CP14). Pathways of auxin transport detected by the pattern of xylem regeneration (white arrowhead) around a wound (W) in a decapitated young internode of *Cucumis sativus* treated with auxin (0.1% IAA in lanolin for 7 days), which was applied to the upper side of the internode immediately after wounding and removing the leaves and buds above it (A), and by *DR5::GUS* expression (blue GUS staining) in an intact hypocotyl of a 3-month-old transformed Arabidopsis (grown under short day conditions), showing localization of GUS activity in the vascular cambium (B) and the phellogen (C). A. Longitudinal view of a polar pattern of xylem regeneration induced by the basipetal polar flow of auxin, observed after clearing with lactic acid and staining with phloroglucinol, x 25. The photograph shows a typical polar regeneration characterized by a dense appearance of regenerative vessel elements (arrow) immediately above the wound that differentiated close to the wound surface. Below the wound there are a few defined files that connect to the damaged bundle at greater distances from the wound. The pattern of xylem regeneration follows the path of auxin movement around the wound. As vascular bundles are preferable pathways for auxin flow, the applied IAA moved basipetally in the damaged bundle to where it was interrupted by the wound and was forced (arrow) to find new pathways around the obstacle. B and C are transverse sections in a well developed Arabidopsis hypocotyl, both have the same orientation; their right side is located towards the stem periphery. B. The internal secondary tissues, showing that auxin (detected by *GUS* expression) moves through the cambium and differentiating vascular elements, (all show blue spots of GUS activity, located in front and beside the arrows), forming radii patterns adjacent to the latest formed secondary vessels (v), likely indicating the sites where new vessels will differentiate, x 600. C. The peripheral secondary tissue, showing the path of free-IAA transport (detected by blue GUS staining) through the phellogen (arrows), x 400.

Regular passages (especially within the auxin streams of the internal route) and environmentally-induced exchanges of IAA occur between the auxin routes following light or gravity stimulation, in response to wounding, at merging points, and via the vascular rays in the secondary body.

The auxin which induces the vascular tissues flows polarly in the internal route, mainly through the vascular meristem (procambium or cambium) and the associated parenchyma sheathes. The constant polar IAA movement through the vascular meristem maintains vascular continuity. When vascular tissues are wounded, the interrupted IAA flow could form bypassing pathways via parenchyma or cambial cells resulting in the regeneration of vascular

tissues around a wound (Fig. 5A). Free IAA (detected by *DR5::GUS* expression) moves via intact vascular cambium in the hypocotyls of well developed Arabidopsis plants, preferably between the differentiating vessels and sieve tubes (Fig. 5B), thus explaining the formation of radial patterns of secondary vessels and sieve tubes.

When a cambium shows low activity, or occurs mainly inside the vascular bundles (fascicular cambium), as in organs of many herbaceous plants, it is likely that the polar auxin descending from leaves and flowers would move preferably through xylem parenchyma cells, as has already been found in the inflorescence of Arabidopsis (18, 28, 44). However, in growing trees possessing a wide vigorous cambium, undergoing active cell divisions, the polar auxin is transported through the cambium (54, 57). This is especially true for conifers, where most of the axial cells in their xylem are dead tracheids. It should be noted, that trees have developed earlier than did the specialized herbaceous species, and that during plant evolution, the cambium activity of trees has been minimized in herbaceous plants. Since the extremely advanced species of the monocots do not produce cambium, the polar IAA transport in their internal route is expected to occur via their xylem parenchyma cells.

The associated sheaths that surround the vascular tissues in the primary body, e.g. the pericycle (in roots) and bundle sheaths (like the starch sheath in shoots), are specialized envelopes of cells through which auxin moves polarly. Their auxin flow could boost auxin concentration locally above sites where the polar IAA transport is interrupted (e.g., by wounding, or local ethylene production), resulting in apical meristem formation (e.g., lateral root initiation from the pericycle, or adventitious root formation associated with vascular bundles in the stem). The auxin flowing in the sheaths as well as in the sieve tubes could promote vascular regeneration in cases of wounding.

Auxin flow in the enveloping sheaths could influence normal vascular differentiation within shoot vascular bundles and the root vascular cylinder. Thus, in primary roots, although the metaxylem and protoxylem vessels start differentiation almost simultaneously, the auxin flow in the pericycle enhances the differentiation of the neighboring protoxylem vessels, which consequently lay down their secondary walls earlier, resulting in narrow vessels. Contrariwise, the metaxylem vessels (differentiating away from the pericycle) have more time to expand before secondary wall deposition and therefore become wide vessels.

In shoots, the polar IAA flow via the bundle associated cells at the xylem pole may induce an internal phloem. While the most common bundle type in gymnosperms and angiosperms is the *collateral* bundle, in which the phloem occurs on one side (phloem toward the outside, xylem toward the inside), there are stem bundles in some angiosperm families (e.g., Cucurbitaceae, Solanaceae) where an internal low-level auxin stream at the xylem pole induces an internal phloem, thus forming a *bicollateral* bundle, which has phloem on both sides of the xylem.

In the peripheral route, the polar IAA flow controls the following major

activities: (a) promotes tropic growth of young organs following modifications in auxin concentrations induced by environmental stimulations; (b) maintains the continuity of the protective tissues by promoting suberin lamellae and cork formation after wounding; and (c) induces and controls phellogen activity.

