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DOI: https://doi.org/10.1111/j.1365-3040.2009.01997.x

Auxin transport-feedback models of patterning in plants

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Abstract

Many patterning events in plants are regulated by the phytohormone auxin. In fact so many things are under the influence of auxin, that it seems difficult to understand how a single hormone can do so much. Auxin moves throughout the plant via a network of specialized membrane-bound import and export proteins, which are often differentially expressed and polarized depending on tissue types. Here we review simulation models of pattern formation that are based on the control of these transporters by auxin itself. In these transport-feedback models, diversity in patterning comes not from the addition of more morphogens, but rather by varying the mechanism that regulates the transporters.

Auxin drives patterning in plants

From the establishment of the first embryonic axis to the formation of leaves and flowers in adult plants, many patterning events in plant development involve the phytohormone auxin (Benkova, Michniewicz et al. 2003; Friml, Vieten et al. 2003; Reinhardt, Pesce et al. 2003; Blilou, Xu et al. 2005; Tanaka, Dhonukshe et al. 2006). In the embryo, plants must initially define the apical/basal axis to correctly position the shoot apical meristem at one end and the root apical meristem at the other. Disrupting auxin transport by mutations or inhibitor treatments compromises this early patterning process, resulting in unviable embryos (Steinmann, Geldner et al. 1999; Friml, Vieten et al. 2003; Vieten, Vanneste et al. 2005; Weijers, Sauer et al. 2005; Weijers, Schlereth et al. 2006; Jenik, Gillmor et al. 2007). As the shoot apex develops, plants use auxin to position the organs, leaves or flowers, at regular angles around the stem axis, resulting in patterns called phyllotaxis (Reinhardt, Pesce et al. 2003; Heisler, Ohno et al. 2005; Kuhlemeier 2007). Equally important is the formation of the vascular system to deliver nutrients, water and photo assimilates throughout the plant. Although the patterns of interconnected veins seen in leaves (Fig. 1B) look very different from phyllotactic patterns (Fig. 1A), vein specification is also thought to be driven by auxin (Sachs 1969; Sachs 1975; Sachs 1981; Scarpella and Meijer 2004; Scarpella, Marcos et al. 2006). Auxin is involved in controlling the formation of lateral roots, which originate post-embryonically, by promoting the recruitment of lateral root primordium founder cells from the pericycle tissue (Casimiro, Marchant et al. 2001; Benkova, Michniewicz et al. 2003; De Smet, Tetsumura et al. 2007; Ditengou, Tealea et al. 2008; Dubrovsky, Sauer et al. 2008; Swarup, Benkova et al. 2008). At a larger scale, auxin is involved in controlling the relatively long distance signaling processes that results in apical dominance (Ongaro and Leyser 2008). The plant hormone has also been shown to trigger major developmental reconfiguration events in response to external developmental cues such as tropic responses of the plant to light and gravity (Marchant, Kargul et al. 1999; Friml, Wisniewska et al. 2002; Tatematsu, Kumagai et al. 2004; Swarup, Kramer et al. 2005; Esmon, Tinsley et al. 2006).

At the core of all these patterning processes is the establishment of auxin gradients (Benkova, Michniewicz et al. 2003). The visualization of these auxin gradients allows us to predict where tissue differentiation will occur. For instance, GFP expression driven by the synthetic auxin responsive promoter DR5 appears in the surface of the shoot apical meristem at the site of organ formation well ahead of any physical signs of organogenesis (Heisler, Ohno et al. 2005; Smith, Guyomarc'h et al. 2006). Similarly, the formation of strands high in DR5 expression in young leaves defines where the vein network will eventually differentiate, nearly a day before the expression of early pro-cambium markers such as ATHB8 (Scarpella, Marcos et al. 2006). Although auxin accumulation at specific sites can induce differentiation, the activation of a certain developmental pathway also depends on additional tissue-specific fate specification factors. For instance the microapplication of auxin in the peripheral zone of the shoot meristem triggers organogenesis whereas application to young leaves induces the formation of additional vascular strands (Reinhardt, Mandel et al. 2000; Scarpella, Marcos et al. 2006). As Ottoline Leyser writes, “When the auxin baton points your way, it’s your turn to play whatever musical instrument you happen to be holding” (Leyser 2005).

If the auxin gradients are indeed the main driving force behind many patterning events in plants, then it is natural to ask how these auxin gradients are established, given they often must start *de novo*. An example of this *de novo* patterning capability can be seen in the regeneration of an entire plant from a single cell isolated from leaf mesophyll (Takebe 1971). As this single cell grows and divides, its progeny must collectively decide which cells will become shoot, and which will become root. These cells all have the same genetic code, but somehow they are able to communicate and self-organize to create the various structures and cell types required in the adult plant. Although not quite as dramatic, similar processes occur during venation in developing leaves and organ primordium initiation in the shoot apex, when a subset of cells is selected from a ground cell population, and subsequently undergoes specific differentiation.

Turing’s reaction-diffusion model can create de novo patterns

The question of how a group of equivalent cells self-organize to create a pattern was addressed by Turing (1952), and his reaction-diffusion model has since become one of the most widely used models for pattern formation in biology (Meinhardt 1982; Murray 2002). Turing’s model fits nicely within the modern

framework of genetic regulatory networks, which are often modeled with differential equations for the production, decay, and interaction of gene products. This was one aspect of Turing's model, the reactions of substances he called morphogens, and the feedbacks and interactions between them. However Turing was interested in pattern formation, and thus his theory also included a spatial component. Whereas genetic regulatory network modeling is often focused on the workings of a single cell, Turing considered a system of multiple cells[1] , where the output from the reaction network in one cell becomes the input to the reaction network in the next. The cell-to-cell communication was in the form of diffusion, hence the name *reaction-diffusion*.

