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Towards the systems biology of auxin-transport-mediated patterning

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Polar auxin transport intimately connects plant cell polarity and multicellular patterning. Through the transport of the small molecule indole-3-acetic acid, plant cells integrate their polarities and communicate the degree of their polarization. In this way, they generate an apical–basal axis that serves as a positional reference anchoring subsequent patterning events. Research in recent years has brought the molecular mechanisms underlying auxin perception and auxin transport to light. This knowledge has been used to derive spectacular molecular visualization tools and animated computer simulations, which are now allied in a joint systems biology effort towards a mathematical description of auxin-transport-mediated patterning processes.

Plasticity and reiteration

How three-dimensional patterns of functionally integrated cell identities are genetically encoded is one of the central questions of developmental biology. Most commonly, cells in patterning processes communicate with each other through specific proteins, but we are now witnessing an amazing new theme in plants: a single simple molecule, in the context of a tightly regulated cellular transport mechanism, reiteratively provides a basic axial reference system in a variety of patterning processes throughout the life cycle.

Plasticity and reiteration have long been known as hallmarks of plant patterning [1,2]. When exposed to experimental or environmental influences, apical meristems or organ tissue patterns can appear highly abnormal, but retain functionality and can regain normal appearance over time. Such plastic features are suggestive of underlying self-stabilizing mechanisms, and it is now becoming apparent that feedback-stabilized auxin-distribution patterns underlie this phenotypic robustness [3]. Reiterative initiation of new growth axes in the form of lateral shoot organs and lateral roots then generates the dazzling diversity of plant morphology (Figure 1a). As discussed below, visualization of auxin-transport routes in the ontogenies of these elements now reveals an even more reductionistic picture. In each case, an auxin-flow-dependent central axis serves as a basic positional reference for organ-specific patterning processes, and the initiation of a new axis is foreshadowed by apparent auxin convergence at specific sites (for detailed reviews see Refs [4–7]).

The currently unfolding story of auxin-transport-driven patterning fascinates with its conceptual simplicity, but complexity is lurking beneath the surface in the difficult transition from plausible concepts to precise mathematical models. Molecular genetics, improved visualization tools and computer simulations are now tied in a systems-analysis approach towards a mathematical description of auxin-flow-derived patterning.

Learning from distortion; experimental and genetic interference

Plant hormones have been increasingly accepted as signals in developmental programs and the polar transport of auxin has been experimentally characterized for some time [6,8,9]. Moreover, the classic model of auxin transport – the chemiosmotic theory [10,11] – is still widely accepted and supported by molecular evidence (reviewed by Jiří Friml and colleagues in this issue of *Trends in Plant Science*). It is built upon the physical properties of indole-3-acetic acid (IAA; the most common auxin in higher plants). As a weak acid, IAA is charged selectively at intracellular pH. Therefore, its export from the cell is dependent upon specific proteins, which are assumed to be concentrated at the basal end of each cell. Interesting consequences arise if the basal localization and concentration of efflux proteins is not only the cause but also a consequence of auxin-flow patterns. As can be seen in Figure 1b, the expression of polarity in one cell then instills polarity in neighboring cells, eventually integrating polarity across cells. The auxin canalization hypothesis [12] extends this idea and suggests that such a feedback mechanism should also increase the disparity of transport properties among cells and should lead to preferred transport routes (canals) made up of highly conductive cells that might later differentiate to become vascular bundles. Alternative self-organizing models, in which the role of auxin-transport is restricted to the removal of a signal from the tip of a growing vascular strand, can also be envisaged [13], but would need to be expanded to account for auxin-mediated patterning in other developmental processes.

Despite such early conceptual projections, how a molecule with such a diversity of functions and a fairly high abundance in certain tissues can instruct patterning within primordia of a few micrometers across has remained a subject of debate. However, evidence for the