We have recently found that IAA moves along the epidermis and phellogen (Fig. 5C), forming longitudinal strips, or a continuous sheath pattern (all around the stem and root circumference). This was observed in the epidermis and phellogen of stems and roots of *Trifolium* (detected by high *GH3::GUS* expression), and the phellogen of well developed hypocotyls of Arabidopsis (detected by strong *DR5::GUS* expression) (R.A.). Recent findings in Arabidopsis indicate polar auxin flow in the outermost tunica layer of the shoot vegetative meristem, the epidermis of young leaf primordia (45) and epidermis near the root tip (27, 55), but these studies have not yet clarified experimentally the direction of the auxin flow in the outermost layer of the shoot (where both AUX1 and PIN1 partially overlapped; 45) and near the root tip (55), which need to be determined by physiological means. From the published photographs (55) it looks as if the AUX1 permease in the epidermis is enhanced near the root tip, where the highest *DR5::GUS* expression is detected (11, 49), possibly indicating that the high IAA concentrations accumulated at the root tip intensify the expression of the specialized auxin transport protein at this region. As mentioned above, developing hydathodes in leaves (11) and tips of floral organs (R.A.) are major sites of auxin production, whereas the root tips are the sites where auxin is accumulated (11, 49). Therefore, both extremities are sites of high auxin concentrations resulting from either free-IAA production (by hydrolysis) in the shoot organs, or auxin accumulation in the root tips. At these shoot and root tips, the local high IAA concentrations could enhance expression of genes associated with the metabolism and transport of auxin.

ORGANIZED DIFFERENTIATION OF VASCULAR TISSUES

Control of Vascular Bundle Formation by a Signal Flow

In an elegant series of experiments using pea seedlings, Sachs (51) provided evidence supporting his hypothesis that canalization of the auxin flux determines the orderly pattern of vascular tissues from leaves to roots. He proposed (51) that auxin flow, which starts by diffusion, induces a polar auxin transport system which promotes auxin movement and leads to canalization of auxin flow along a narrow file of cells. The continuous polar transport of auxin through these cells induces a further complex sequence of events which terminates in the formation of a vascular bundle. Once developed, a vascular bundle remains the preferable pathway of IAA transport. Consequently, new streams of auxin produced by young leaves are directed towards the developed vascular bundles. A preexisting vascular bundle that is not supplied with auxin (e.g., one descending from an old leaf) acts as a sink for a new stream of auxin (50) and therefore, a new bundle will differentiate towards the preexisting

bundle that has a low supply of IAA. Thus, the canalization of auxin flow through meristematic, or parenchyma cells, induces the orderly pattern of continuous vascular bundles from leaves to roots.

It has also been shown that IAA could have an inhibitory effect on vascular tissue formation, where by a newly induced vascular bundle will not interlink with a preexisting vascular bundle that is well supplied with high auxin concentrations (50). A naturally-occurring instance of such an inhibition was recently encountered in gynoecia of Arabidopsis flowers, where the short veinlets (arrowheads in Fig.. 3C; Fig. 8C) induced by the ovules did not form connections with the longitudinal bundles descending from the IAA-producing stigma (Fig. 3B,C) because these longitudinal bundles were well supplied with auxin.

Application of auxin transport inhibitors increased the number of vessels in the midvein and secondary vascular bundles of Arabidopsis leaves (40, 53) and in the petals (Fig. 6B). However, when an exogenous auxin (NAA) was applied (on and off with TIBA, but not on the same day), drainage of the extra auxin did occur, consequently resulting in narrow secondary bundles with a limited number of vessels (Fig. 6C). Furthermore, when only auxin (40μM NAA) was applied daily, it did not exert any effect on vascular differentiation (R.A.). These findings demonstrate that both movement and drainage of auxin are required for normal bundle formation, and that neither presence of the hormone, nor its concentration is sufficient *per se*.

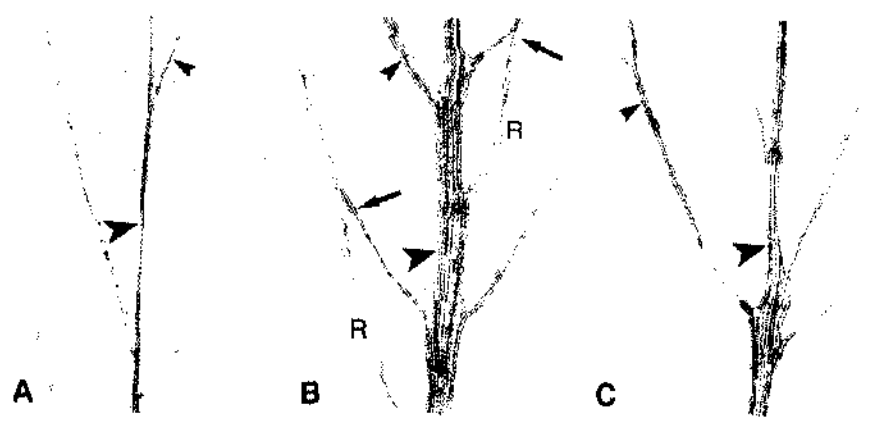

Figure 6. Effects of auxin transport inhibitor (2,3,5-triiodobenzoic acid, TIBA) and auxin (NAA) spray applications (over a 14-d period) on vascular differentiation in wild-type Arabidopsis petals, all x 85. A. Typical delicate vein system in the middle of an intact petal, consisting of two to three vessels in the midvein (large arrowhead) and one vessel in each secondary vein (small arrowhead). B. The auxin transport inhibitor (20μM TIBA) induced a very wide midvein (comprised of 10 to 12 vessels), and wide secondary veins (2 to 4 vessels). In addition, random sites of auxin retardation (arrows) in the secondary bundles, promoted new bypassing pathways resulting in additional regenerative bundles (R). C. Repeated alternate-day treatments of 20μM TIBA (on one day) and 40μM NAA (on the next), caused an increase in vessel number at the base of the midvein and a moderate effect in the secondary veins.

Control of Vessel Size and Density Along the Plant Axis

Auxin acts as a morphogenetic signal forming polar concentration gradients along the plant, from the shoot organs to the root tips. These gradients provide directional and locational information to differentiating cells along the morphogenetic fields. A decreasing gradient of IAA concentrations from leaves to roots may result in polar patterns of growth and differentiation along the plant axis (4, 9, 49).

