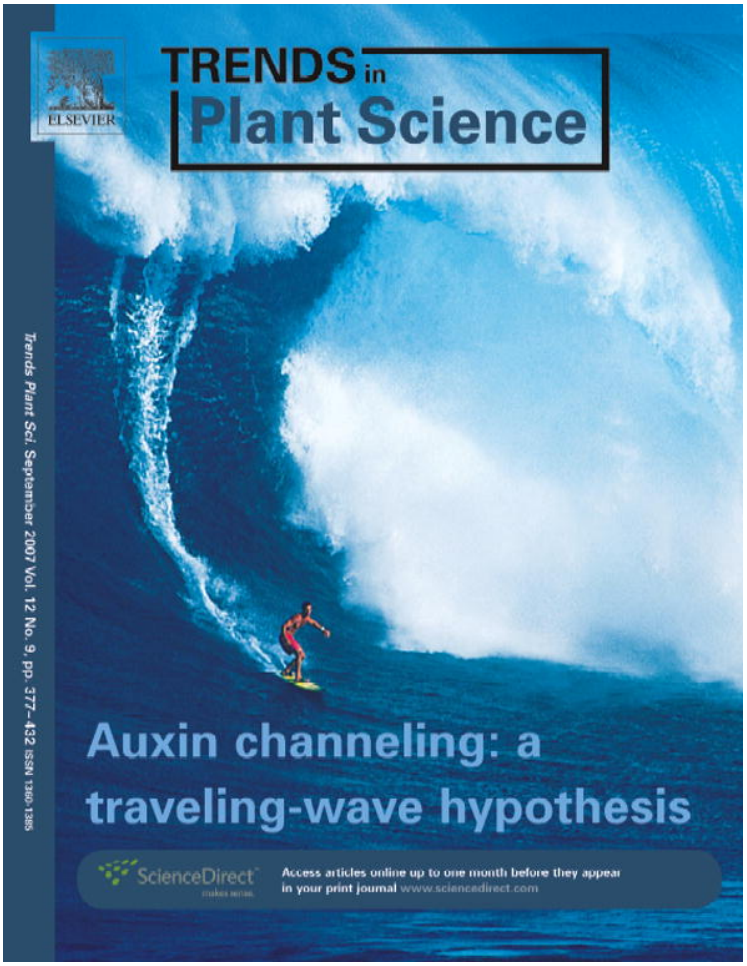

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Canalization without flux sensors: a traveling-wave hypothesis

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In 1969, Tsvi Sachs published his seminal hypothesis of vascular development in plants: the canalization hypothesis. A positive feedback loop between the flux of the phytohormone auxin and the cells' auxin transport capacity would canalize auxin progressively into discrete channels, which would then differentiate into vascular tissues. Recent experimental studies confirm the central role of polar auxin flux in plant vasculogenesis, but it is unclear if and by which mechanism plant cells could respond to auxin flux. In this Opinion article, we review auxin perception mechanisms and argue that these respond more likely to auxin concentrations than to auxin flux. We propose an alternative mechanism for polar auxin channeling, which is more consistent with recent molecular observations.

Canalization hypothesis: existing views

How auxin regulates venation patterning in the leaf and other organs is one of the classic problems in plant biology. In 1969, Tsvi Sachs proposed a mechanism for venation patterning: the canalization hypothesis [1]. In this view, cells enhance their auxin transport capacity depending on the strength and direction of the auxin flux that they perceive. This mechanism, which resembles the way that streaming water carves out a river system (hence the name *canalization*), can canalize a uniform apical-to-basal flux of auxin into discrete transport channels. A series of mathematical models have shown that canalization and derived flux-dependent mechanisms can indeed form venation-like patterns, including linear veins [2–4], branched patterns [5,6] and even closed loops and networks [6–8] (Box 1).