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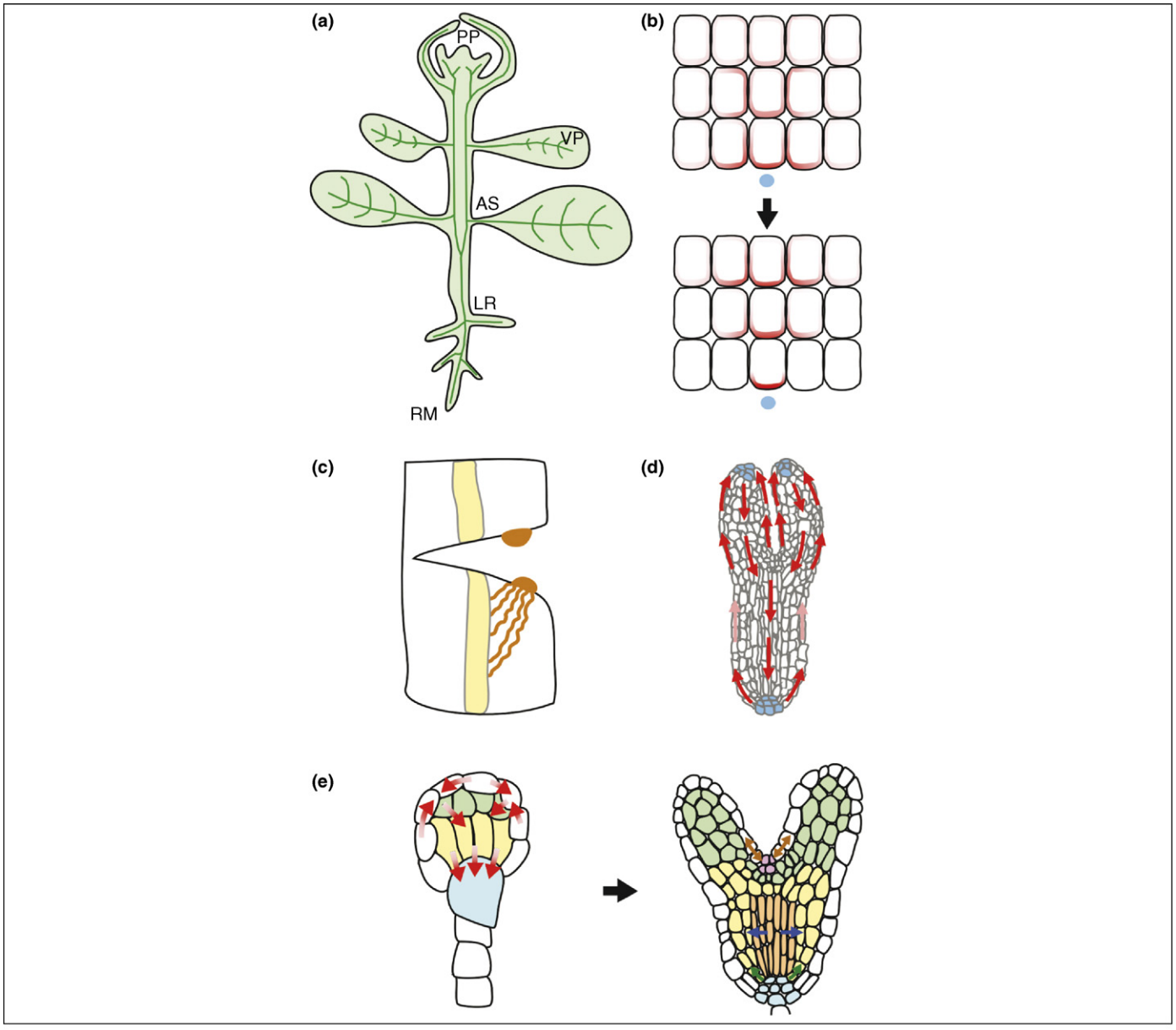

Figure 1. Instances of auxin-transport-mediated patterning. **(a)** Positions of auxin-transport-mediated patterning processes. Abbreviations: AS, axillary shoot growth; LR, lateral root positioning; PP, phyllotactic pattern; RM, root meristem patterning; VP, vascular (venation) pattern. **(b)** Gradual adjustment of auxin-transport polarities under the influence of a localized sink (blue dot) and self-enhancing transport properties indicated by the position and intensity of efflux proteins (red), from weak polarization close to the sink (e.g. pre-existing vasculature) towards an extension of an auxin-flow route. The same feedback mechanism that integrates polarities across a field of auxin transporting cells, will also lead to conductivity diversification among cells and to the formation of a preferred route of auxin transport (depicted as a strong-red label in the vertical cell file in center, bottom panel). **(c)** Auxin triggering vascular strand formation in already polarized tissue by selecting narrow lines of cells basal to the application site (brown dot). **(d)** Torpedo-stage embryo with positions of auxin maxima (blue) and directions of auxin flow (red arrows) as derived from DR5 expression patterns [41] and PIN1 polarities in postembryonic organs [19], respectively. Auxin-transport continuity throughout the embryo (light-red arrows) has not been demonstrated, but appears possible based on the inverse transport directions in root and shoot. **(e)** Left: formation of the main body axis and of lateral shoot organ axes. Globular-stage auxin-flow (red arrows) and basal auxin accumulation (blue). As outlined in the text, these features are persistently associated with the formation of lateral shoot organs and root meristems. Right: axis anchored patterning in torpedo embryo. Mutual promoting influences between the adaxial side of lateral shoot organs and the shoot meristem [81] (brown arrows), stele-derived signaling in endodermis and quiescent center specification (blue arrows). Root stem cell promoting influence around the QC (green arrows).

instructive patterning potential of auxin had already been derived from early auxin application experiments [12]. Local auxin application not only induces the formation of organized structures, such as vascular bundles, but it also does so in direct correlation to the application site and to the direction of auxin transport, indicating that IAA has the capacity to trigger all activities necessary to guide the patterning of a structure as complex as a vascular bundle (Figure 1c). Regenerative patterning in these examples co-opts polarities already present in the recipient tissue, but there is evidence that those polarities are themselves auxin-transport mediated. Proper auxin transport turns out to be a crucial prerequisite not only for regenerative vascular strand formation but also for the acquisition of apical–basal embryo polarity, the formation of new growth axes and for vascular patterning during organ development [14–18].

