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Top-Down and Inside-Out: Directionality of Signaling in Vascular and Embryo Development

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Abstract

Although most aspects of vascular tissue patterning remain elusive, the alignment of vascular cells into continuous strands is becoming amenable to molecular genetic analysis. Recent data are consistent with an apical-basal signal-flow model underlying vascular strand formation, but also body axis formation in embryos. Directional, top-down auxin flow could set up a basic axial coordinate system upon which a second tier of patterning cell interactions could elaborate the cell patterns in embryos and meristems. Most of these pattern-elaborating activities may still be unknown, but recent examples illustrate how inward-out signaling from the center could regulate the development in the overlying tissue layers, relative to the position of vascular tissue. A two-tiered control of cellular patterns, in which fine-tuning cell interactions generate a reproducible cell pattern on the basis of an underlying robust, feed-back stabilized axial architecture, could also account for the amazing pattern regeneration capacities of embryos and meristems.

Key words: Auxin; Embryo pattern formation; Polar auxin transport; Root meristem; Shoot meristem; Vascular development

Introduction

The formation of vascular tissues expanded the architectural options of plant life. It allowed plants to generate rigid bodies of enormous size with reliably connected specialized organs in remote terminal positions. Now emerging molecular insights into plant patterning processes bear the promise of understanding the integrated development of vascular plants and their vascular systems in mechanistic detail.

In the past years, plant vascular tissue research has made great progress, which is reflected in recent reviews on anatomical, physiological, cell biological, and developmental aspects of vascular development (Nelson and Dengler 1997; Fukuda 1997a, b; Sachs 2000; Berleth and others 2000; Dengler and Kang 2001). An unresolved issue to be discussed is the possible relationship between vascular and overall body patterning, which is reflected in at least two observations: First, mutations disrupting directional growth—in the formation of the embryo axis or in the establishment of new growth axes—usually also interfere with vascular strand formation, suggesting common axial cues. Second, there is an increasing number of examples in which vascular tissues seem to serve as sources for positional information directing the patterning in overlying tissues. This review will first provide a brief summary of recent genetic approaches towards the identification of vascular patterning genes with a short summary of findings on apical-basal (top-down) signaling in axis forma-

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tion. It will then discuss the question of whether there is also an inside-out directionality, through which vascular-derived signals direct the development of other tissues. The subject of this discussion will be restricted to a few recent publications; the larger context can be found in the reviews listed above.

Vascular Pattern Genes

Over many years, vascular mutants had been found serendipitously in a variety of plant species, and only recently, systematic screens for mutants with abnormal leaf vascular patterns have been introduced in *Arabidopsis* (Carland and others 1999; Deyholos and others 2000; Koizumi and others 2000). Up to 10 new genes implicated in the promotion of vascular continuity, proper structure of vascular bundles, and vascular cell biology may have been identified in these screens, and the forthcoming years will probably reveal whether some of these genes have genuine functions in vascular tissue patterning. This will require integrated phenotypic and molecular studies as there are already examples that weak allele mutations in genes with more general roles can create the impression of a vascular specific gene function (Carland and McHale 1996; Cnops and others 2000; Zhong and Ye 1999; Ratcliff and others 2000). With recent mutant screens in *Arabidopsis* approaching saturation, the persistent scarcity of vascular mutants needs to be explained and could be due to (a) early lethality of vascular defective individuals, (b) redundancy in gene function, or (c) low genetic complexity of the genuine vascular patterning process.

Each of the three interpretations may explain to some extent the small number of identified *Arabidopsis* vascular pattern mutants. Severe vascular defects are conceivably lethal however, (a) one could argue that some of the screens were performed at the seedling stage, and vascular function seems to be dispensable during embryogenesis (see below). Further, (b) widespread redundancy of gene function has indeed turned out to pose severe problems to genetic analysis in most model species (Miklos and Rubin 1996), but there is no reason to suspect that vascular functions are so exceedingly redundant that hardly any mutants can be found. Thus, (c) we may have to consider the possibility that, at least in *Arabidopsis*, there may not be very many specific vascular (strand) pattern genes. Rather, vascular strand patterns (this is what is monitored in current mutant screens) could be inextricably linked to body pattern formation principles. This would limit the number of specific vascular pattern genes that can be identified at vegetative stages, but should be revealed by the identification of embryo mutants with correlated vascular and body axis defects. As will be discussed below, the latter class of mutants has indeed been identified and their properties support the notion of a dominating influence of an apical-basal signal flow in setting up the pattern of vascular strands along the main axis of the plant (Figure 1).