The control of vessel diameter is an important parameter for assessing the ascent of water and minerals from roots to leaves and the adaptation of plants to their environment (2). The specific purpose of research on this topic is to develop a reliable concept for understanding and explaining the mechanisms that control vessel size and density (number of vessels per transverse-sectional area) along the plant. Such a concept may also serve as a tool for analyzing developmental patterns of vessel size and density in specific sites (e.g. below the stigma, see Fig. 3C) in the vascular system.

The longest known gradient in cell size along plants is encountered in their vascular tissues (as much as 100 m long in very tall trees), where vessel diameter (or tracheid size in conifers) along the plant axis increases gradually with increasing distance from the leaves (9, 37). The narrow vessels differentiate near the leaves, where the highest auxin concentrations are expected, while the widest vessels are formed in the roots, at the greatest distance from the auxin sources. The gradual increase in vessel diameter from leaves to roots is associated with a gradual decrease in vessel density. Hence, vessel density is generally greater in branches, where the vessels are narrow, than in roots, where they are wide (9, 37).

We proposed that the general increase in vessel size and decrease in vessel density along the plant axis is due to a gradient of decreasing auxin concentrations from leaves to roots (9). This is based upon the assumption that the steady polar flow of IAA from leaves to roots controls these polar changes in the vascular system. High auxin concentrations near the young leaves induce narrow vessels because of their rapid differentiation, allowing only limited time for cell growth. Contrariwise, low IAA concentrations further down result in slow differentiation, which permits more cell expansion before secondary wall deposition, and thereby results in wide vessels. Vessel density is controlled by auxin concentration, to wit: high concentrations (near the sites of IAA production) induce greater density, while low concentrations (further down, towards the roots) diminish density. Consequently, vessel density decreases from leaves to roots (4, 9). This hypothesis was experimentally confirmed by showing that various auxin concentrations applied to decapitated bean stems induce substantial gradients of increasing vessel diameter and decreasing vessel density from the auxin source towards the roots (9). High auxin concentration yielded numerous vessels that remained small because of their rapid differentiation. Low auxin concentration resulted in slow differentiation and therefore in fewer and larger vessels. Auxin concentration also influenced the patterns of vessels in the secondary xylem of bean.

Immediately below an auxin source the vessels were arranged in layers. Further down along the stem, where lower levels of auxin were expected, the vessels grouped into bundles (9).

Studies on transgenic plants with altered levels of IAA confirmed the general relations between IAA concentration and vessel size and density. Auxin-overproducing plants (i.e., ones overexpressing the *iaaM* gene) contain many more vessel elements than do control plants, and their vessels are narrow (34). Conversely, plants with lowered IAA levels (i.e., expressing the *iaaL* gene as an anti-auxin gene) contain fewer vessels of generally larger size (48).

In spring, the first wide (up to 500 μm) earlywood vessels in temperate deciduous ring-porous trees initiate a few weeks (up to 6 weeks) before the onset of leaf expansion. These wide vessels are induced by low-level streams of auxin produced by dormant looking buds (before swelling), only because the cambium of these trees requires extremely low auxin levels for reactivation (4).

Control of Phloem and Xylem Relationships in Axial and Foliar Organs

A major problem in studying vascular differentiation is the difficulty of observing the phloem (2, 3, 47). Not surprising, then, that information on phloem differentiation or on gene expression in the phloem is limited, with most of the studies focusing on xylem differentiation in organized vascular bundles (2, 17, 51, 56, 61) and in isolated *Zinnia* tracheary elements (36).

The first organized vascular system encountered in lower members of the plant kingdom consists of phloem with no xylem (3). Thus, we find the first developed sieve tubes in members of the brown algae. Much later in the evolution of plants, during their transition from aquatic to terrestrial habitats, the water conducting system developed.

In tissue cultures, low IAA concentrations induced sieve elements but not tracheary elements, while high auxin concentrations resulted in the differentiation of both phloem and xylem (1, 2), but even in these cultures, at the surface farther away from the auxin-containing medium, only phloem with no xylem developed (1). In the course of vascular development along the plant axis, phloem differentiation precedes that of xylem (25) and therefore vascular bundles consisting of phloem with no xylem are common in young internodes. In very young internodes of *Coleus*, there are more phloem-only bundles than collateral bundles (47). During internode development, vessels will differentiate in some of these phloem bundles.

Plant vascular systems are usually composed of phloem and xylem, and insofar as the relative proportions of phloem and xylem are concerned, there is a major difference between foliar and axial organs. Thus, along the plant axis, xylem does not differentiate in the absence of phloem, though bundles of phloem (with no xylem) and phloem anastomoses are common in stems of many plant species (5, 6, 7, 8, 47). To emphasize the abundance of these phloem ramifications we pointed out that in a mature internode of *Cucurbita maxima* (150 mm long) there are about 10,000 phloem anastomoses between

the vascular bundles (5), which means that the phloem may form dense reticulated patterns. The anastomoses are variable in size, consisting of one or more sieve tubes that are difficult to visualize in conventional light-microscope sections because of their sinuous nature. It has been suggested that the differentiation of phloem bundles and phloem anastomoses between the bundles is induced by streams of low-IAA levels (2, 3). High auxin concentration applied to decapitated *Luffa* stem induced xylem differentiation in its phloem anastomoses (3) indicating the need for high hormonal stimulation for xylem differentiation. These networks of phloem anastomoses operate as an emergency system which enables the plant to respond to damage by providing alternative pathways for assimilates around the stem (7). They also enable xylem regeneration within phloem anastomoses in mature internodes (after the parenchyma cells between the bundles had lost the ability to redifferentiate to vessel elements) before they produce interfascicular cambium (5).