To see how reaction-diffusion can create a pattern, it is instructive to look at the *activator-inhibitor* model developed by Geirer and Meinhardt (1972). They proposed that patterning requires the cooperation of two components: *local activation*, to select a subset of cells for differentiation, combined with a *longer range inhibition*, to suppress the activation of neighboring cells. In their model a substance called the activator enhances its own production, as well as that of second substance, termed the inhibitor. The inhibitor inhibits production of the activator. This system can be seen as a simple genetic regulatory network operating in each cell. The cells communicate via diffusion of the two substances. A small local maximum in activator concentration in one cell due to random variation leads to a local increase in production of both the activator and the inhibitor. The inhibitor diffuses away more quickly than the activator, reducing its effect on local activator self-enhancement, while suppressing activator self-enhancement in neighboring cells. In a system of identical cells, each operating with identical reaction rules, this destabilization can create a spatial pattern of peaks in activator concentration, which can trigger selective differentiation leading to patterning (Fig. 2A). By using interactions of multiple substances, combined with multiple cascading interactions, Meinhardt has used this basic idea to account for a wide variety of patterning processes observed in nature (Meinhardt 1982; Meinhardt 1995), including the appearance and interpretation of morphogen gradients (Wolpert 1969). Much of Meinhardt's work was done before the widespread availability of molecular data about the morphogenetic substances involved. However, reaction-diffusion models are now appearing as genetic regulatory networks linked directly to identified gene products in both plants and animals (Jönsson, Heisler et al. 2005; Sick, Reinker et al. 2006; Bouyer, Geier et al. 2008; Digiuni, Schellmann et al. 2008).

Auxin is different from Turing's morphogens

Although Turing's reaction-diffusion model can account for a wide variety of biological patterning and *de novo* pattern formation, auxin differs from the morphogens considered by Turing and Meinhardt in two important ways. First, the movement of auxin through plant tissue does not occur primarily by diffusion, but by polar transport. Auxin carrier proteins move auxin from cell to cell in ways diffusion cannot, for example, up a concentration gradient. Second, it appears that in many cases auxin gradients that are established in plant tissue do not result from the local production of auxin, but rather by redistributing auxin from surrounding tissue via polar transport. It is thus the control of the rate and the direction of auxin transport that is central to auxin-based patterning.

It has been known for some time that plants transport auxin in a polar fashion (Goldsmith 1966; Leopold and Hall 1966). To explain this phenomenon, the *chemiosmotic model* of auxin transport was proposed (Rubery and Sheldrake 1974; Raven 1975; Goldsmith and Goldsmith 1981; Goldsmith, Goldsmith et al. 1981). Auxin (indode-acetic-acid or IAA) is a weak acid, and in the neutral pH inside cells it is largely dissociated. In this ionic form, auxin is hydrophilic and unable to diffuse across the plasma membrane. In order for auxin to leave a cell, it requires the activity of carriers located at the plasma membrane. One of these carriers, the PIN1 export protein in Arabidopsis, is so important in the shoot that the *pin1* mutant is unable to generate floral organs, and produces a pin-shaped inflorescence instead (Okada, Ueda et al. 1991). Once outside the cell, in the lower pH of the extracellular space, a significant portion (approx 20%) of the auxin becomes protonated, making it lipophilic and able to cross the plasma membrane and diffuse back into cells. However there is evidence that this diffusion is not always enough for reliable patterning. Although the phenotypes are not as severe as the PIN1 mutant, Arabidopsis plants missing several import carriers show major disruption in organ positioning (Bainbridge, Guyomarc'h et al. 2008). Fig. 3 shows a

[1] Turing also presented a continuous version of his theory based on PDEs.

schematic representation of how auxin is thought to move through cells. Import carriers located in the plasma membrane import auxin into the cytosol from extracellular space. Once inside a cell, auxin moves via diffusion, and then is transported out of the cell by export carriers. The export carriers are often polarly localized to one side of the cell, and when coordinated over multiple cells, this polarity results in a directional flux of auxin through the tissue.

Since auxin gradients are created by active transport, the patterning mechanism behind the establishment of these gradients results from the control of the abundance and/or polarity of the auxin transporters at the plasma membrane. Experimental work suggests that auxin itself has a major effect on regulating its own transport, and that the asymmetric auxin distribution patterns seen in plant tissues are formed as a result of a feedback loop between auxin and its transporters. For example it has been shown that auxin can regulate the expression of auxin export carriers (Heisler, Ohno et al. 2005; Vieten, Vanneste et al. 2005). Auxin also modulates sub-cellular localization of its exporters, since Arabidopsis PIN1 and PIN2 proteins can re-localize upon auxin microapplication (Sauer, Balla et al. 2006; Bayer, Smith et al. 2009). High auxin levels inhibit endocytosis, locking export carriers at the plasma membrane thus promoting auxin efflux (Paciorek, Zazimalova et al. 2005). Moreover, the upregulation of the auxin importer AUX1 by auxin helps to establish an auxin maximum that triggers lateral root formation (Laskowski, Grieneisen et al. 2008). This suggests that plants have found a variety of ways to use the feedback of auxin on its own transport to generate patterns, creating a class of *transport-feedback* patterning mechanisms.