The Apical-Basal Signal-Flow Model

The concept of a dominating influence of an apical-basal (top-down) signal flow was proposed many years ago by Tsvi Sachs, based on vascular and organ regeneration experiments (Sachs 1981, 1991). Central to its explanatory power is its ability to account for the enormous plasticity of plant development and for the capacity of plant cells to generate biologically meaningful cell patterns in multiple ways. These adaptive capacities exclude rigid pattern specification mechanisms and suggest that result-controlled feed-back mechanisms form the basal most level of the pattern-forming process. The signal flow interpretation considers axis formation as a basic principle in higher plant pattern formation. The axis is formed by alignment of cell differentiation with the axis of an apical-basal signal flow, further elaborated by differential conductivity leading to vascular differentiation along the preferred routes of signal flow and, eventually, the initiation of root meristems at basal sites of signal accumulation (Figure 1E). Stable, feed-back mechanisms are thought to reestablish apical-basal polarity, a directional signal flow, and the selection of routes of vascular differentiation even after major distortions (Sachs 1981). What could be the cell biological nature of these feedback controls? This question can only be answered by detailed cell biological and molecular studies in the future. Present and past models serve the mere purpose of highlighting the need for such controls to explain the self-adjusting abilities of embryonic, meristematic, and vascular patterns. In its most basic form a directional signal flow through each cell could occur via specific import and export molecules in the plasma membrane. If the flow of the signal positively influences the polar position (at opposite poles of the cells) and abundance of these molecules, once established apical-basal polarity would be reinforced and, by restricting the flow to preferred channels, different cell identities in the radial dimension could be established (Figure 1E).

Biological patterns are usually not generated through the minimal number of molecules predicted in computer models and therefore even these basic feedback controls may involve an amazingly com-