Among the gene families implicated in the cellular efflux of IAA (reviewed by Jiří Friml and colleagues in this issue of *Trends in Plant Science*), six genes in the PIN-FORMED (PIN) family of integral plasma membrane proteins exert highly redundant functions, and their subcellular

localizations mark the presumed auxin-efflux side of cells in highly dynamic, yet robust patterns [19] (reviewed in Ref. [3]). Mutations in *PIN1* directly resemble mild levels of auxin transport inhibition, whereas multiple mutations in *PIN* genes dramatically interfere with embryo axis formation and apical–basal polarity acquisition. Because PIN proteins are permanently reshuffled from endosomal compartments, their subcellular positions can be dynamically regulated [20,21]. Beyond self-stabilizing polarity acquisition (Figure 1b), it has become increasingly evident that tissue-specific factors influence PIN positions [22], and at least one gene, *PINOID* [23], has been implicated in redirecting subcellular PIN polarities. Tissue-specific features of auxin transport patterns have been observed in shoots [24,25] and roots [26]. Such additional regulatory inputs allow auxin flow to be differentially targeted in different tissue layers and might explain how auxin flow could be directed along continuous loops with apparently opposite polarities in shoots and roots (Figure 1d) [19]. At root tips, auxin appears to be redirected into outer layers and away from the tip, whereas in lateral shoot organs an opposite redirection seems to take place. At least in the small dimensions of an embryo, all major transport routes might be integrated into a single loop, similar to the field around a magnet (Figure 1d). Interestingly, this metaphor holds when the polarized tissue is cut and the poles are separated. Under suitable conditions, each tissue piece will develop shoot and root pole [27] because the system owes its properties to the polarities of its constituent cells.

It is a challenge for future research to explain how such a system can reiteratively generate new growth axes in the form of leaves or lateral roots. Adding to the complexity, auxin transport capacity also appears to be crucial for meristem activity in leaf axils. Axillary bud outgrowth is limited by the auxin-transport capacity of the stem, which in turn is restricted by the activity of *MAX* genes. If signaling through *MAX* genes is defective, increased auxin transport leads to abnormally high branching, which can be normalized by moderate auxin transport inhibition (reviewed in Ref. [28]).

The patterning role of auxin is also reflected in the phenotypes of certain mutations in the core machinery of auxin-dependent gene regulation, which comprises Auxin Response Factors (ARFs), their co-regulators of the Aux/IAA family and genes involved in the auxin-dependent control of Aux/IAA protein stability, through which auxin affects ARF activity [29]. Apparently because of widespread functional redundancy, informative mutant phenotypes in these gene classes are rare and severe embryo pattern defects have only been found associated with mutations in the ARF *MONOPTEROS*/*ARF5* [30] the AUX/IAA gene *BODENLOS*/*IAA12* [31,32], and the cullin gene *AUXIN RESISTANT6* [32]. The common defects in the three mutants indicate the importance of auxin signaling for the formation of an axial core (central embryonic procambium) as a positional reference that anchors subsequent patterning processes (Figure 1e). Overlapping functions of other ARFs support this notion, because *arf5 arf7* double mutants do not form any lateral organs [33] and *arf7 arf19* double mutants fail to initiate lateral roots [34,35].

If auxin flow primarily generates new growth axes, how is this basic architecture translated into the real patterns of shoots and roots? The single axis of the early embryo can illustrate how the auxin-mediated generation of a central procambial strand can serve as a positional reference to anchor further pattern elaboration in shoots and roots (Figure 1e). In analogy to the proposed mechanism underlying post-embryonic phyllotaxis [24], the positioning of cotyledons is expected to depend on efficient auxin transport through subtending vasculature. This interpretation is supported by the apical defects in auxin-transport-inhibited embryos [15,18]. Furthermore, ground tissue patterning is positionally anchored in the procambium as the source tissue of the endodermis-inducing SHORT-ROOT (SHR) protein [36]. Finally, auxin transported through the central procambial strand triggers the expression of the auxin-inducible PLETHORA transcription factors at its bottom end, which, in conjunction with SHR signaling, is crucial for the formation of the stem cell niche in the root meristem [37]. Because the embryo comprises prototypes of shoot and root organs, it is plausible that similar auxin-transport-anchored patterning continues to operate in the reiterative generation of new growth axes throughout the life of the plant.

Although occasionally highly suggestive, current observations are still of insufficient resolution for distinguishing tests of mathematical models. This is where continuously improving visualization tools become essential. Visualization tools are being used to generate a three-dimensional view of auxin flow at increasingly higher resolution and are now moving towards the generation of a complete picture in space and time.