Modern formulations of the canalization hypothesis assume that mesophyll cells localize auxin transporters (including PINs, a class of membrane bound auxin efflux transporters), along local auxin fluxes, thus enhancing further auxin flux and, consequently, further cell polarization [5–9]. A range of classic and recent experimental observations support the canalization hypothesis. In many plant tissues, auxin moves in files of polar, differentiating cells, and existing vascular strands induce and orient new strands by acting as auxin sinks (reviewed in Ref. [10]). More recent experimental evidence includes the observation that auxin transport inhibitors, including 1-naphthylphthalamic acid (NPA), restrict vascular differentiation and auxin response to the leaf margin [11,12], which is the putative site of auxin influx into the leaf mesophyll [13]. Many of the molecular components required for auxin canalization have been identified, including auxin transporters and mechanisms by which auxin itself feeds back on the polar localization of these transporters (reviewed in Vieten *et al.* [14]). During early leaf development, PIN1 expression is the first marker for vascular development, forming threads of cells where PIN1 expression is polarized along the putative direction of auxin flux [15,16]. Together, these data pinpoint a central role for polar auxin flux in leaf venation patterning. The key requirement for canalization is that plant cells polarize along a perceived auxin flux. Thus, a crucial question is if and how cells can measure the rate at and direction according to which auxin flows through them. Although it is not impossible that plant cells respond to auxin fluxes, it remains unclear how confirmed and putative auxin receptors, including the recently identified nuclear F-box transport inhibitor response 1 (TIR1) protein [17,18], would do that.

Is canalization the only possible explanation for polar auxin flux? We propose a mechanism for polar auxin channeling that does not require cells to sense flux, like the canalization hypothesis does. Our proposal derives from a computer model of phyllotaxis that Henrik Jönsson and coworkers proposed recently [19]. According to their model, PIN1 auxin efflux transporters localize preferentially at cell membranes adjacent to neighboring cells with the highest auxin concentrations, thus pumping auxin upstream into auxin maxima. In contrast to Jönsson *et al.* who assumed constitutive PIN1 expression, we also take into account that PIN1 expression is auxin-induced. We show how this hypothetical mechanism generates traveling waves of polar auxin transport and PIN1 expression, producing auxin channels, and mimics the main experimental manipulations of polar auxin transport (Box 2).

Canalization hypothesis: new insights

A frequently raised objection against the canalization hypothesis concerns the fact that it should form continuous, uninterrupted vasculature. Several knockout phenotypes, including *van-3*, produce isolated islands of vascular tissue, which canalization cannot explain. This led Koji Koizumi and coworkers [20] to argue that the existence of discontinuous vein mutants falsifies the canalization hypothesis, and proposed that the reaction–diffusion (RD) hypothesis (Box 1) should be reconsidered. This class of mechanisms can produce patterns strikingly similar to leaf vasculature (Figure 1 and Movie S1 in the supplementary

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Box 1. Computational studies of venation patterning

Several classic and recent simulation studies demonstrated that Tsvi Sachs' canalization mechanism [1] can produce simple channels, tree-like structures, loops and networks. G.J. Mitchison [2,3] was the first to implement Sachs' hypothesis in a computational model. In a first, facilitated diffusion model, auxin diffuses passively from the leaf edge to the leaf base. Auxin flux increases wall permeability, whereas walls slowly become impermeable in the absence of flux. After a small perturbation, this model produces distinct auxin channels. A second, active transport canalization model assumes that flux polarizes the distribution of transporters. This model produced similar results, in addition to a pattern of circular loops [2,3]. Through the addition of auxin point sources at specific positions and times, Anne-Gaëlle Rolland-Lagan and coworkers [8] reproduced a secondary vein loop similar to the early venature in *Arabidopsis thaliana*.

François Feugier and collaborators [5,7] presented an in-depth analysis of active-transport canalization models. They showed how vascular patterns vary with the type of response function used to link auxin flux to transporter localization. Hironori Fujita and Atsushi Mochizuki [6] studied a related canalization-type mechanism, and introduced a hypothetical, diffusible enhancer molecule produced according to the net auxin flux. Their model produces branching venation patterns like Feugier's model and reticulate patterns.

The recent 'constant production hypothesis' by Pavel Dimitrov and Stephen Zucker [4] is a variant of the facilitated diffusion interpretation of the canalization hypothesis [3]. The model explains side-vein formation as the shortest, steepest path draining an auxin maximum, formed in the center of the areoles in a balance between constitutive auxin production and drainage by the veins. Adam Runions and collaborators [45] proposed another auxin production hypothesis, in which veins grow toward auxin sources in the leaf blade that appear iteratively at even spacing from the venature and pre-existing auxin sources.