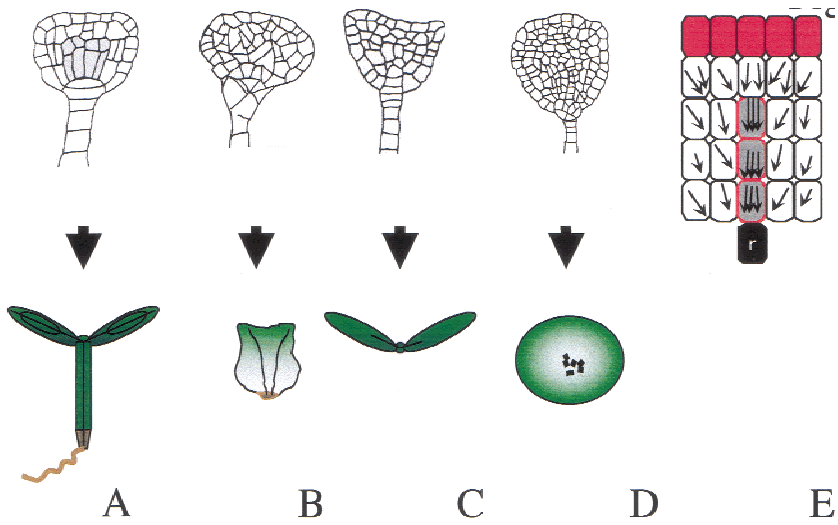

Figure 1. Embryo axis formation and vascular continuity. (**A–D**) Cell patterns in triangular stage embryos (top) and schematic seedling patterns (bottom). Lines in seedlings **A to C** symbolize continuous vascular strands, dark dots in **D** isolated, randomly oriented vascular cells. (**A, B**) Early embryo cell patterns of *Arabidopsis* are normally invariable (**A**, provascular tissue in grey), but mutants (**B to D**) demonstrate that the patterning cues underlying the basic axial organization of the seedling operate irrespective of cell patterns in early embryos. For example, mutations in the *FASS* gene distort cell dimensions and result in irreproducible, chaotic cell patterns in early embryos, from which, however, functional seedlings develop (**B**). These are short and stout, but all major tissues are functional and properly positioned. Numerous mutations, affecting primarily cell shape, confirm the independence of embryo axis formation from the appearance of defined cell patterns during early embryo development (drawn after Torres and Juergens 1994). (**C, D**) The axial patterning mechanism could involve the apical-basal flow of auxin (**E**), which has also been implicated in the formation of vascular strands. This interpretation is supported by the phenotypes of *Arabidopsis* mutants, which suggest that auxin perception and auxin transport are essential for both embryo axis formation and vascular strand formation. In mutants of the 'auxin response' transcription factor *MP* (**C**), auxin-dependent vascular strand formation is reduced and the oriented cell differentiation initiating the formation of the hypocotyl-root body axis is missing. Mutations in the gene *EMB30/GN* (**D**) interfere with the vesicle-transport dependent polar localization of the presumptive auxin efflux carrier component AtPIN1. Mutant seedlings may completely lack apical-basal polarity and vascular cells remain randomly oriented (drawn after Mayer and others 1993; Berleth and Juergens 1993). (**E**) Highly schematic illustration of a feedback-stabilized axial pattern. Rectangles represent cells, the signal being produced either in all cells, or predominantly in apical cells (red/dark grey), leading to preferred route (grey) of signal flow (arrows), which can be associated with overt cell differentiation (red/dark grey cell walls) and root differentiation at positions of signal accumulation (black). A self-reinforcing signal flow could result from a feedback mechanism, in which flow enhances the signal conductivity of cells. If the conductivity is unidirectional (through localized influx and efflux mechanisms in individual cells) this would stabilize polarity and, by gradually enhancing initial conductivity differences among cells (numbers of arrows), generate a crude radial pattern (drawn after Sachs 1991). Colors/grey values refer to online/print versions of images, respectively.

plex cellular machinery. Nevertheless, many findings in recent years support the notion of a robust, basic patterning mechanism that is intimately linked to polarity, axis formation, and vascular differentiation in continuous strands, and may therefore constitute entries to molecular genetic analyses. Although the often debated chemical identity of the flowing signal is not an essential component of the signal flow concept, most of the recent findings support the idea of auxin (IAA) being the apical-basally transported signal substance. These findings are consistent with a scenario, in which feedback-stabilized polar auxin flow establishes a coarse axial coordinate system that serves as an underlying scaffold for fine-tuning tissue patterning processes. This two-tiered control could account for the amazing adaptive capacities of plant cell patterns.

Apical-Basal Signaling in Embryos

Early *Arabidopsis* embryogenesis is characterized by the apparent paradox of reproducibility and flexibility. Under normal circumstances, early cell divisions in *Arabidopsis* embryos are perfectly reproducible, however, *Arabidopsis* mutants as well as embryo culture experiments demonstrate that this high degree of order is largely gratuitous (Laux and Juergens 1997; Berleth 1998). If embryos are forced to abandon this strict path of cell divisions, for example, due to altered cell dimensions or experimental manipulation, they are able to normalize development even from extremely distorted cellular patterns (Figure 1B). This observation implies that basic features of embryo architecture are signaled irrespective of cell arrangements. Most interestingly, it follows that it

should be possible to classify mutants with highly distorted early embryonic cell patterns by the criterion of whether or not their development is readjusted at subsequent embryonic stages. Where this is the case, mutations seem not to affect basic patterning mechanisms (Figure 1B), as opposed to a minority of mutations which cause lasting characteristic pattern distortions, which may even be observed in tissue culture or at postembryonic stages (Figures 1C, D). Conceivably, the high degree of order observed in normal development should require additional cellular interactions, most of which are presently not genetically defined. However, the extreme flexibility of manipulated embryo development suggests that these cell interactions comprise only the 'fine-tuning' layer of a two-tiered mechanism of embryo pattern control. Beneath these precise cues, there seems to be a rather coarse coordinate system, which remains intact even in embryos with highly distorted cell arrangements.