Conversely, in leaves, the differentiation of xylem in the absence of phloem is a common feature and occurs in freely ending veinlets (32, 38) and hydathodes (4). In *Oxalis stricta* there are virtually no sieve tubes in any terminal vein, while *Polygonum convolvulus*, at the other extreme, has sieve tubes extending to the tips of most terminal veins (32). In most of the studied species freely ending veinlets may display disparate relations between phloem and xylem in the same plant, e.g., vein endings lacking sieve tubes or having them up to the tip, and sieve tubes that end at some intermediate point (32, 38). In leaves, the proximity between the sites of IAA production and the sites of differentiating vascular cells probably results in relatively high local auxin concentrations at the differentiating sites (4), which may explain why xylem can differentiate in the absence of phloem at the freely ending veinlets (32, 38) and hydathodes. However, when Arabidopsis plants are subjected to limiting conditions, like very 'short' days (4h light / 20h darkness), some of their tertiary veins, which had developed near the leaf base, produce phloem-only bundles (R.A.). This finding demonstrates that limited hormonal stimulation at the base of these small leaves grown under very short days may not be enough to produce xylem in the phloem-only bundles.

Control of Discontinuous Xylem and Phloem Patterns during Early Stages of Bundle Maturation in Wild-Type Plants

A major issue in vascular differentiation deserving of clarification is the process of xylem maturation during bundle formation. It should be emphasized that during early stages of bundle formation in wild-type plants, normal vessel differentiation initiates and progresses in discontinuous basipetal and acropetal patterns (Fig. 7C-E). Although the incipience of xylem and phloem discontinuities during early stages of leaf and shoot morphogenesis is well known (25), there is no understanding of how these discontinuities are controlled. Understanding these basic vascular patterns is important for correct interpretations and they therefore need detailed explanation. The direction of

xylem maturation by itself does not provide enough information to determine the source of IAA production, or IAA transport direction. Although xylem and phloem regeneration is induced by IAA originating in the young leaves above a wound (4, 33, 51), the regeneration of xylem and phloem around the injury is characterized by basipetal, acropetal and discontinuous developmental patterns (5,6), emphasizing that the basipetal polar auxin flow can induce vascular maturation in opposite directions and may also result in discontinuous patterns.

Generally in shoots, a basipetal maturation pattern starts from the free-IAA production site, whereas an acropetal maturation pattern results from auxin accumulation above a specific location. At the end of the maturation process, these acropetal and basipetal vascular patterns will join into a functional bundle (R.A.).

In leaves, the naturally-occurring discontinuous xylem patterns follow the configuration of the vascular meristem. Loops of procambium develop basipetally (Fig. 7A,B) in very young leaf primordia of Arabidopsis. The first procambium loop commences at the tip (Fig. 7A) and additional loops will gradually differentiate basipetally towards the developing leaf base (Fig. 7B). This very early basipetal pattern of provascular development is likely induced by incipient low auxin stimulation produced by the tip hydathode (11, 39, 60). The late formed basal loops may also be supplied with additional auxin, which is produced by the developing hydathodes in the lobes (11, 39). This basic procambium framework (midvein and loops) determines the patterns of xylem and phloem differentiation, which will follow the procambium pattern during leaf morphogenesis (4).

Naturally-occurring discontinuous basipetal patterns of early-differentiating vessel fragments can be detected at the developing hydathode of the tip (Fig. 7C) and at the differentiating hydathodes of the lobes (Fig. 7D-E). The initiation of fast differentiating isolated vessel elements in the lobes of wild-type plants is promoted by the high local IAA concentrations produced by the differentiating hydathodes (Figs. 1B-D, 2A). Analogously, normal discontinuous basipetal patterns also characterize the early stages of vessel differentiation in the floral organs, where the initiation of vessel elements starts from the sites of high auxin production at the anthers (Fig. 8A) and immediately below the auxin-producing stigma (Fig. 8B). Hence, it is crucial to realize that normal vascular differentiation in shoot-organ primordia of wild-type plants starts from the sites of IAA production and progresses basipetally in discontinuous patterns, which should never be confused with possible vascular discontinuities found in mature organs of defective mutants.

On the other end, acropetal vascular development may progress from the base of shoot organs upward. Such acropetal progress of xylem and phloem differentiation (which characterizes the midvein and the base of secondary bundles) is suggested to result from a local build-up of IAA concentration above a local interruption to the basipetal auxin flow, which slows IAA movement locally. Thus, above locations which slow auxin movement (e.g., at the base of the petioles of leaves, where the abscission zone will develop) a local IAA accumulation (11) will induce an acropetal pattern of xylem