A transport-feedback mechanism for vein formation: Sachs' canalization hypothesis.

Sachs was perhaps the first to suggest that auxin could feed back on its own transport (Sachs 1969; Sachs 1975; Sachs 1981). By performing microapplication experiments on pea hypocotyls he observed that auxin was sufficient to trigger the differentiation of vascular strands from the source (application site) to the intact central vein which he believed was acting as a sink for auxin. The fact that such treatments led to the formation of narrow vascular strands could not be explained by diffusion alone, and he proposed the existence of a “canalization” mechanism for auxin flux. He suggested that a plant cell’s ability to transport auxin increases with auxin flux, and that this feedback of auxin on its own transport is responsible for selecting strands of tissue that later differentiate into vascular bundles. He drew the analogy with the way erosion leads to the formation of discrete channels with the flow of water. Any initially dominant path, however small, would undergo increased erosion, which would subsequently attract more flow. This would cause the path to enlarge (local activation), while simultaneously suppressing the formation of competing paths nearby (longer-range inhibition).

The involvement of auxin transport in vein formation has since been confirmed by complementary approaches. In mutants impaired in auxin transport machinery (Galweiler, Guan et al. 1998; Deyholos, Cordner et al. 2000; Sieburth, Muday et al. 2006), auxin signaling (Przemeck, Mattsson et al. 1996; Hardtke and Berleth 1998; Hobbie, McGovern et al. 2000) and auxin biosynthesis (Cheng, Dai et al. 2007), the leaf venation pattern is highly disrupted. A similar effect is seen when treating leaves with auxin transport inhibitors (Mattsson, Sung et al. 1999; Sieburth 1999; Mattsson, Ckurshumova et al. 2003). Moreover, GUS or GFP expression driven by the auxin responsive promoter DR5 marks the initiating leaf vein (Mattsson 2003; Scarpella, Francis et al. 2004; Scarpella, Marcos et al. 2006) prior to the expression of the early pre-procambium marker ATHB8. This early DR5 expression occurs concomitantly with the expression of the auxin exporter PIN1, and both are currently the earliest known markers for vascular initiation (Scarpella, Marcos et al. 2006).

Mitchison’s simulation models of the canalization.

Mitchison explored the plausibility of Sach’s canalization hypothesis with computer simulation studies (Mitchison 1980; Mitchison 1981). He proposed two main variants of the model, facilitated diffusion and polar transport, each suggesting different molecular mechanisms. In his facilitated diffusion model, the transporters were passive channels, which simply increase the diffusion rate between cells. This increase is bidirectional, as each interface is symmetric, with flux in either direction causing an increase in the ability of auxin to move in both directions. Mitchison suggested plasmodesmata as potential candidates for these channels although experimental support this hypothesis is lacking. In his polar transport model, the

abundance of transporters at the cell periphery could vary on each side of the interface, and it was therefore necessary to consider the direction of flux when adding carriers. Only a net efflux of auxin from a cell across an interface induced the addition of carriers based on flux. If the efflux was less than zero (influx) then only the background amount of carriers was added. Mitchison showed that both models were able to produce discrete canals of auxin flow in a uniform field of cells that included a small amount of noise. He also reported that in order for the canalization of auxin to occur, the relationship between the addition of transporters (channels or carriers) and auxin flux must be non-linear, and used a quadratic function in his models. Feugier et al. (2005) confirmed this result when exploring Mitchison's polar transport model and tried various alternatives for the flux response function.

Rolland-Lagan and Prusinkiewicz (2005) performed simulations based on both the facilitated diffusion and the polar transport versions of Mitchison's model, comparing them with experimental data on vein formation in Arabidopsis leaves. They noted that the polar transport model was more consistent with the current theory of auxin transport (Fig. 3) and PIN1 expression data, in that it predicts a polarity of the carriers in the cells from source to sink. This can be seen during the initiation of the midvein, where the auxin exporter PIN1 displays an overall polarity directed away from the auxin maximum at the leaf tip towards the stem vasculature below (Reinhardt, Pesce et al. 2003; Scarpella, Marcos et al. 2006; Bayer, Smith et al. 2009). This process is repeated during the formation of the first secondary veins, where convergence points of PIN1 and DR5 expression appear in the leaf margin and then extend into the leaf and connect to the midvein at the base of the leaf. Thus, as in the case of midvein initiation, PIN1 polarizes away from the source of auxin towards existing veins.

Finding the sink.

Sachs suggested that preexisting vascular strands were acting as sinks for auxin, creating an auxin concentration gradient in the surrounding tissue, thereby attracting initiating veins towards them. Since the preexisting vascular strand have high DR5 expression and presumably high auxin concentration (Scarpella, Marcos et al. 2006), it is not clear how they can act as a sink for auxin as suggested by Sachs. This raises the question how the developing vein is able to find preexisting veins. Heisler and Jonnson (2006) suggest that these cells may still act as sinks if they express high levels of auxin import carriers, an idea supported by the simulation models of Kramer (2004). In order to explore the ability of Mitchison's models to find a sink, we performed simulations using the equations and parameters from Rolland-Lagan and Prusinkiewicz (2005) with both a point source of auxin, representing the convergence point at the leaf tip, and a point sink, representing the pre-existing vasculature. We found that the facilitated diffusion model could much more reliably connect the source to the sink than the polar transport model (Fig. 4). On a grid of cells, moving the sink just a few cells to the side causes the vein in the polar transport simulation to go past, and hit the end of the grid, before it turns and connects to the sink. We also found that the facilitated diffusion model is less sensitive to parameter values. With the polar transport model, too much carrier production or too much auxin in the system often creates small cycles of cells that pump auxin around in circles. In the facilitated diffusion model carriers can only transport auxin down its concentration gradient and thus cycles do not form. Sachs has observed such cycles experimentally after wounding, but notes that they are rare in intact plants (Sachs 1982).