New visualization tools; conceptual insights from auxin-transport polarities

Although auxin remains invisible at the cellular level, two powerful tools are currently available to visualize auxin transport routes and accumulation patterns. It has recently been shown that PIN proteins are instrumental in defining the polarity of auxin flow [38,39] and, therefore, its directions can be inferred from the asymmetric localization of PIN proteins during development (Figure 2g,h). By monitoring the subcellular localization of PIN1 in shoot apical meristems (SAMs) through immunolocalizations using antibodies directed against PIN1, Reinhardt *et al.* [24] proposed a model for regulating leaf primordium formation and phyllotaxis by auxin transport. According to this hypothesis, auxin is transported in epidermal cells towards the SAM. There, auxin further induces PIN1 expression, which, in turn, promotes auxin accumulation at the site of incipient primordium formation (Figure 2a). Leaf primordia, once established, are postulated to drain auxin through their central midveins, with resulting auxin depletion in their vicinity. Through a combination of positive feedback (i.e. auxin accumulation) and lateral inhibition (i.e. depletion of auxin from adjacent tissues), auxin would accumulate at a certain distance from the pre-existing primordia, enabling the phyllotactic patterning cycle to start again. Consistent with this hypothesis, auxin overload (through direct auxin application or auxin transport inhibition) enlarges primordia or reduces their

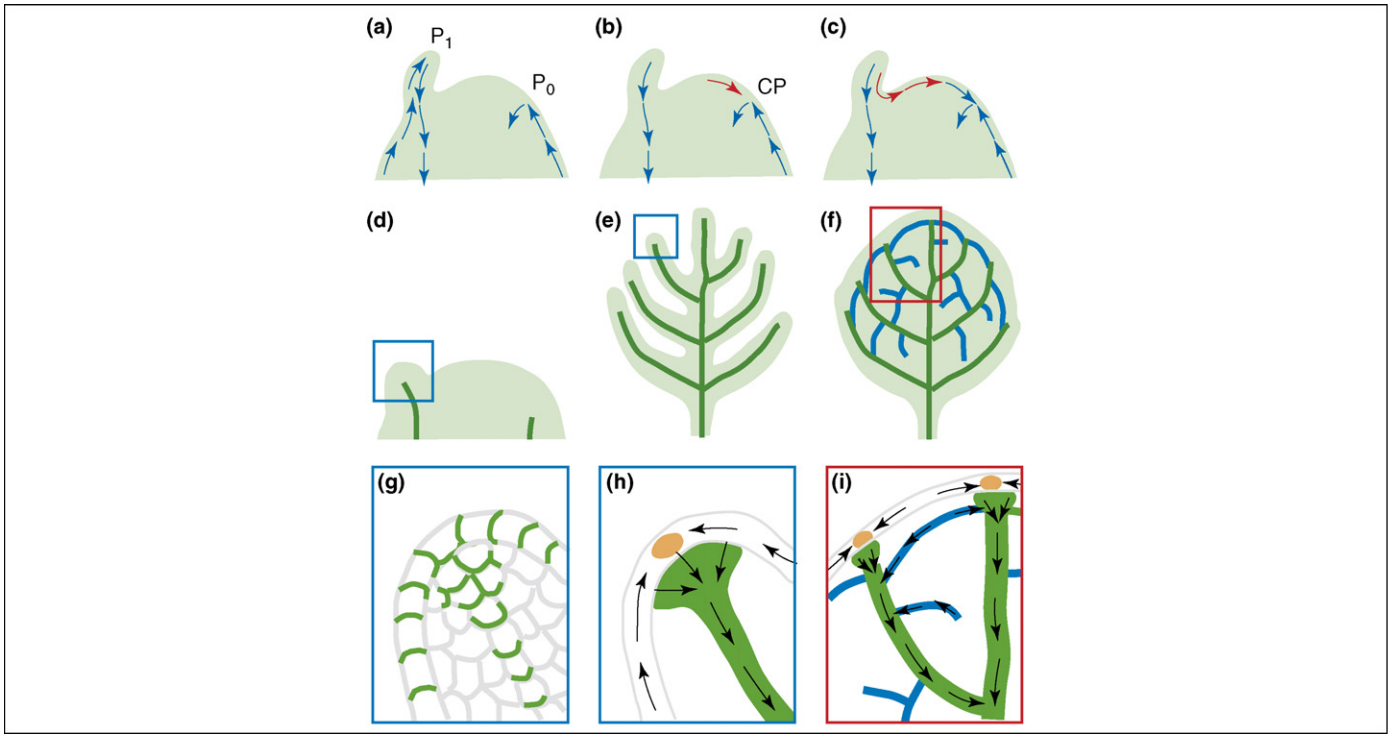

Figure 2. Visualization of directions of auxin flow in plant development. **(a–c)** Refinement of models of auxin transport in phyllotaxis as a result of advances in visualization tools and techniques. **(a)** Immunolocalizations with antibodies against the PIN1 auxin efflux protein [24] suggest that auxin flows in a base-to-tip direction in epidermal cells of the stem, basal to the site of primordium formation (P_0). There, auxin is transported in internal tissues where it gradually induces formation of a vascular strand, which transports auxin in a tip-to-base direction. Primordia (P_1) deplete the surrounding area of auxin through highly efficient tip-to-base transport of auxin through their differentiated vascular strands. **(b)** Imaging of a functional translational fusion between PIN1 and GFP (PIN1:GFP) [19] suggests that auxin also flows in a tip-to-base direction in epidermal cells of the SAM apical to the site of primordium formation (red arrows), with resulting formation of a point of converging auxin flows (CP). **(c)** Time-lapse imaging of PIN1:GFP [45] suggests that auxin flows in a tip-to-base direction in all the epidermal cells of the SAM apical to the site of primordium formation, and that older primordia additionally deplete the surrounding area of auxin through its transport in epidermal cells towards the site of new primordium formation (red arrows). **(g)** Subcellular localization of PIN1:GFP (green), and **(h)** inferred routes of auxin transport (black arrows) and convergence point of auxin flows (orange) in leaf primordium **(d)** and lateral organ **(e)** formation. Leaves with broad lamina and closed vein patterns composed of major veins (green) and minor veins (blue) **(f)** are thought to have evolved from simple branching systems with open vein patterns composed of major veins only (e). All the major veins in (d–f) are associated with epidermal convergence of auxin flows (g–i); minor veins that form the upper part of each vein loop in (f) represent the extension of minor veins that otherwise end freely in the lamina (i).