The last 5 years have generated some evidence as to the molecular cues establishing a coarse axial coordinate system. One of its principal elements is apical-basal polarity and a common axial orientation of cells along the apical-basal axis. In *Arabidopsis,* there seems to be a class of mutants with generally impaired capacities to align cell differentiation with the apical-basal axis. In embryos of the *monopteros* (*mp*) mutant, cells remain isodiametric and hardly any provascular tissue is formed (Berleth and Juergens 1993). Mutations in *MP* do not seem to be associated with general cellular abnormalities, but the axis formation defect persists not only through embryogenesis, but is also reflected in vascular continuity defects in vegetative organs (Przemeck and others 1996). Tests revealed reduced auxin transport capacity and impaired auxin-induced adventitious root formation in *mp* mutant tissue. Consistent with a function in auxin signaling, the *MP* gene turned out to encode an Auxin Response Factor (Hardtke and Berleth 1998). Auxin Response Factors constitute a family of *Arabidopsis* transcription factors that can bind to conserved 'auxin-response elements' in the promoters of auxin-inducible genes and confer rapid auxin-dependent transcriptional regulation (Guilfoyle and others 1998). More recently, two new mutants, *bodenlos* (*bdl*) and *auxin resistant 6* (*axr6*), with strikingly similar embryonic and vascular defects have been isolated (Hamann and others 1999; Hobbie and others 1999). Although the respective gene products remain to be identified, the auxin-insensitivity of both mutants suggests functions in auxin signal transduction.

Mutant embryos of the *mp, bdl, axr6* class fail to form the apical basal cell files that give rise to the hypocotyl, but retain some axial organization in the apical domain of the embryo. Although the basal embryo domain is most affected in those mutants, the genes may have functions throughout the embryo. *MP* is expressed in apical as well as basal positions (Hardtke and Berleth 1998) and in double mutants of *mp* and *bdl,* directional growth is abolished throughout the embryo (Hamann and others 1999). Therefore, the formation of the hypocotyl may be particularly sensitive, but eventually directional growth in the entire embryo seems to depend on auxin signaling. Interestingly, a single mutation that can abolish all axial cell elongation in the embryo has recently been identified in the *Arabidopsis* gene encoding the Auxin-Binding-Protein 1 (ABP1) (Chen and others 2001). Mutant embryos develop normally up to the early globular stage. From then on, they continue to grow in size for a little while, but fail to produce files of elongated cells. Eventually, they become arrested at the late globular stage. Auxin binding and other properties of ABP1 have been characterized extensively (summarized in Venis and Napier 1995) and overexpression of the *ABP1* gene in tobacco leaf strips resulted in increased auxin-mediated cell expansion (Jones and others 1998), raising the possibility that *ABP1* encodes an auxin receptor. Because ABP1 seems to be a unique gene in *Arabidopsis,* it will be extremely interesting to see whether and to what extent the *abp1* mutant tissue is impaired in auxin responses and whether the *ABP1* gene is primarily involved in controlling cell elongation or axis-oriented differentiation.

Although the above genes may act in auxin signal transduction, *Arabidopsis* genetic analysis also provides candidate genes for functions in auxin transport. The *Arabidopsis PIN FORMED1* (*AtPIN1* or *PIN1*) gene is believed to encode a component of polar auxin efflux carriers (Galweiler and others 1998) and the *EMB30/GN* gene encodes a guanosine nucleotide exchange factor involved in vesicle formation, which in turn seems to be critical for the coordinated polar localization of the *PIN1* protein (Steinmann and others 1999). *PIN1* is a member in a large gene family, raising the possibility that some other members have overlapping functions in auxin transport and cell polarization. *EMB30/GN* gene activity could be required for the proper polar localization of several proteins of the PIN family, which could explain why *emb30/gn* mutants display vascular defects far more severe than those in *pin1* mutants. Vascular cells in *emb30/gn* mutants are disconnected and not aligned along an axis (Figure 1D). In extreme cases, *emb30/gn* embryos are completely apolar, resembling *Brassica juncea* embryos grown in the presence of auxin transport inhibitors (Mayer and others 1993;