In their simulation of midvein formation with a variation of the canalization model, Bayer et al. (2009) also found that the initiating vein could not reliably find the sink. However experimental results strongly support the view that existing vasculature guides initiating veins to connect to it (Sachs 1981; Bayer, Smith et al. 2009). Because the sink effect of existing vasculature does not seem to be enough to attract the initiating veins, especially if the latter are high in auxin, they included an additional vein attracting factor in their model.

High flux or high concentration?

A long standing criticism of Mitchison's model is that it predicts high flux, but low concentration in emerging veins. This seems in contradiction with experimental evidence that suggests that auxin concentration is high in developing veins. Vascular strands can be induced when exposed to ectopic auxin microapplication (Sachs 1981; Sauer, Balla et al. 2006), and several auxin-induced genes are upregulated in

initiating veins (Mattsson 2003; Scarpella, Francis et al. 2004; Scarpella, Marcos et al. 2006). Feugier tried to address this problem by exploring different ways of allocating the carriers to the cell periphery. If carriers were allocated from a fixed pool in each cell, the saturating effect of limited carriers led to auxin accumulation in the veins compared to surrounding tissue (Fig. 5) (Feugier, Mochizuki et al. 2005; Fujita and Mochizuki 2006; Fujita and Mochizuki 2006). In Mitchison's model, carriers were added to each wall independently, with the amount of carriers added at an interface only depending on the flux across that interface. The allocation of carriers from a pool is in agreement with experimental data showing that auxin carriers cycle between the plasma membrane and the endosomal compartment and that this cycling is important for dynamic polarization (Steinmann, Geldner et al. 1999; Geldner, Friml et al. 2001; Geldner, Anders et al. 2003; Dhonukshe, Aniento et al. 2007; Dhonukshe, Tanaka et al. 2008; Kleine-Vehn 2008). However in Feugier's model, carrier (PIN1) expression domains initiate from a discrete sink cell at the base of the leaf, and are initially low in auxin. High concentration emerges after the canals become collectors for larger areas, supplied by smaller, feeder canals (Fig. 5). Note that high DR5::GFP signal is observed in Arabidopsis at the earliest stages of vein initiation, when the PIN1 expression domains are first forming (Scarpella, Marcos et al. 2006; Bayer, Smith et al. 2009), which suggests that the PIN1 expression itself is in response to high auxin levels . Kramer (2004) proposes that the presence of auxin importers may also be required to maintain a high concentration in developing veins.

Making loops.

The canalization hypothesis can account for the formation of open vein branching patterns, however many species, including Arabidopsis, display reticulate venation. After the primary midvein has formed, higher order secondary veins create loops by connecting back to the primary midvein. During this initial loop formation, cells with bipolar PIN1 expression were found within the loop in young Arabidopsis leaves (Scarpella, Marcos et al. 2006). These bipolar cells occur at the point where PIN1 polarity switches direction, suggesting that the upper part of the loop directs auxin towards the connection at the top of the midvein, with the lower portion draining towards the connection with the midvein at the base of the leaf. Heisler and Jönsson (2006) note that this is consistent with the canalization model if this bipolar cell was a source of auxin, although Scarpella et al. (2006) do not report an upregulation of DR5 expression at the bipolar cell. Exploring this aspect of vein patterning Rolland-Lagand and Prusinkiewicz (2005) were able to create loops by including discreet auxin sources, and in some case moving them as veins form. A role for discreet auxin sources is also supported by the more abstract model of Runions et al. (Runions, Fuhrer et al. 2005) which was able to generate highly realistic reticulated venation patterns. Feugier took a different approach, creating loops by adding an additional factor to his model, and did not assume discrete auxin sources (Feugier and Iwasa 2006). Another possibility was suggested by Sachs; loops in venation might be the result of alternating directions of auxin flow. He induced loop formation in pea internodes by alternating the application of auxin to two different sites over a period of 5 days (Sachs 1975). This idea, however, does not easily fit with our current understanding of directional auxin transport, and would be much easier to reconcile with a facilitated diffusion model of canalization.

A transport-feedback model closely related to the canalization model for venation was proposed by Kramer (Kramer 2002; Kramer, Lewandowski et al. 2008) for wood grain patterning. In this model, cells in the cambium responsible for creating the grain pattern orient themselves in response to the flux of auxin, following the lines of auxin flux. Since the orientation of the cells is also included in the calculation of flux, there is a feedback of auxin on its own transport. With this model Kramer was able to reproduce realistic grain patterns around obstructions such as branch junctions and experimental verification of the model was provided by the simulation of the changes in grain patterning in response to wounding (Kramer, Lewandowski et al. 2008). However, it was not a cellular model, but rather a continuum model (Kramer 2007) with the discretization used for simulation being larger then single cells. Although at a different level of abstraction, the patterns created by Kramer's model appear qualitatively similar to the laminar flow patterns that can be produced with Mitchison's model if the feedback of flux on the addition of carrier is linear (Feugier, Mochizuki et al. 2005; Stoma, Lucas et al. 2008). In Kramer's model for wood grain patterning the polar transport of auxin is restricted to the vector representing what is normally the long (apical-basal) axis of the cells, so that the changing grain orientation can be calculated based on the auxin gradient perpendicular to this vector. To what extent this is equivalent to the flux-based feedback used in the canalization model is unclear, however it is interesting that the formulation of the model uses auxin

concentration to calculate flux.