The reaction–diffusion hypotheses derive from classic work by the British mathematician Alan Turing in 1952 [46]; in their basic form, these assume a relatively immotile chemical, called the activator, and a rapidly diffusing inhibitor, which form periodic patterns via short-range activation and long-range inhibition, respectively. Hans Meinhardt [43] extended this system with additional chemicals to produce mobile activator peaks tracing out vein-like patterns (see Figure 1 in the main text). His alternative venation model [47] produces reticulate patterns by assuming an additional chemical whose concentration peaks in the activator's valleys.

material online), but chemicals matching those in the RD models have not been identified experimentally. Moreover, experimental observations of the role of polar auxin transport in leaf development (for example, see Refs [15,16]) are not easily reconciled with the RD concept. Enrico Scarpella and coworkers [15] looked at PIN1::GFP expression in the *van-3* mutant. Surprisingly, *van-3* initially shows normal PIN1::GFP patterning, whereas later on it fragments into discontinuous vascular islands. These findings indicate that discontinuous venature mutants do not necessarily falsify canalization.

How could cells polarize in response to the rate and direction according to which auxin flows through them? Cells can perceive auxin with the recently identified F-box TIR1 protein [17,18]. TIR1 participates in the SCF^{TIR1} complex, which binds auxin and tags the AUX/IAA repressor of auxin response factors (ARFs) for degradation by the proteasome [21]. Although we cannot rule out the possibility that this mechanism is sensitive to the rate of auxin supply, TIR1's nuclear localization makes it unlikely that it can sense the direction of auxin flow. Another putative auxin receptor is the membrane-bound auxin-binding protein-1 (ABP1) [22,23]. Although ABP1 has a motif that allows for its targeting to the endoplasmic reticulum, it is also secreted to the cell membrane [23]. Protoplast swelling experiments suggest that ABP1 binds auxin at the apoplastic side of the cell membrane [22]. A receptor binding extracellular auxin is unlikely to respond to auxin flux, because it cannot assess the amount of molecules crossing the membrane. Auxin transporters might also signal their activity at cell walls and recruit more transporter proteins to enhance the transport rate. Rainer Hertel [24] proposed that paralogs of auxin carriers located at intracellular membrane compartments are responsible for auxin-induced cell elongation by cotransporting calcium ions. A similar dual transporter/receptor function was also proposed for sugar transporters, including SUT1, which has a cytosolic loop that might act as a signaling domain [25]. Thus, although it remains possible that plant cells have a mechanism to perceive auxin flux, it seems unlikely that the nuclear auxin receptor TIR1 and the membrane-bound, putative auxin receptor ABP1 can do it.

Venation patterning: a variation on a theme?

Do plants require a molecular sensor for auxin flux to construct polar auxin transport channels? We set out to determine if it were possible to define a simple mechanism that produces auxin channels with cells only sensitive to auxin concentration. For reasons of parsimony, venation