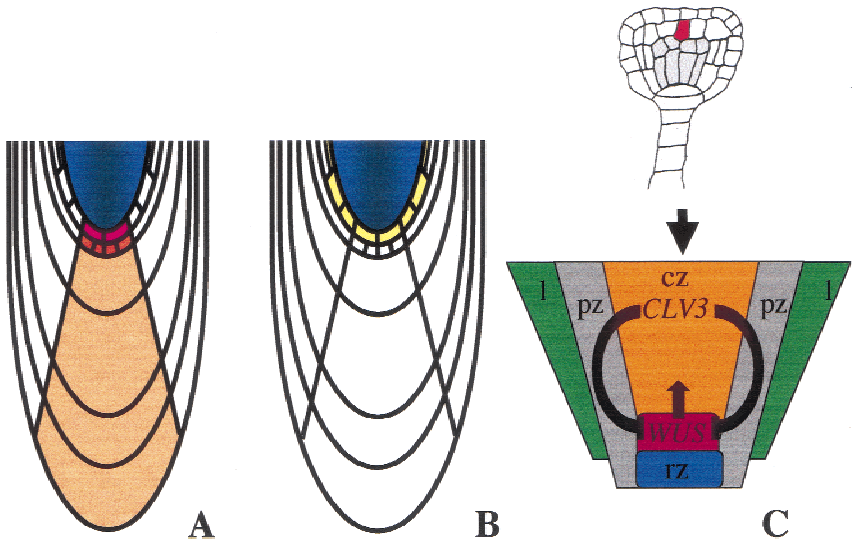

Figure 2. Control of apical meristem cell patterns through activities in the center. (**A**) The position of a maximum auxin response (auxin 'peak'), in normal development at the position of the columella initials (brown, dark grey), influences the pattern in the entire distal root. When the position of this 'peak' is altered (by mutation or inhibition of auxin transport), corresponding changes of cell patterns in the root meristem are observed, suggesting an instrumental role of auxin in root meristem patterning. Auxin could be derived from the shoot through the vascular cylinder (blue, shaded). (Sabatini and others 1999). Pink/grey: quiescent center cells; light orange/light grey: columella. (**B**) The *SHR* gene is expressed exclusively in the vascular cylinder (blue/shaded), but its activity is required for the expression of *SCR* in the adjacent cortical initials and in the endodermis (*SCR* expression domain, yellow/very lightly shaded). *SCR* acts downstream of *SHR* and is required for the periclinal divisions of ground tissue initials to produce two-layered ground tissue and, possibly, for maintaining endodermis identity in the inner ground tissue layer. Ectopic overexpression of *SHR* leads to *SCR* expression throughout the outer layers and unscheduled cell division and specification (reference: Helariutta and others 2000). (**C**) *WUS* expression is already detectable in apical, subepidermal positions in the embryo (red/dark grey cell; top) and is continuously expressed in the quiescient center of the shoot meristem (bottom). *WUS* acts positively on the activity of several layers of stem cells, from where a counteracting signal through the *CLV* pathway restricts *WUS* expression. Ectopic *WUS* expression demonstrates that *WUS* is sufficient to promote cell division throughout the meristem. The positive regulators positioning *WUS* expression domain relative to preexisting tissue are not known (drawn after Schoof and others 2000; for details and further references see text). (green, leaf primordia; grey, pz, peripheral zone; orange, cz, central stem cell zone; blue, central rib zone, producing the pith). Colors/grey values refer to online/print version of images, respectively.

Hadfi and others 1998). In summary, for an increasing number of genes, phenotypic and molecular features are consistent with functions in perception or transport of an apical-basally transported auxin signal (reviewed in further detail Berleth and others 2000). Mutations in these genes seem to affect coordinated cell differentiation at various stages of development. Unlike mutations affecting unrelated cell parameters, these defects are not normalized in later development, suggesting that they affect the establishment of a basic coordinate system in embryo development.