A transport-feedback mechanism for phyllotaxis

Perhaps no other patterning process in plants has been studied more than phyllotaxis. The exquisite patterns of intersecting spirals combined with a curious mathematical connection to Fibonacci numbers and the golden ratio has inspired mathematicians and biologists for over a century (Jean 1994; Adler, Barabe et al. 1997). Phyllotaxis begins in the shoot apical meristem, a dome-shape structure at the tip of the shoot. The central zone of the meristem contains a self-sustaining group of undifferentiated stem cells, that provide founder cells for all of the aerial structures of the plant (Sussex 1989; Laux 2003). Surrounding the central zone is the peripheral zone, a narrow band of cells with the potential to differentiate and generate new leaf or floral organs upon receiving the appropriate signal. In order to create highly ordered phyllotactic patterns, organ primordium formation must be tightly controlled, both spatially and temporally (Jean 1994). Thus, a central question in the regulation of phyllotaxis is to understand how the plant knows where and when to position the next primordium at the shoot apex.

The first insight into the regulation of organ positioning at the shoot apex came from Hofmeister (1868) who observed that new organ primordia form periodically, as far as possible from preexisting ones. This observation led to the inhibition model of phyllotaxis which postulates that existing organs prevent the formation of new organs in their vicinity. Most models of phyllotaxis are in some way based on this idea, a point echoed by Jönsson et al; that phyllotaxis can be explained "… by any regular spacing mechanism superimposed on a gradually enlarging generative region" (Jönsson, Heisler et al. 2006). To see how a simple spacing mechanism can lead to the variety of phyllotactic patterns observed in nature, see Douady and Couder (1992) or Smith et al (2006). Yet despite its long research history, the details of the spacing mechanism behind phyllotaxis and its dependence on the polar transport of auxin have only recently come to light. This is perhaps in part because, unlike leaf venation, the problem of how to create regularly spaced spots could be easily explained by diffusing inhibitors (Hellendoorn 1974) and reaction-diffusion models (Meinhardt 1982; Meinhardt 1998), and thus there was little reason to expect a transport driven process. However, experimental support for reaction-diffusion or inhibitors in phyllotaxis patterning has not materialized. The wealth of new data about auxin and its transporters point to a transport-feedback mechanism instead (Reinhardt 2003; Heisler, Ohno et al. 2005; Reinhardt 2005; de Reuille, Bohn-Courseau et al. 2006; Jönsson, Heisler et al. 2006; Smith, Guyomarc'h et al. 2006).

The involvement of polar auxin transport in organs formation first became evident from experiments where the auxin transport machinery was impaired. Inhibition of auxin movement by chemical treatment or mutation in the auxin exporter PIN1 arrests flower formation without affecting stem growth and meristem maintenance, resulting in a pin-like inflorescence devoid of floral organs (Okada, Ueda et al. 1991; Reinhardt 2000; Reinhardt 2003). Exogenous application of a microdroplet of auxin to such pin-formed meristems restores organ formation at the site of microapplication (Reinhardt 2000; Reinhardt 2003), demonstrating the role of auxin as an inducer of organogenesis. Using the synthetic auxin responsive promoter DR5, auxin was subsequently shown to accumulate at the sites of organ formation, forming a spot-like pattern that displays a phyllotactic arrangement (Benkova, Michniewicz et al. 2003; Smith, Guyomarc'h et al. 2006). This pattern appears well ahead of other signs of primordium development, up to several days before physical bulging from the meristem surface. Based on their data, Reinhardt et al. (2003) proposed an auxin transport-based model for the regulation of phyllotaxis, suggesting that PIN1 in the surface layer of the meristem directs auxin fluxes to convergence points which then trigger organogenesis. Bulging primordia inhibit new organ formation in their vicinity, not by emitting an inhibitor molecule, but by draining the activator of organogenesis, auxin, via their initiating midvein. As a result, a spacing mechanism is created by transport, preventing new organs from forming too close existing one as observed by Hofmeister (1868).

Given that the formation of auxin maxima is largely controlled by the exporter PIN1, a key question is what determines the orientation of PIN1 within a cell. A hypothesis came from simulation modeling studies (Jönsson, Heisler et al. 2006; Smith, Guyomarc'h et al. 2006), where it was proposed that cells are able to sense the auxin concentration in neighboring cells, and orient their PIN1 proteins preferentially towards neighbors with higher concentration. As in the case of reaction-diffusion, random fluctuations cause some

cells to have slightly higher auxin concentration then others, producing a slight bias of the PIN1 polarization in neighboring cells. The additional transport caused by this bias increases the cells' concentration of auxin leading to the recruitment of even more PINs in the neighboring cells. This results in a convergence point of both PIN1 expression and auxin accumulation (local activation), while the draining of auxin from surrounding tissue prevents the formation of other convergence points nearby (longer range inhibition). Thus the up-the-gradient PIN1 polarization causes a positive feedback loop of auxin on its own transport, and creates regularly spaced peaks in much the same way as reaction-diffusion (Fig 2B). Implemented on simulation models of a growing shoot meristem, this mechanism was able to create a variety of the phyllotaxis patterns observed in nature (Jönsson, Heisler et al. 2006; Smith, Guyomarc'h et al. 2006). Some support for the existence of an up-gradient polarization mechanism has since been provided by auxin microapplication experiments on tomato vegetative apices (Bayer, Smith et al. 2009).