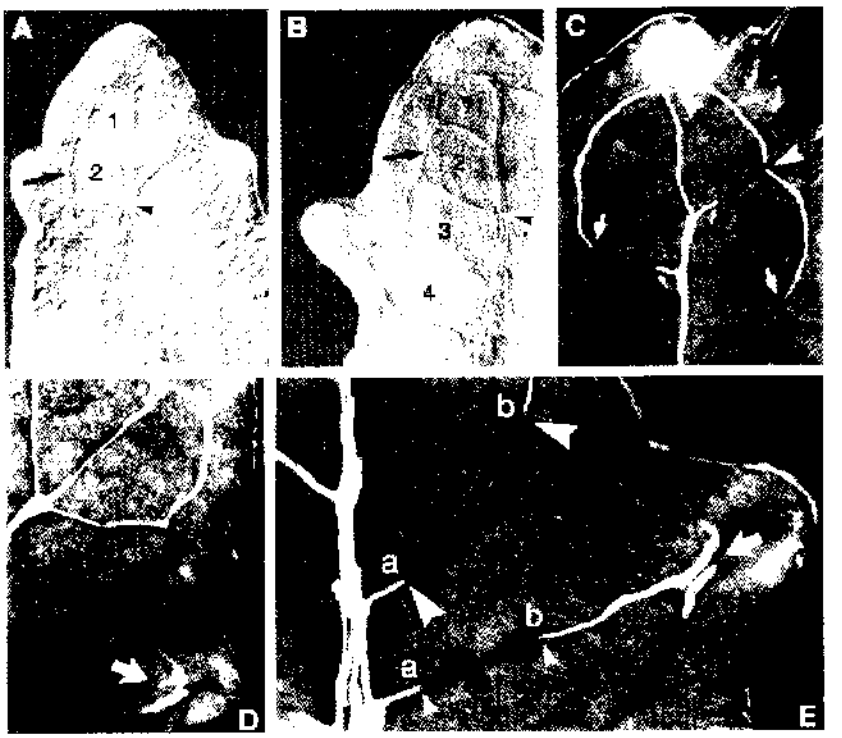

Figure 7. Procambium development (A,B) and naturally-occurring discontinuous vein patterns (C-E) in cleared wild-type Arabidopsis leaf primordia, photographed under Nomarski illumination. Under this illumination the vessels have a white appearance. Magnifications: A-D x 35; E x 50. A. A very young primordium with two procambial loops (loop 1 differentiated before loop 2). B. A more developed primordium with four procambial loops numbered according to their basipetal development. The black arrowhead marks the analogous site in A and B, emphasizing the basipetal development of the procambium (arrow). C. Discontinuous xylem (arrowheads) at the tip, showing basipetal (arrows), and acropetal differentiation (small arrows). D. Typical discontinuous xylem initiation (arrow) in a lobe, where the high auxin concentration is produced by the differentiating hydathode. E. Typical early stages of secondary bundle maturation, showing two discontinuous vessels (each vessel is marked by the same size arrowheads) progressing acropetally (a) and basipetally (b).

development. This is true also in junctions between the midvein and the secondary bundles (Fig. 7C,E), where the auxin streams originating in the marginal hydathodes merge with that of the midvein. Only because the midvein is well supplied with the IAA descending from the tip hydathode, does the junction become a site of elevated auxin concentration which promotes fast local acropetal vessel development at the base of the secondary bundle. Such typical acropetal patterns of xylem development (Fig. 7C,E) are normally detected during early stages of leaf-primordium ontogeny.

At the end of the bundle-maturation process, the vessel fragments which develop in acropetal and basipetal directions (Fig. 7) will join into one functional vessel. This gradual and fragmented bundle maturation, progressing

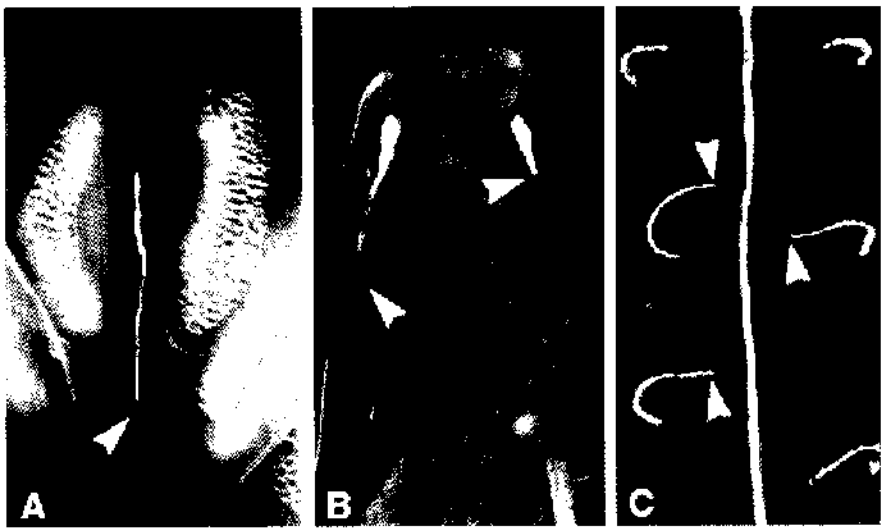

Figure 8. Naturally-occurring discontinuous vein patterns in cleared wild-type Arabidopsis flowers photographed under Nomarski illumination, all x 70. Xylem maturation starts immediately beneath the sites of free-IAA production at the tips of very young floral-organ primordia (A,B), whereas in mature gynoecium (C) the typical short ovule veinlets never connect with the central vascular bundle. A. In a young stamen primordium, a discontinuous vessel (arrowhead) commences maturation from the anthers, and progresses basipetally. B. Two sites of xylem initiation in a very young gynoecium, starting beneath the stigma and progressing downward (arrowheads). C. In a mature gynoecium, the short veinlets (arrowheads) originating from the ovules do not connect with the central bundle of the gynoecium.

in opposite directions, demonstrates that a bundle is composed of a population of differentiating cells that may have different rates of maturation. Those procambial cells exposed to high IAA concentrations (either at the hydathodes, or at the midvein junctions) will differentiate faster than the intervening procambial cells.

To avoid any possible confusion between naturally-occurring and defective-mutant discontinuities, the latter should be analyzed in mature organs (at a time when mature wild-type organs do not show vascular discontinuities). Xylem discontinuities in mature organs of defective mutants (19, 20, 24, 35, 46) can be regarded as early developmental stages that have become fixed during differentiation owing to a failure to complete the maturation process, or due to other possible interruptions to vascular differentiation. Similar discontinuities in mature phloem have been observed also in defective mutants of Arabidopsis (19, 46). Such discontinuous phloem patterns have been correlated with analogous discontinuities in xylem patterns (19).

Genetic Approaches Provide New Insights on the Mechanisms that Control Patterned Vascular Differentiation

Molecular biology techniques are powerful tools for studying vascular differentiation and vein pattern formation (17, 56, 61). In order to identify

genes that determine venation pattern formation and control vascular differentiation, mutants of Arabidopsis and other model plants are being screened for altered vascular patterns. Such genetic screening may uncover mutations in genes that specifically disrupt the normal pattern of vascular differentiation (20, 24, 31, 35, 46).