Inside-Out Signaling in the Root Meristem

A polar signaling mechanism along the apical-basal axis could serve as a mere vectorial input, aligning cell differentiation events with the axis of auxin flow. However, there are indications that in root apical meristems, signals originating from the vascular center impinge on patterning in overlying tissue layers. Just as in the embryo as a whole, the *Arabidopsis* root meristem (embryonic as well as postembryonic) is characterized by both reproducibility and flexibility, which could again reflect a two-tiered patterning mechanism. The promoting influence of auxin on root meristem formation is well established and further supported by the failure of *axr6*, *bdl* and *mp* mutants to initiate a primary root in the early embryo. The expression pattern of an 'auxin-response'-reporter gene used in a new study seems to provide a molecular explanation for the auxin-dependence of root meristem initiation, but it also reveals a role of auxin as a positional signal in meristem patterning (Sabatini and others 1999). A particular synthetic 'Auxin Response' element genuinely reflected auxin distribution and was therefore used to monitor the distribution of 'perceived' auxin in the growing root. A local auxin perception maximum ('auxin peak'), which is normally positioned just distal to the quiescient center (QC) in normal root development, was manipulated in size and position by interference with auxin perception and auxin transport. Any shift in the localization of this peak was found to be associated with correlated shifts in the pattern of distal cell fates in the root meristem (Figure 2A). Most strikingly, long-term inhibition of

auxin transport through the auxin efflux inhibitor NPA generated a centrally positioned auxin peak, flanked on either side by inversely polarized root segments. In conclusion, manipulations of the position of the auxin peak by various methods were associated with corresponding changes in the cellular pattern, suggesting that auxin distribution has an instrumental role in root meristem patterning beyond the vascular system (Figure 2A). Auxin-dependent specification of meristem cell fates by a centrally positioned 'organizer' also makes sense from the perspective of a signal coordinating shoot and root development (discussed in Berleth and Sachs 2001). In normal plant development, the peak is located at the distal end of the vascular cylinder and its formation would probably depend on shoot-derived auxin. Shoot and root development could thereby be coordinated by a long-range auxin signal, resulting in a localized response, which simultaneously constitutes the initial cue to trigger patterning cell interactions in the entire distal root (Figure 2A).

Arabidopsis embryo and meristem patterning has been studied for years. If auxin has a fundamental role in these processes, why is it that this influence has not been revealed earlier? For example, it may be surprising that the identification of tissue-specific patterning functions in the root meristem preceded the observation of an organizing role of auxin. However, it seems that this sequence of discovery reflects the normal course of genetic analysis. At an early stage of investigation, highly specific defects, which are usually of intermediate severity, can most easily be discerned, whereas the interpretation of severe, often pleitropic distortions as well as the identification of subtle defects are less obvious and therefore follow at a later stage.

In roots, most of the early-discovered root pattern mutations affect the number and identity of individual root tissue layers (reviewed in Heidstra and Scheres 1999). Now, molecular details are being added and reveal an interesting directionality in the flow of patterning information. Two GRAS domain transcription factors, SHORT ROOT (SHR) and SCARECROW (SCR), are required for the formation of double-layered ground tissue in the root (Scheres and others 1995; Di Laurenzio and others 1996; Helariutta and others 2000). *SHR* seems to act upstream of *SCR,* as its activity is required for *SCR* expression, but not *vice versa* (Helariutta and others 2000). In the double mutant as well as in *shr* mutants only a single layer of ground tissue is formed and this layer has the characteristics of the outer, cortical layer. *SHR* but not *SCR* gene activity is required for the expression of endodermal markers in this single ground tissue layer. However, it requires the activity of both genes to ensure separation of the two tissue layers through an unequal division of a common initial and to suppress cortical markers in the inner of the two layers, thereby establishing two layers of different identity (Figure 2B). If it takes the hierarchy of at least two genes to carve out a separate endodermis tissue layer from a cortical ground state, with *SCR* being expressed in the endodermis under *SHR* control, where is *SHR* expressed? If it were also expressed in the endodermis (or in the single ground tissue layer in both mutants), how could this expression impose radial polarity to separate the two layers? Recent molecular analysis demonstrates that *SHR* is expressed exclusively in the stele, where it does not seem to have any function. Further, ectopic expression of *SHR* is sufficient to induce unscheduled cell proliferation and inappropriate cell specification in the outer layers of the root meristem. Thus, in normal development, the restriction of *SHR* expression to the vascular cylinder is instrumental for the non-cell-autonomous control of ordered development in the two overlying tissue layers. This observation provides further molecular evidence for the control of tissue patterns beyond the vascular system through a centrally expressed gene.