It is likely that an up-gradient transport-feedback mechanism is behind other patterning events in plants. The convergence points of auxin that appear in the margin of young Arabidopsis leaves that initiate the bottom portion of the first vein loops are also accompanied by a convergence of PIN1 expression in the margin that is consistent with the up-gradient mechanism (Scarpella, Marcos et al. 2006). Such mechanism may also account for the specification of incipient leaflet primordia in compound leaves (Barkoulas, Hay et al. 2008).

Can a single mechanism of PIN polarization explain both leaf venation and phyllotaxis?

Phyllotaxis and leaf venation patterns appear very different, and require mechanisms with different properties. Phyllotaxis requires a mechanism capable of making regularly spaced peaks, and venation requires a mechanism that can create a connected network of strands. However, at the molecular level, these two patterning processes involve the same components. High auxin levels (based on the DR5 data) as well as the upregulation of PIN1, are the earliest known markers for both processes (Reinhardt, Pesce et al. 2003; Heisler, Ohno et al. 2005; Scarpella, Marcos et al. 2006; Smith, Guyomarc'h et al. 2006). Simulation models suggest that both types of patterns can be created with auxin transport-feedback models, the main difference being the strategy for orienting PIN. The canalization model uses with-the-flux PIN orientation, whereas the phyllotaxis models orient PIN up-the-gradient. This raises the question of how the plant chooses between these two possibilities.

The simplest explanation would be that different tissue types use different strategies for orienting PIN. In the Arabiodopsis root, it has been shown that in some cases PIN orientation can be a function of cell type (Vieten, Vanneste et al. 2005; Wisniewska, Xu et al. 2006). When PINs not normally found in certain cell types are expressed ectopically, they can follow the orientation of other PINs normally expressed in that cell type. In this scenario, cells in the surface layer of the shoot apex, or in the margin of the developing leaf would use up-the-gradient PIN1 polarization to form convergence points. In the interior of the shoot apex and the young growing leaf, PINs would polarize with-the-flux and form strands. **Fig. 6** shows a simulation model based on this idea.

Two recent simulation studies propose that both types of patterns can be explained by a single strategy for PIN polarization. In simulations where PIN production is promoted by auxin, the auxin peaks created by the up-gradient mechanism were not always stable and could move around in the tissue (Heisler and Jönsson 2006). Merks et al (2007) exploited this observation, and suggested that the convergence point of auxin that initiates leaf primordium formation subsequently moves as a traveling wave into inner tissue. In their model, PIN polarity follows this moving peak of auxin, leaving behind a strand of PINs polarized in the direction of the traveling wave. This strand would then differentiate into a vein.

Stoma et. al. took the opposite approach and proposed a model to explain phyllotaxis based on the with-the-flux mechanism. In their model, when auxin reaches a threshold concentration in the surface layer of cells in the shoot apex, it initiates a primordium, and auxin is allowed to flow into internal tissue. This flux causes the PINs in neighboring cells to polarize towards the primordium center, giving the characteristic PIN1 convergence points around primordia observed experimentally (Reinhardt, Pesce et al. 2003). In internal tissue, flux-based PIN1 orientation causes strands to form as in the canalization model. They proposed that PIN1 responds differently to flux in the surface layer of cells than in the internal tissue of the

shoot apex. They show with simulations on sheets of cells that broad PIN1 expression domains can be created when the PIN response to auxin flux is linear, and that canalization occurs with a non-linear response (Mitchison 1980; Feugier, Mochizuki et al. 2005). This would explain why strands of PIN expression do not appear in the surface layer of cells in the shoot apex.

In an effort to choose between the possibilities, Bayer et al. (2009) focused their attention on the early events in the initiation of the midvein. This is the point where the two patterning processes intersect, and through a careful analysis of PIN expression and polarity, they came to the conclusion that both with-the-flux and up-the-gradient polarization must be operating at the same time. When a convergence point that initiates a primordium first appears in the surface layer of the shoot apex, the cells around it are all polarized towards a few cells in its center. This includes cells in the inner layers of the meristem directly below the convergence point. This is consistent with an up-the-gradient mechanism for PIN1 polarization, as DR5 data (and the orientation of the PINs themselves) suggest that this area is high in auxin. As development proceeds, a strand of cells with high PIN1 expression extends from the convergence point in the surface layer into inner tissue. The PIN1 polarity of the cells in the center of the convergence point, and those immediately below, switches towards the direction of the extending vein, suggesting a transition to a different polarizing mechanism, such as with-the-flux. Note that this could also occur if the auxin peak were to move, however such movement has not been observed experimentally. In fact the peak in DR5 expression at the leaf tip gets brighter as leaf development progresses (Mattsson, Ckurshumova et al. 2003; Scarpella, Marcos et al. 2006), likely due to the activation of auxin biosynthesis genes (Schwendener 1878; Cheng, Dai et al. 2007; Zhao 2008). In addition, the extending midvein is accompanied by strong lateral PIN1 polarization in adjacent cells, pointing towards the central cells in the forming midvein. Scarpella et al. (2006) observed similar PIN1 localization in young leaves, where DR5 expression in the vein occurs concomitantly with PIN1 expression, suggesting that auxin levels are high during these early stages. It is thus difficult to reconcile strong lateral polarization towards the center of the midvein with a mechanism based on flux alone. These observations led Bayer et al. (2009) to propose a combined model in which PINs can transition from polarizing up-the-gradient at low auxin levels, to with-the-flux at higher levels. Simulations of their model reproduced the switch from early apical to basal polarity, as well as strong lateral PIN1 polarity. In addition, their model maintained high auxin levels throughout the simulations, as the peak of auxin in the surface layer was extended to a strand in the forming vein.