Molecular biology tools are very useful for studying early events in vascular differentiation at the stages where it is difficult to observe procambium formation and initiation of vascular cells. Handy markers for this purpose are the *AtHB8*, *TED3* and *VH1* genes which are expressed during procambium and early stages of tracheary element differentiation (14, 22, 39). When these reporter genes are fused with the *GUS* gene, their activity can be visualized before clear anatomical features can be detected. Likewise, uncovering genes which are specifically expressed during phloem differentiation would be an important contribution for analyzing sieve-tube formation.

Vascular differentiation is induced and controlled by the basipetal polar flow of the auxin signal and, therefore, it is important to understand the molecular basis of auxin influx and efflux and the genes involved in this process. Polar auxin flow is attributed to an asymmetric distribution of specific transport proteins involved in the efflux of auxin (15). These and genes regulation their biosynthesis, location and recycling are described in chapter E1.

Recent findings indicate that sterols may play a crucial role in vascular differentiation; there is now evidence that sterols may be involved in the establishment of plant cell polarity, by regulating positioning of proteins in the plasma membrane. Sterols which regulate fluidity and interact with lipids and proteins within the plasma membrane can modulate the activity of membrane-bound proteins required for correct auxin signaling (20, 60).

Mutants with defective vascular patterns in their mature cotyledons and leaves may fail to establish uniformly aligned vascular cells and xylem discontinuities may occur in their mature leaves (19, 24, 35, 46). The genes detected in these mutants may be involved in auxin signal transduction during organized bundle formation. The most studied mutant with discontinuous patterns is *monopteros (mp)*, which is characterized by discontinuous bundles and improperly aligned vessel elements especially in the leaves (17, 46). The *MP* gene, which has been cloned, encodes a transcriptional factor capable of binding to auxin response elements in the control regions of auxin regulated genes (30).

Additional to the above, novel patterns of vascular networks may emerge in defective mutants (24, 35), indicating modifications in the auxin pathways and transport during the process of vascular differentiation. Mutants defective in their ability to form continuous vascular networks may result from mutations in genes encoding components of the polar auxin transport machinery, e.g., genes causing defected basipetal transport of IAA (21), reduced capacity for polar transport of auxin (46), or modified sensitivity to IAA (24). Genes affecting early stages of vascular patterning, prior to

provascular network formation, may promote differentiation along wide pathways rather than narrow canals, owing to failure to establish efficient canals of IAA flow. An ineffective broad pathway resulting from a defect in the polar auxin transport machinery may fail to establish continuous wide xylem strands and can therefore result in fragmented patterns of vascular islands (24, 35).

SUMMARY AND CONCLUSIONS

Differentiating hydathodes (water secreting glands), that develop in the tip and later also along the leaf margins, are the primary sites of free-IAA production. In a leaf primordium, the basipetal polar auxin flow descending from the tip hydathode induces the midvein, and IAA streams from the marginal hydathodes induce the secondary vascular bundles. Trichomes and mesophyll cells, which produce low free-IAA concentrations, induce the minor tertiary and quaternary veins, as well as the freely ending veinlets. Similarly, the stigma of a young gynoecium is a major site of free-auxin production which induces a wide xylem fan pattern immediately below it, whereas the ovules with their low auxin content induce short veinlets. The polar auxin flow from the shoot organs to root tips induces the entire plant vascular system.

From the sites of free-IAA production in shoot organs, the auxin moves along the plant through three main transport pathways: (1) the vascular tissues, (2) protective tissues, and (3) sieve tubes. The continuous polar auxin flow through the vascular tissues controls their differentiation and regeneration. In vascular tissues of trees, the IAA flows mainly via the cambium. However, during plant evolution, because the cambium activity of trees has been minimized in herbaceous plants, I suggest that in advanced herbaceous species, with minimized, or absence (in monocots) of cambium activity, the polar IAA transport in the vascular tissues occurs mainly through their xylem parenchyma cells. The polar transport of auxin through parenchyma sheaths around the vascular tissues affects vascular pattern formation (e.g., by controlling the diameter of primary vessels in roots and internal phloem formation in bicollateral bundles) and could promote vascular regeneration and root initiation.

Polar IAA transport that induces vascular differentiation is attributed to asymmetric distribution of specific transport proteins regulated by vesicle trafficking, which are usually confined to the apical/basal end-poles of auxin-transporting cells. The fast non-polar IAA flow inside the sieve tubes is likely a house-keeping signal, which controls callose levels and possibly polarizes the actin cytoskeleton along the apical-basal axis in these phloem conduits.

Auxin acts as a morphogenetic signal, forming polar concentration gradients along the plant from the free-IAA producing hydathodes to the root tips and inducing polar patterns of increasing vessel diameter and decreasing vessel density form leaves to roots.

Phloem differentiation is induced by low-auxin streams, while xylem

differentiation requires higher IAA concentrations. Consequently, near sites of high-auxin concentrations in a leaf primordium, veins of xylem (with no phloem) differentiate frequently. Conversely, away from the sites of IAA production, phloem-only bundles and phloem anastomoses between bundles are common along the stem in many plant species.

Early stages of vein maturation in wild-type plants are characterized by initiation and development of discontinuous basipetal and acropetal patterns. Generally, in shoots the basipetal maturation patterns start at the free-IAA production sites, whereas acropetal maturation patterns result from auxin accumulation above sites where IAA movement is locally retarded. At the end of this gradual process, the discontinuities will join into one functional vein. In order to avoid possible confusion between the naturally-occurring and defective-mutant discontinuities, the latter should be analyzed in mature organs.

Molecular approaches are expected to provide further new insights into the mechanisms that control vascular differentiation and vein pattern formation, and they should be integrated with physiological analyses at the cellular and organismic levels for correct interpretations.