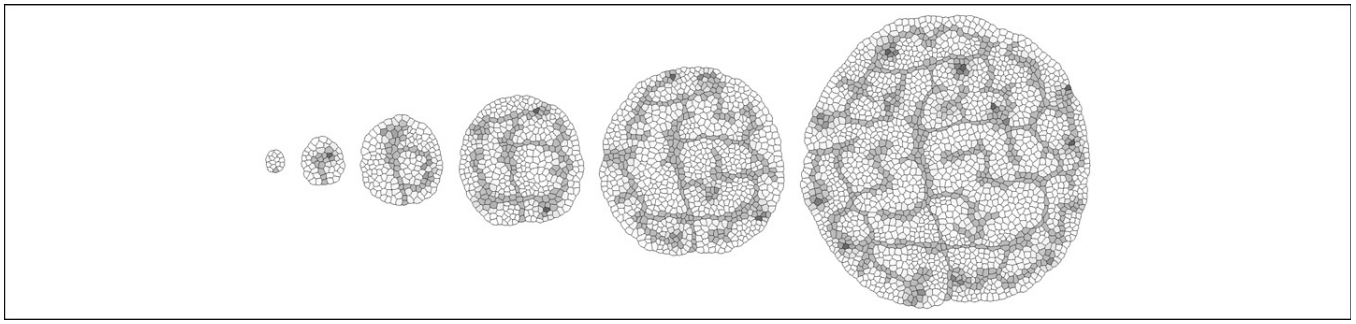

Figure 1. Reaction–diffusion model of leaf-venation patterning. In 1976, Hans Meinhardt [43] proposed a reaction–diffusion model to explain patterning of branching and network-like structures. Cells differentiate into vascular tissue (shown in light grey) in response to the activator peaks (dark grey), which is produced by autocatalysis and lateral inhibition. The vascular tissue consumes a third chemical that is required for activator production (Box 1). This pushes the activator peak to a neighboring cell, producing a strand of differentiated cells. The vascular tissue slowly produces activator, initiating lateral veins at a distance from the tip. Here, we reproduce this model in a piece of growing, simulated plant tissue. New lateral veins appear at a critical distance from surrounding vein tips when the tissue has grown sufficiently. Reproduced, with permission, from Ref. [44]. See also Movie S1 in supplementary material online.

Box 2. Detailed model description

Our model derives from the phyllotaxis model by Henrik Jönsson and coworkers [19]. The model considers active cell-to-cell auxin transport by PIN1, and a small diffusive transport term:

$$\frac{dA_i(t)}{dt} = T_{active}\sum_j\left(\frac{P_{ji}A_j(t)}{k_a + A_j(t)} - \frac{P_{ij}A_i(t)}{k_a + A_i(t)}\right) + T_{diffusive}\sum_j L_{ij}\left(A_j(t) - A_i(t)\right) \quad \text{[Equation I]}$$

In Equation I, the sum is over all neighbor cells, $A_i(t)$ is the auxin concentration in cell *i*; $P_{ji}(t)$ and $P_{ij}(t)$ are the amounts of PIN1 in cell *i* pumping auxin into cell *j* and *vice versa*, and $T_{diffusive} \ll T_{active}$ are passive and active transport coefficients, $k_a = 1$ is a Michaelis-Menten constant for PIN-mediated auxin transport, and *Lij* is the surface area length (or length in our two-dimensional simulation) of the wall between cell *i* and cell *j*. Auxin enters the primordium at its tip (purple walls in Figure 3) with constant flux φ_{tip}, while PIN1 molecules localize preferentially toward the shoot apical meristem to pump the auxin away (flat bottom row of cell walls). The other boundaries are impermeable to auxin (zero-flux boundary condition). The model currently neglects the apoplast, so auxin moves directly from one cell into the next.

We assume that recycling between the endosome and the membrane, and the formation and dissociation of the auxin-receptor complex are much faster processes than PIN1 internalization and auxin-inhibited PIN1 endocytosis. This allows us to eliminate two equations from the full system, by assuming that after any change of auxin and PIN1 concentrations, the levels of the complexes practically instantly reach a steady state; this so-called quasi steady-state assumption is a common mathematical technique, which is used, for example, to derive the Michaelis-Menten equations [48]. Thus we arrive at the following, saturated dynamics for PIN1 recycling:

$$\frac{dP_i(t)}{dt} = -k_1\sum_j\frac{P_i(t)\,f\left(A_j(t)\right)}{k_m + P_i(t)} + k_2\sum_j P_{ij}(t) + \alpha A_i(t) - \delta P_i(t) \quad \text{(Equation II)}$$

$$\frac{dP_{ij}(t)}{dt} = k_1\frac{P_i(t)\,f(A_j(t))}{k_m + P_i(t)} - k_2 P_{ij}(t) \quad \text{(Equation III)}$$

In Equations II and III $P_i(t)$ represents the number of PIN1 molecules in the endosome of cell *i*; $P_{ij}(t)$, the number of molecules at cell wall *j*, and k_m is the Michaelis-Menten constant shown in Equation IV:

$$k_m = \frac{k_{-3} + k_1}{k_3} \quad \text{(Equation IV)}$$

The function $f(A_j(t))$ as defined in Equation V represents the number of receptor molecules activated by auxin in cell *j*, with $R = 100$, the total level of receptors and $k_R = 100$, a Michaelis-Menten constant:

$$f(A_j(t)) = \frac{A_j(t)R}{k_R + A_j(t)} \quad \text{(Equation V)}$$

In contrast to Jönsson *et al.* [19] who neglected PIN1 production and breakdown, here we assume auxin-dependent PIN1 production and independent PIN1 decay. Also we do not put the PIN1 cycling equations to quasi steady state.