A transport-feedback mechanism involved in lateral root initiation.

Just as the shoot apex is responsible for the specification of the aerial structure of the plant, the root apex creates the plant's subsurface architecture. In Arabidopsis the root tip is a highly ordered structure, with a network of several members of the PIN protein family directing auxin in a reflux loop about the quiescent center where the root stem cells reside (Scheres 2002; Grieneisen, Xu et al. 2007). This results in an auxin maximum at the center of the root tip that is thought to be a major player in root apical meristem organization and maintenance (Sabatini, Beis et al. 1999; Blilou, Xu et al. 2005; Bansal 2006). As in the shoot, the root also produces lateral organs to expand its structure, and experimental data suggest that this process is likewise triggered by elevated auxin levels (Blakely, Blakely et al. 1988; Laskowski, Williams et al. 1995; Sussex, Godoy et al. 1995; Dubrovsky, Sauer et al. 2008). High DR5 expression in founder cells is currently the earliest known marker for lateral root initiation (Dubrovsky, Sauer et al. 2008). These early peaks in DR5 expression, however, do not appear to be accompanied by major changes in PIN expression or polarity, as is the case with organ formation in the shoot apex. This raises the question as to how cells in the pericycle are able to accumulate auxin preferentially over their neighbors, given that they are in the middle of a basally directed auxin transport stream (Sauer, Balla et al. 2006).

Noticing that lateral roots often appear on the outside of a curve in the root (Fortin, Pierce et al. 1989), Laskowski et al. (2008) proposed that changes in the shape of cells could affect auxin transport and cause certain cells to preferentially accumulate auxin. Mitchison (1981; 1981) had also suggested a geometrical influence on auxin transport involving the position of the vacuole as a possible explanation for gravitropism. Using simulation studies, Laskowski et al. showed that under suitable conditions, pericycle cells on the outside of a bend in the root developed higher auxin levels, simply due to the geometry of the cells. Although this mechanism provides a plausible explanation for an initial bias for auxin accumulation, it does not explain why straight roots produce lateral root primordia (Fortin, Pierce et al. 1989) or how

auxin accumulation becomes restricted to a few pericycle founder cells.

Laskowski et al propose a transport-feedback mechanism involving auxin importers to supplement the initial bias in auxin accumulation due to cell geometry. Members of the AUX/LAX family have been shown to be important in lateral root initiation (Marchant, Bhalerao et al. 2002; Swarup 2008), and can be upregulated in response to auxin in the root (Laskowski, Biller et al. 2006; Laskowski, Grieneisen et al. 2008; Paponov, Paponov et al. 2008). Thus they added auxin-induced production of AUX1 in the pericycle to their model. Cells with slightly higher auxin levels produce more AUX1, causing them to retain even more auxin from the transport stream, thus magnifying any initial bias (local activation). As in the transport-feedback mechanism in the shoot, inhibition of neighbors is caused by a reduction of surrounding auxin levels due to the movement of auxin to the activated cells (long range inhibition). A closely related model was proposed by Lucas et al. (Lucas 2008) whereby polar transport causes auxin to build up in initiation zone of the root until it hits a threshold, which then causes lateral root primordia to initiate. The primordia then consume auxin, reducing the levels in the initiation zone and thus suppressing the formation new ones nearby. Although at a different level of abstraction, it is likely that the transport-feedback mechanism of Laskowski et al. based on the upregulation of AUX1 by auxin could provide a molecular basis for the Lucas et al model. A simplified version of this importer driven transport-feedback model is shown in Fig. 2C on a line of cells. In this simulation the cell geometry was uniform, and the symmetry-breaking initial bias was provided by introducing a small amount of noise to the auxin concentrations. As in the case with the activator-inhibitor system (Fig. 2A) or the up-the-gradient PIN polarization model (Fig. 2B), the mechanism based on the upregulation of AUX1 by auxin is also able to create a pattern of peaks (Fig. 2C).

In contrast to the mechanism based on auxin exporters in the shoot, the transport-feedback model based on importers proposed for the root does not require shifts in transporter polarity. The lateral root primordium founder cells which become activated and express elevated levels of AUX1 distribute the importer uniformly on their plasma membrane (Laskowski, Grieneisen et al. 2008), and exporter polarities are largely unaffected in the early stages of founder cell specification (Benkova, Michniewicz et al. 2003). The overall levels of the exporters at the plasma membrane do, however, have an effect on the spacing of lateral root primordia, playing a more passive role in patterning in the root, as seems to be the case with importers in the shoot.