References

1. Aloni R (1980) Role of auxin and sucrose in the differentiation of sieve and tracheary elements in plant tissue cultures. Planta 150: 255-263
2. Aloni R (1987) Differentiation of vascular tissues. Annu Rev Plant Physiol 38: 179-204
3. Aloni R (1995) The induction of vascular tissues by auxin and cytokinin. *In* Plant Hormones: Physiology, Biochemistry and Molecular Biology, PJ Davies, *ed*, Kluwer, Dordrecht, pp 531-546
4. Aloni R (2001) Foliar and axial aspects of vascular differentiation - hypotheses and evidence. J Plant Growth Regul 20: 22-34
5. Aloni R, Barnett JR (1996) The development of phloem anastomoses between vascular bundles and their role in xylem regeneration after wounding in *Cucurbita* and *Dahlia*. Planta 198: 595-603
6. Aloni R, Jacobs WP (1977) The time course of sieve tube and vessel regeneration and their relation to phloem anastomoses in mature internodes of *Coleus*. Amer J Bot 64: 615-621
7. Aloni R, Peterson CA (1990) The functional significance of phloem anastomoses in stems of *Dahlia pinnata* Cav. Planta 182: 583-590
8. Aloni R, Sachs T (1973) The three-dimensional structure of primary phloem systems. Planta 113: 345-353
9. Aloni R, Zimmermann MH (1983) The control of vessel size and density along the plant axis - a new hypothesis. Differentiation 24: 203-208
10. Aloni R, Raviv A, Peterson CA (1991) The role of auxin in the removal of dormancy callose and resumption of phloem activity in *Vitis vinifera*. Can J Bot 69: 1825-1832
11. Aloni R, Schwalm K, Langhans M, Ullrich CI (2003) Gradual shifts in sites of free-auxin production during leaf-primordium development and their role in vascular differentiation and leaf morphogenesis in *Arabidopsis*. Planta 216: 841-853
12. Aloni R, Wolf A, Feigenbaum P, Avni A, Klee HJ (1998) The *Never ripe* mutant provides evidence that tumor-induced ethylene controls the morphogenesis of *Agrobacterium tumefaciens*-induced crown galls on tomato stems. Plant Physiol 117: 841-847
13. Avsian-Kretchmer O, Cheng J-C, Chen L, Moctezuma E, Sung ZR (2002) IAA distribution coincides with vascular differentiation pattern during *Arabidopsis* leaf ontogeny. Plant Physiol 130: 199-209
14. Baima S, Nobili F, Sessa G, Lucchetti S, Ruberti I, Morelli G (1995) The expression of the *Athb-8* homeobox gene is restricted to provascular cells in *Arabidopsis thaliana*.

Development 121: 4171-4182
15. Baluška F, Šamaj J, Menzel D (2003) Polar transport of auxin: carrier-mediated flux across the plasma membrane or neurotransmitter-like secretion? Trends Cell Biol 13: 282-285
16. Barker-Bridgers M, Ribnicky DM, Cohen JD, Jones AM (1998) Red-light regulated growth. II. Changes in the abundance of indoleacetic acid in the maize mesocotyl. Planta 204: 207-211
17. Berleth T, Mattsson J, Hardtke CS (2000) Vascular continuity and auxin signals. Trends Plant Sci 5: 387-393
18. Booker J, Chatfield S, Leyser O. (2003) Auxin acts in xylem-associated or medullary cells to mediate apical dominance. Plant Cell 15: 495-507
19. Carland FM, Berg BL, FitzGerald JN, Jinamornphongs S, Nelson T, Keith B (1999) Genetic regulation of vascular tissue patterning in *Arabidopsis*. Plant Cell 11: 2123-2137
20. Carland FM, Fujioka S, Takatsuto A, Yoshida S, Nelson T (2002) The identification of CVP1 reveals a role for sterols in vascular patterning. Plant Cell: 14: 2045-2058.
21. Carland FM, McHale NA (1996) *LOP1*: a gene involved in auxin transport and vascular patterning in *Arabidopsis*. Development 122:1811-1819
22. Clay NK, Nelson T (2002) VH1, a provascular cell-specific receptor kinase that influence leaf cell patterns in *Arabidopsis*. Plant Cell 14: 2707-2722
23. Dengler N, Kang J (2001) Vascular patterning and leaf shape. Curr Opin Plant Biol 4: 50-56
24. Deyholos MK, Cordner G, Beebe D, Sieburth LE (2000) The *SCARFACE* gene is required for cotyledon and leaf vein patterning. Development 127: 3205-3213
25. Esau K (1965) Vascular Differentiation in Plants, Holt, Rinehart and Winston, New York
26. Friml J, Benkova Eilou I, Wisniewska J, Hamann T, Ljung K, Woody S, Sandberg G, Scheres B, Jürgens G, Palme K (2002) AtPIN4 mediates sink-driven auxin gradients and root patterning in *Arabidopsis*. Cell 108: 661-673
27. Friml J, Plame K (2002) Polar auxin transport - old questions and new concepts? Plant Mol Biol 49: 273-284
28. Gälweiler L, Guan C, Müller A, Wisman E, Mendgen K, Yephremov A, Palme K (1998) Regulation of polar auxin transport by AtPIN1 in *Arabidopsis* vascular tissues. Science 282: 2226-2230
29. Geldner N, Friml J, Stierhof Y-D, Jürgens G, Palme K (2001) Auxin transport inhibitors block PIN1 cycling and vesicle trafficking. Nature 413: 425-428
30. Hardtke CS, Berleth T (1998) The *Arabidopsis* gene *MONOPTEROS* encodes a transcription factor mediating embryo axis formation and vascular development. EMBO J 17: 1405-1411
31. Hobbie L, McGovern M, Hurwitz LR, Pierro A, Yang Liu N, Bandyopadhyay A, Estelle M (2000) The axr6 mutants of *Arabidopsis thaliana* define a gene involved in auxin response and early development. Development 127: 23-32
32. Horner HT, Lersten NR, Wirth CL (1994) Quantitative survey of sieve tube distribution in foliar terminal veins of ten dicot species. Amer J Bot 81: 1267-1274
33. Jacobs WP (1952) The role of auxin in differentiation of xylem around a wound. Amer J Bot 39: 301-309
34. Klee HJ, Horsch RB, Hinchee MA, Hein MB, Hoffmann MB (1987) The effects of overproduction of two *Agrobacterium tumefaciens* T-DNA auxin biosynthetic gene products in transgenic petunia plants. Gene Dev 1: 86-96
35. Koizumi K, Sugiyama M, Fukuda H (2000) A series of novel mutants of *Arabidopsis thaliana* that are defective in the formation of continuous vascular network: calling the auxin signal flow canalization hypothesis into question. Development 127: 3197-3204
36. Kuriyama H, Fukuda H (2001) Regulation of tracheary element differentiation. J Plant Growth Regul 20: 35-51
37. Leitch MA (2001) Vessel-element dimensions and frequency within the most current growth increment along the length of *Eucalyptus globulus* stems. Trees 15: 353-357
38. Lersten NR (1990) Sieve tubes in foliar vein endings: review and quantitative survey of *Rudbeckia laciniata* (Asteraceae). Amer J Bot 77: 1132-1141