lateral separation, eventually leading to primordium fusion [40].

A functional translational fusion of PIN1 and a Green Fluorescent Protein (GFP) tag [19] confirmed and extended observations by Reinhardt *et al.* [24], according to which PIN1 polarity in epidermal cells of the SAM is directed towards discrete points at positions of incipient primordium formation [19] (Figure 2b). Furthermore, converging auxin flow, as deduced from PIN1 polar localization, coincided with apparent auxin accumulation at the incipient primordium tip. This was monitored using another important visualization tool, an endoplasmic reticulum-targeted version of GFP driven by a DR5 (DR5Rev) [41] promoter. The DR5 promoters are synthetic promoters composed of tandem repeats (typically seven or nine) of the CCTTTTGTCTC sequence (or of its inverted sequence GAGACAAAAGG in the Rev variants) [42], which includes an ARF binding site (underlined) [43]. Expression of DR5 transcriptional fusions have been shown to be proportionally responsive to a range of auxin concentrations [44] and to overlap spatially with auxin accumulation patterns as detected by immunolocalizations with antibodies directed against IAA [41]. As such, DR5 reporters are currently used to visualize patterns of auxin distribution, whether they are the result of regulated auxin metabolism or transport.

The generation of a DR5 fluorescent reporter that is spectrally separable from PIN1:GFP signals [45] and, most importantly, a time-lapse imaging system for the SAM [46,47], enabled phyllotaxis models to be refined further. Time-lapse visualization of auxin concentration maxima (as visualized by a nuclear-localized, rapidly folding variant of the Yellow Fluorescent Protein driven by the DR5Rev promoter) and directions of auxin flow (as inferred from subcellular localizations of PIN1:GFP) in live SAMs supports the hypothesis that auxin is drained by developing leaf primordia [45]. However, instead of auxin depletion occurring only through auxin transport into the midvein, time-lapse visualizations suggest that it might also result from reversals of epidermal PIN1 polarity in the SAM away from developing primordia and towards the site of incipient primordium formation (Figure 1c). This finding raises the possibility that the patterning mechanism that positions leaf primordia might act primarily within the epidermis, because it has previously been suggested by laser ablation experiments [48].

Similar visualization tags have recently been used to monitor the formation of vein patterns in leaf primordia [25]. In striking similarity to leaf initiation at the SAM, the outgrowth of the lamina and the formation of major lateral veins within the developing leaf primordia are associated with convergence points of auxin transport in epidermal