Conclusion

Over the last 30 years experimental evidence has been accumulating that the feedback of auxin on its own transport is responsible for a significant amount of patterning in plants. Simulation studies of transport-feedback mechanisms have demonstrated their plausibility, and in many cases provide the best fit between experimental data and emergent model behavior. Although auxin transport-feedback models represent a common framework for many patterning events in plants, the means by which auxin feeds back on its own transport appears to vary depending on the developmental event considered. The specification of leaf or floral organ primordia in the shoot apex depends on coordinated changes in exporter polarity, resulting in a spacing mechanism that can create phyllotactic patterns. Lateral root primordium initiation is often much more irregular than organ initiation in the shoot, but nevertheless still requires a spacing mechanism. A transport-feedback model based on the upregulation of importers by auxin has recently been proposed, which does not appear to require changes in transporter polarity. Leaf venation, which has the completely different patterning requirement to create a connected network of strands, is also best explained by a transport-feedback process. Using the same molecular machinery as the mechanism proposed for phyllotaxis, a simple change in the strategy for auxin exporter polarization, from up-the-gradient to with-the-flux, can switch the outcome of the mechanism from the production of uniformly spaced spots to a system of connected strands.

It is unclear as to whether transport-feedback mechanisms can create as wide a variety of patterns as has been possible with reaction-diffusion. By using multiple substances and interactions, reaction-diffusion has been has able to reproduce many patterns observed in biology. With auxin-based patterning, it seems is hard to imagine that such a variety of patterning events can be controlled by one hormone. However transport is a much more sophisticated means of movement than diffusion, and the diversity of methods by

which it can be controlled may provide a clue as to how auxin can do so many different things.

Acknowledgements

We thank Kath Bainbridge for the providing Fig. 3C. We thank Naomi Nakayama, for critical reading of the manuscript. We thank Eric Kramer, Naomi Nakayama, Saiko Yoshida, Adam Runions, Przemek Prusinkiewicz, and Cris Kuhlemeier for discussions. We gratefully acknowledge support from SystemsX.ch project “Plant Growth in a Changing Environment” and the Swiss National Science Foundation.

Figure Captions

Figure 1. Examples of auxin-induced patterning in plants. (A) Transversal section through Arabidopsis vegetative meristem showing spiral phyllotactic pattern. Green signal is the auxin exporter PIN1 and red signal is calcofluor staining of the cell walls. (B) PIN1 (green) immunolocalization of young Arabidopsis leaf showing developing vein network. Scale bars, 50 µm.

Figure 2. Different patterning mechanisms can create similar patterns on a line of cells. (A) Turing style reaction-diffusion patterning based on Gierer and Meinhardt's (1972) activator-inhibitor system (activator shown in green, inhibitor shown in red). (B) Transport-feedback patterning based on up-gradient PIN1 orientation (auxin shown in green, PIN1 shown in red). (C) Transport-feedback patterning based on the upregulation of AUX1 (auxin shown in green, AUX1 shown in red). Simulations performed with wrap around boundary conditions. For simulation equations and parameters see the supplemental materials.

Figure 3. The polar transport of auxin through cells. (A) Schematic representation of auxin transport. From the extracellular space, the plant hormone auxin enters the cells by diffusion and/or via auxin importers (yellow) located at the plasma membrane. Once inside the cell, auxin moves through the cytosol by diffusion and is then transported out of the cell by export carriers (red) which are often polarly localized to one side of the cell. The coordinated polar localization of the auxin exporters over multiple cells determines the overall direction of the auxin flux within tissue (adapted from Smith 2008). (B) Immunolocalization for PIN1 (red) in tomato shoot apex showing polarly localized exporters. (C) Immunolocalization for AUX1 (yellow) in Arabidopsis shoot apex showing the uniform non-polar localization of the importer at the plasma membrane (picture courtesy Katherine Bainbridge). Scale bars, 5 µm.

Figure 4. Feugier's polar transport model of the canalization of auxin flux based on the allocation of PIN (red) to cell membrane sections from a pool of PIN in the cytosol. Blue indicates auxin concentration, black arrows indicate the direction and magnitude of auxin flux. Note the development of high auxin concentration in collector veins. For simulation equations and parameters see the supplemental materials.

Figure 5. Comparing Mitchison's canalization models' ability to find a sink. Simulations of Mitchisons polar transport (A) and facilitated diffusion (B) models of canalization. Blue indicates auxin concentration, red indicates carrier density at the cell membrane sections, and the black arrows indicate the strength and prominent direction of auxin flux. If an auxin sink (outlined in red) is placed immediately below an auxin source (outlined in green) both models will make a direct connection from source to sink. However, if the sink is moved just a few cells to one side, the strand in the polar transport model (A) extends past the sink, whereas the facilitated diffusion model (B) is able to find the more direct route. For simulation equations and parameters see the supplemental materials.

Figure 6. Simulation of early steps in vein formation on a growing leaf. (A). PIN1:GFP picture of a young Arabidopsis leaf showing PIN proteins in margin cells orienting towards the tip to create a convergence point there. A PIN expression domain extends from this convergence point into the interior of the leaf where the midvein (m) will form (image reproduced with permission from Scarpella 2006) (B-C) Simulation model of a growing leaf with up-the-gradient PIN orientation in the margin cells and with-the-flux orientation of PIN in interior cells. PIN1 localization at the plasma membrane is shown in red, auxin levels in green. The up-the-gradient mechanism causes self-organizing peaks of auxin appear in the margin. When the auxin at these peaks builds up to a threshold concentration, it leaks into internal cells and the with-the-flux mechanism causes vein strands to appear. As the leaf grows (C), more space is created in the margin allowing more convergence points appear. This in turn causes more veins to be initiated. Dark cells at the bottom are sinks for auxin and represent the existing vasculature of the plant. For simulation equations and parameters see the supplemental materials. Scale bar, 10 µm.

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