39. Mattsson J, Ckurshumova W, Berleth T (2003) Auxin signaling in *Arabidopsis* leaf vascular development. Plant Physiol 131: 1327-1339
40. Mattsson J, Sung ZR, Berleth T (1999) Responses of plant vascular systems to auxin transport inhibition. Development 126: 2979-2991
41. Morris DA, Kadir GO, Barry AJ (1973) Auxin transport in intact pea seedlings (*Pisum sativum* L.): the inhibition of transport by 2,3,5-triiodobenzoic acid. Planta 110: 173-182
42. Müller A, Düchting P, Weiler EW (2002) A multiplex GC-MS/MS technique for the sensitivity and qualitative single-run analysis of acidic phytohormones and related compounds, and its application to *Arabidopsis thaliana*. Planta 216: 44-56
43. Nelson T, Dengler N (1997) Leaf vascular pattern formation. Plant Cell 9: 1121-1135
44. Palme P, Gälweiler L (1999) PIN-pointing the molecular basis of auxin transport. Curr Poin Plant Biol 2: 375-381
45. Reinhardt D, Pesce E-R, Stieger P, Mandel T, Baltensperger K, Bennett M, Traas J, Friml J, Kuhlemeier C (2003) Regulation of phyllotaxis by polar auxin transport. Nature 426:255-260
46. Przemeck GKH, Mattsson J, Hardtke CS, Sung ZR, Berleth T (1996) Studies on the role of the *Arabidopsis* gene *MONOPTEROS* in vascular development and plant cell axialization. Planta 200: 229-237
47. Roberts I.W, Gahan PB, Aloni R (1988) Vascular Differentiation and Plant Growth Regulators. Springer-Verlag, Berlin
48. Romano CP, Hein MB, Klee HJ (1991) Inactivation of auxin in tobacco transformed with the indoleacetic acid-lysine synthetase gene of *Pseudomonas savastanoi*. Genes Dev 5: 438-446
49. Sabatini S, Beis D, Wolkenfelt H, Murfett J, Guilfoyle T, Malamy J, Benfey P, Leyser O, Bechtold N, Weisbeek P, Scheres B (1999) An auxin-dependent distal organizer of pattern and polarity in the *Arabidopsis* root. Cell 99: 463-472
50. Sachs T (1969) Polarity and the induction of organized vascular tissues. Ann Bot 33: 263-275
51. Sachs T (1981) The control of patterned differentiation of vascular tissues. Adv Bot Res 9: 151-262
52. Sachs T (2000) Integrating cellular and organismal aspects of vascular differentiation. Plant Cell Physiol 41: 649-656
53. Sieburth LE (1999) Auxin is required for leaf vein pattern in *Arabidopsis*. Plant Physiol 121: 1179-1190
54. Sundberg B, Uggla C, Tuominen H (2000) Cambial growth and auxin gradients. *In* RA Savidge, JR Barnett, R Napier, *eds*, Cell and Molecular Biology of Wood Formation, BIOS Scientific Publishers, Oxford. pp 169-188
55. Swarup R, Friml J, Marchant A, Ljung K, Sandberg G, Palme K, Bennett M (2001) Localization of the auxin permease AUX1 suggests two functionally distinct hormone transport pathways operate in the *Arabidopsis* root apex. Genes Dev 15: 2648-2653
56. Turner S, Sieburth LE (2002) Vascular patterning. The Arabidopsis Book, CR Somerville, EM Meyerowitz, eds, American Society of Plant Biologists. Rockville, MD. http://www.aspb.org/publications/arabidopsis/toc.cfm
57. Uggla C, Moritz T, Sandberg G, Sundberg B (1996) Auxin as a positional signal in pattern formation in plants. Proc Nat Acad Sci USA 93: 9282-9286
58. Ullrich CI, Aloni R (2000) Vascularization is a general requirement for growth of plant and animal tumours. J Exp Bot 51: 1951-1960
59. Ulmasov T, Murfett J, Hagen G, Guilfoyle TJ (1997) Aux/IAA proteins repress expression of reporter genes containing natural and highly active synthetic auxin response elements. Plant Cell 9: 1963-1971
60. Willemsen V, Friml J, Grebe M, van den Toorn A, Palme K, Scheres B (2003) Cell polarity and PIN protein positioning in *Arabidopsis* require *STEROL METHYLTRRANFERASE1* function. Plant Cell 15: 612-625
61. Ye Z-H (2002) Vascular tissue differentiation and pattern formation in plants. Annu Rev Plant Biol 53: 183-202