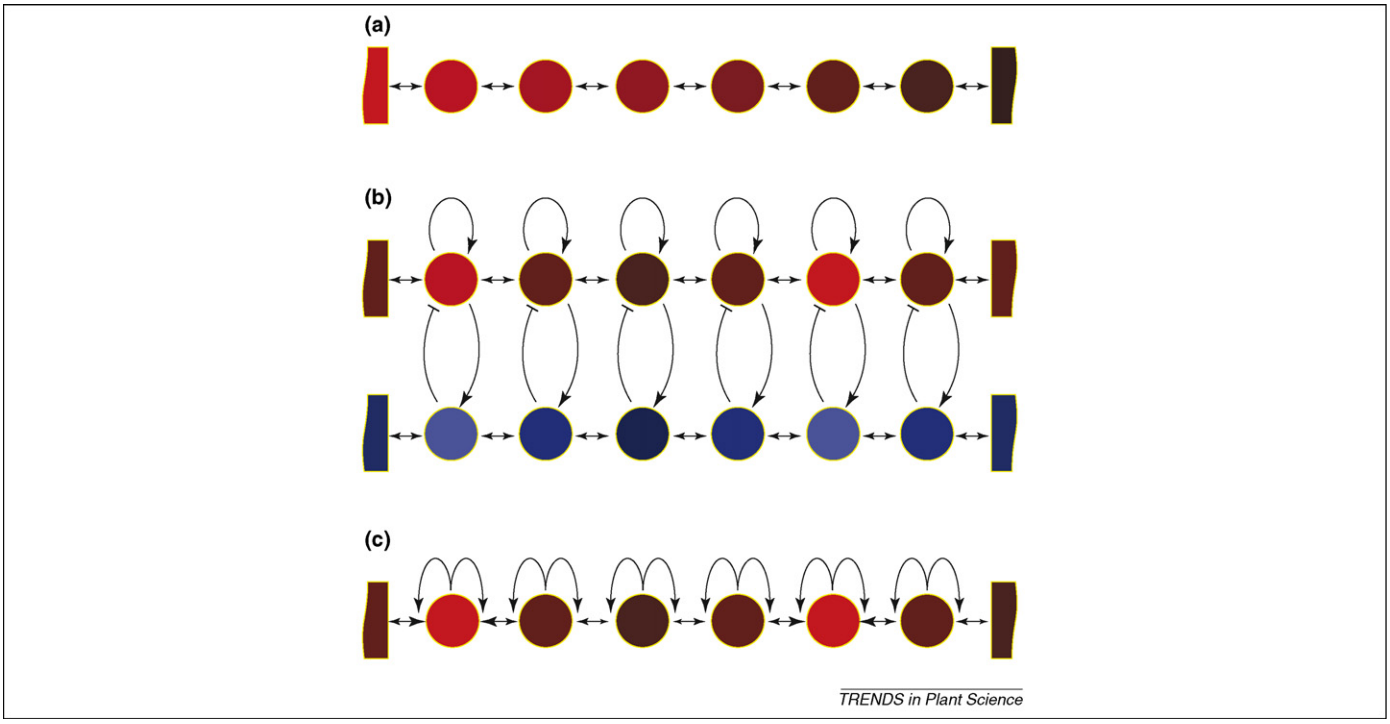

Figure 3. Selected models of morphogenesis. **(a)** Gradient-based model of positional information. Cells (circles) acquire information about their position within a tissue by sensing the concentration of one or more morphogens (one substance shown in red) that diffuse through the tissue (two-sided arrows) and form a gradient between its boundaries (broken rectangles). **(b)** Reaction-diffusion. Diffusing morphogens are locally produced or turned over in a feedback loop of interactions that can range from simple chemical reactions to complex biological mechanisms such as control of gene expression. In the example shown, the activator (red) upregulates its own expression and the expression of the inhibitor (blue), while the inhibitor downregulates the activator. The resulting distribution of concentrations forms a pattern that might be largely independent of the boundary conditions. **(c)** Regulated transport. The pattern results from the redistribution of a substance that is actively transported under the control of cells (arch-shaped arrows regulating horizontal arrows).

cells at the primordium margin (Figure 2i). Furthermore, excess auxin resulting from local auxin application or systemic auxin transport inhibition reduces lateral spacing of epidermal convergence points and leads to the formation of supernumerary veins in leaf primordia [16,25], suggesting functional similarity between major vein positioning and leaf primordium formation. If convergence point patterning in SAMs and leaf primordia share a common, reiteratively used axis-initiation process that depends on auxin supply and transport, its mechanistic dissection might benefit from the one-dimensional arrangement of epidermal cells in the leaf margin.

Epidermal convergence of auxin flow in the formation of lateral shoot organs and of major veins within them is a new, extreme example of the reiterative use of auxin-transport-mediated principles. It is also consistent with the prevailing hypothesis of how leaves formed during the evolution of land plants. Fossil evidence suggests that leaves with broad lamineae evolved from simple, leafless branching systems [49,50] (Figure 2d–f). Obviously, leaf venation is more than a series of branching veins, but comprises closed networks of minor veins, where most veins are connected to others at both ends with no obvious polarity. Visualization of early auxin transport routes during lamina formation also provided a possible explanation that would reconcile the polarity of auxin flow with a closed vein network [25]. Closed veins originate from open-ended precursors with uniform auxin-transport polarity towards pre-existing veins but they switch to bipolarity as they become connected at both ends (Figure 2i).

From intuitive concepts to computational simulations

The organization of data into cause–effect relations is the domain of modeling. The models can range from conceptual models that are stated verbally to formal models stated in mathematical terms. Mathematical models capture ideas with greater precision, expose inconsistencies in underlying assumptions, and might bring unexpected system properties to light. Furthermore, these models lend themselves to computer simulations, which can be used to test the plausibility of proposed mechanisms, refine hypotheses, and make predictions that guide further experiments. Simulations are particularly important in studies of pattern formation and morphogenesis because the dynamics of the intervening spatio-temporal processes are often difficult to grasp intuitively.

From a range of patterning models [51], two classes have historically played a particularly important role. Classic positional-information models are based on the concept that 'there is some cell parameter that reflects distance from a boundary' of an organ or tissue [52] (Figure 3a). This information is typically associated with a concentration gradient of one or more substances, termed morphogens*, which diffuse between the boundaries of the system [52,53]. Reaction-diffusion models [54–56] introduce the concept of reactions between morphogens* (Figure 3b). Gradient-based and reaction-diffusion models successfully explain a variety of developmental processes in animals [57], but another type of mechanism, for which

* We use the term 'morphogen' both to denote Wolpert's positional signals [52], and Turing's reacting morphogen pairs [54].

we suggest the term 'regulated-transport', appears to be responsible for key aspects of plant development [3,6,58]. The central notion is that patterns result from redistribution of a substance that is actively transported in a regulated manner by the cells (Figure 3c), properties that apply to auxin. In pure, regulated-transport models, auxin is assumed to be generally available, for example, because of ubiquitous production. In hybrid models, patterns are assumed to result from a combination of local production and the regulated transport of auxin.