Inside-Out Signaling in the Shoot Meristem

Apical meristems are amazing stem cell populations because they have to maintain their overall cell pattern, despite the fact that the individual cells are continuously displaced from the center to the periphery. Maintaining the balance between pools of proliferating and differentiating cells is particularly critical in the shoot meristem, where differentiation in the periphery involves the formation and positioning of new organs. Depletion of the proliferative cell pool would terminate meristem activity, whereas its extreme enlargement would distort spatial dimensions and organ positioning. As with the cell patterns in embryos and roots, the shoot meristem zonation seems to be highly flexible, capable of readjusting after even extreme experimental distortion. The genes crucial for this readjustment process should be revealed by mutants with characteristic shifts in the balance between proliferating and differentiating cell pools. These types of genes have been identified and their genetic and molecular characterization has been summarized in a number of excellent reviews (Lenhard and Laux 1999; Waites and Simon 2000; Clark 2001). Briefly, a single gene *WUSCHEL* (*WUS*) has been identified to be essential for maintaining the proliferating stem

cell pool, whereas at least three genes (*CLAVATA 1, 2, 3*) interact to generate a counteracting activity, limiting meristem size (Figure 2C). *CLV1* encodes a receptor kinase (Clark and others 1997) expressed in the center of the meristem and *CLV3*, its ligand, is produced by cells at the meristem surface on top of the CLV1 expression domain (Fletcher and others 1999; Trotochoud and others 2000). *CLV* signaling and the activity of the *WUS* transcription factor (Mayer and others 1998) influence each other and form a feed-back control mechanism that maintains the balance between proliferating and differentiating cell pools. Interestingly, however, *WUS* activity is sufficient to overcome all counteracting activities and can induce proliferation throughout the apical dome, when ubiquitously overexpressed (Brand and others 2000; Schoof and others 2000). Further, *WUS* is not expressed in the proliferating cells themselves, but in the nearly quiescent center of the shoot meristem, from where it seems to influence the behavior of the distal proliferating cells (Figure 2C). Proper positioning of the *WUS* activity can be considered the defining step in the positioning of a shoot meristem, but at present, it is not known what specifies the position of the *WUS* expression domain in its normal subapical position on top of each shoot axis. There might be numerous inputs into the regulation of *WUS*, as suggested by its negative control through the *CLV* pathway and its dynamic embryonic expression. Nevertheless, the mechanism that maintains the stable association of the *WUS* expression domain with the center of a shoot growth axis and the directionality of *WUS* action itself suggests ways through which signals from the center could direct development of overlying tissues.

Conclusions

Vascular tissues are fascinating because of many of their features. Their anatomy and physiology form an amazing chapter in its own right and mechanisms underlying their beautiful ramified patterns in leaves will continue to challenge developmental and molecular biologists. The most recent observations in embryo, meristem, and auxin signal transduction research converge to support earlier models, whereby vascular tissue continuity and the overall axial organization of the plant are based on the cell biological interpretation of the same polarly transported signal, which might be IAA. The body pattern of a plant is clearly more than a mere axis and the genetic complexity underlying the three-dimensional patterning in embryos and meristems may go far beyond our expectations. The basic axial organization, possibly through signals derived from vascular tissues, could nevertheless form the anchoring coordinate system for these patterning activities, and recent research has revealed possible examples. In several instances, crucial activities sufficient to trigger downstream events in meristem patterning have to be strictly confined to defined central positions and could therefore use the axial architecture of the vascular system as a positional reference. The speculative nature of all implicated molecules remains to be emphasized. Successive molecular models can be expected for the near future and should be evaluated by their ability to explain the outlined developmental correlations.

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