Although the molecular biology of auxin transport is by no means resolved (reviewed by Jiří Friml and colleagues in this issue of *Trends in Plant Science*), several models for characterizing auxin flux in mathematical terms already exist. For description purposes, it is convenient to compare them with Fick's law, according to which the flux Φ of a substance diffusing between cells i and j is proportional to the difference of concentrations c_i and c_j of this substance in the cells: $\Phi = D(c_i - c_j)$. Through a mathematical analysis of the chemiosmotic theory of auxin transport [59,60], Mitchison [61–63] and Goldsmith *et al.* [64] arrived at a simple formula for polar transport, according to which the flux across a boundary is a linear combination of the auxin concentrations on the two sides of the boundary: $\Phi = P_i c_i - P_j c_j$. Formally, this formula can be viewed as the result of separating the diffusion constant D into two separate coefficients, P_i and P_j. If $P_i \neq P_j$, auxin can be transported against the concentration gradient. Variants and extensions of this idea include the separation of diffusive and polar-transport components [65], the introduction of a saturation term intended to capture the limited availability of efflux carriers according to Michaelis–Menten kinetics [62,66,67], and the introduction of other non-linearities [68,69]. Kramer [70] incorporated intercellular space into his model of auxin transport, which made it possible to separate the effects of efflux and influx carriers. In addition, Kramer's model takes into account the diffusion of auxin within intercellular space. Recently, the relevance of this process has been analyzed from a quantitative point of view [71].

At the heart of regulated transport models is a postulated feedback mechanism, according to which the parameters of auxin transport are controlled by auxin distribution or flow. The cause–effect relations underlying this process, which can assume different forms in different tissues or organs, can be investigated using simulation models. Models of phyllotaxis and leaf vein formation have received the most attention to date.

Recently proposed simulation models of phyllotaxis [67,69] are based on the hypothesis that cell polarization orients PIN1 proteins toward the neighboring cell with the highest auxin concentration. This hypothesis is consistent with the observation that PIN1 proteins in the L1 layer of the SAM are oriented toward incipient new primordia [19,24] and with experimental data and simulation results indicating that the primordia are co-located with apparent auxin concentration maxima [19,65–67]. The simulations by Jönsson *et al.* [67] and Smith *et al.* [69] have shown that the feedback between auxin transport and PIN1 localization, acting on the growing surface of a SAM, is capable of placing primordia in spiral and other phyllotactic patterns (Figure 4b). One of the differences between the two models concerns the formulas used to characterize auxin transport by PIN1 proteins and PIN1 localization by auxin: the formulas of Jönsson *et al.* [65] follow the existing theories of auxin transport and potential mechanisms of PIN1 localization more closely, whereas the strongly non-linear formulas proposed by Smith *et al.* [67] appear to yield more stable patterns.

After converging at primordia tips, auxin is assumed to flow basipetally through immature internal tissue within the primordia, where it initiates leaf vasculature [19,24]. This assumption is supported by the observation that PIN1 proteins are localized towards an auxin convergence point

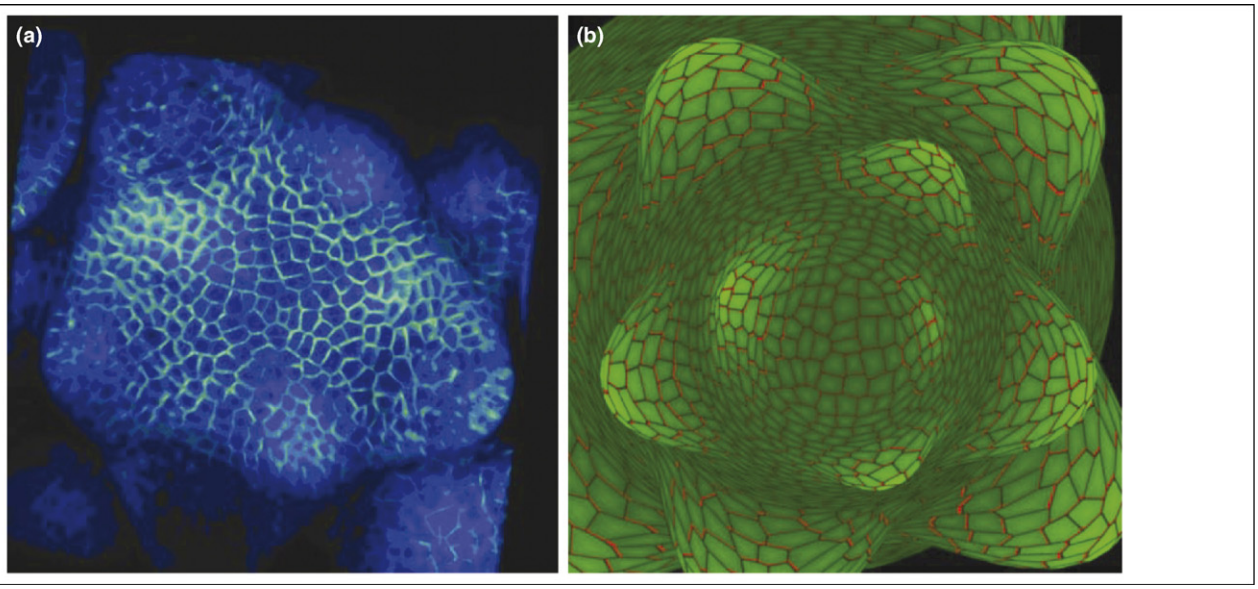

Figure 4. Comparative images of the *Arabidopsis* shoot apical meristem (from above). **(a)** Confocal-microscopy-based volume rendering of PIN1:GFP expression and localization in the meristem [45]. Increasing signal intensities are coded blue to yellow. Image courtesy of Marcus Heisler, California Institute of Technology, USA. **(b)** Computer simulation model of a developing meristem [69]. Localization of PIN1 proteins shown in red, concentration of auxin shown in green. Image courtesy of Richard Smith, University of Calgary, Canada.

in L1, but away from it in subepidermal tissues (Figure 2g,h). Currently, the most plausible explanation for this difference is that PIN1 protein localization depends on cell type and, as in the root, different mechanisms might operate in the epidermal and inner cell layers. The canalization hypothesis, postulates that the inner cells in a leaf primordium are polarized by auxin flow, in a manner that promotes further flow in the current direction [12,13,72,73] (Figure 1b). Cells with an increased auxin flow differentiate into veins. The plausibility of the canalization hypothesis was first tested mathematically by Mitchison [61,63]. Recently his simulations were reproduced and re-examined in light of new experimental data by Rolland-Lagan and Prusinkiewicz [65], who showed that the model extends to patterns resulting from auxin transport inhibition or mutations affecting vein continuity. However, the concentration of auxin in the canals predicted by Mitchison's model is inconsistent with the experimental data: the model produces canals with high auxin flux but low concentrations, whereas data suggest that the concentrations of auxin in vascular precursor cells is high [74]. Analyzing this discrepancy, Feugier *et al.* [66] and Fujita and Mochizuki [75] showed that the concentration of auxin in the canals crucially depends on the detailed properties of the mechanism that distributes efflux carriers within a cell. According to Feugier *et al.* [66], if the number of efflux carrier proteins is controlled independently in different parts of a cell, as is the case in Mitchison's model, the canals will have a lower concentration of auxin than the surrounding tissue. By contrast, if the number of carrier proteins in a cell is fixed or slowly changing and different sides of a cell compete for the allocation of carrier proteins from the available pool, the canals might have a higher auxin concentration that the adjacent tissue. Feugier *et al.* [66] also showed that if auxin is produced uniformly in a sufficiently large grid of cells, both versions of the canalization model yield branching patterns. However, these patterns lack important features of real venation, such as the presence of closed loops or hierarchical vein orders, which underscores the continued need for more refined models. Similar results have been obtained by Fujita and Mochizuki [75], who postulated a hypothetical diffusible substance that localizes PIN1 protein within cells.

One possibility is that the regulated, non-homogenous distribution of auxin sources [76,77] and/or convergence points [25] play an important role in the formation of venation patterns. Using simulation models, Mitchison [61,63] and Rolland-Lagan and Prusinkiewicz [65] showed that such sources, appearing at the proper time and place in the simulated leaf blade, can initiate veins and yield vein loops. Furthermore, even if auxin is produced uniformly throughout the leaf blade, maxima of auxin concentration might emerge near the centers of regions surrounded by veins that drain auxin away [78]. These centers might play a role that is similar to discrete auxin sources. An alternative model of loop formation has been proposed by Feugier and Iwasa [79], who introduced a special factor that attracts vein segments with a high concentration of carrier proteins.

The development of natural vein patterns in their full complexity was considered by Runions *et al.* [80]. According to the proposed model, veins grow towards auxin sources (or maxima of auxin concentration), which are continuously emerging at some distance from existing veins as well as from other sources as the leaf blade expands. The model by Runions *et al.* [80] addresses the diversity of venation patterns found in dicot and monocot leaves. It abstracts from details pertinent to the formation of canals at the cell level, but its results should stimulate experimental research through a variety of approaches.

Conclusions and prospects

Phyllotaxis, vein pattern formation and the internal organization of the root meristem are presently the best-explored examples of auxin-transport-mediated patterning. Processes in other plant organs and in various types of cultured conditions are soon to follow. Increasingly accurate theoretical descriptions can be expected, but it will be a continuing challenge to independently visualize the intercellular signals and cellular mechanisms postulated in them, including the true complexity of the auxin transport machinery (see parallel review by Jiří Friml and colleagues in this issue of *Trends in Plant Science*). Because the systems properties of the underlying patterning mechanisms can no longer be captured intuitively, they are most productively explored in a tight interaction of genetic analysis, auxin-flow imaging, and computer simulation. The progress that has been made using these complementary approaches is already reflected in matching features of experimentally and mathematically generated images (Figure 4) and is likely to be extended towards the precise quantification of events at the cell and molecular levels in the future.

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