Parameter estimation

Maximum membrane permeabilities of PIN-facilitated auxin-transport range from about $p_{active} = 3 \times 10^{-7}$ m s^{-1} to about $p_{active} = 3 \times 10^{-6}$ m s^{-1} [49]. We estimate the size of mesophyll cells at $V \approx 1000$–$8000\ \mu m^3$. With auxin transporting over walls of about $a = 100\ \mu m^2$, the transport parameter T_{active} (Equation VI; note that we divide by cell volume to convert to auxin concentration values) should be in the range of 0.01 s^{-1} to 0.3 s^{-1}.

$$T_{active} = \frac{1}{V}p_{active}a \quad \text{(Equation VI)}$$

In all simulations presented, we used $T_{active} = 0.08$ s^{-1}, but the reported phenomena hold for values within and well outside this range. We also assumed a small diffusive membrane permeability for auxin of $p_{diffusive} \approx 1.5 \times 10^{-10}$ m s^{-1}, corresponding to $T_{diffusive} = 1.5 \times 10^5$ m^{-2} s^{-1} (Equation VII), with $L_{ij} \approx 100\ \mu m^2 = 10^{-10}$ m^2.

$$T_{diffusive} = \frac{p_{diffusive}}{V} \quad \text{(Equation VII)}$$

We set the auxin influx at the leaf tip to $\varphi_{tip} = 1.5 \times 10^6$ m^{-2} s^{-1}.

Because we currently only have limited, qualitative data for the intracellular amounts of PIN and auxin in leaf cells and for the transport activity of individual PIN molecules, we prefer to express auxin and PIN levels in arbitrary units. However, for a typical protein production rate of 3 min^{-1} and a protein degradation rate of 0.003 min^{-1} [50], one arbitrary unit would amount to about 5000 PIN molecules. For Figure 3a, we assume a constant level of PIN1, with the initial levels $P_i(0) = 1$ and $P_{ij}(0) = 0$, by setting the rates of PIN1 production and decay to zero ($\alpha = 0$ and $\delta = 0$). This fixes the total PIN1 level per cell at 1, as in (8)Equation VIII:

$$P_i\left(t\right) + \sum_j P_{ij}\left(t\right) = 1 \quad \text{(Equation VIII)}$$

whereas, for Figure 3b–j, we assume a small auxin-dependent PIN1 production rate $\alpha = 1.0 \times 10^{-5}$ s^{-1} (i.e. arbitrary units per second), and independent PIN1 breakdown of $\delta = 1.0 \times 10^{-8}$ s^{-1}. The initial PIN1 level is $P_i(0) = 0$. For all simulations we set the rate of auxin-induced vesicle internalization to $k_1 = 2 \times 10^{-4}$ s^{-1}, the endocytosis rate $k_2 = 5 \times 10^{-7}$ s^{-1}, and the Michaelis-Menten constant for PIN1 vesicle docking to the membrane $k_m = 100$, unless stated otherwise in the figure captions.

patterning would be most probably a slight modification of a patterning mechanism operating elsewhere in the plant. In the model plant *Arabidopsis thaliana* (thale cress), auxin gradients determine the position of the initiation sites of leaves, lateral roots, cotyledons, floral organs and ovules [26]. In primordia of shoot-derived lateral organs, auxin flows according to an inverse fountain: in the epidermal layers, PINs converge to an auxin maximum [27], from where auxin drains centrally toward the vasculature. Thus auxin accumulation via PIN-mediated polar auxin transport seems a common developmental module for lateral organ initiation [26].

Recently, two computational models, one by Henrik Jönsson and coworkers and one by Richard Smith and coworkers have suggested a simple mechanism for producing auxin maxima in the shoot apical meristem [19,28]. These models hypothesize that cellular auxin concentrations regulate PIN1's localization in adjacent cells, where most PIN1 localizes toward cells with the highest concentration of auxin. In this way, PIN1 tends to pump auxin upstream of auxin gradients, which produces auxin maxima. These models correctly simulate the intracellular localization of PINs: they point toward the auxin maximum in the shoot apical meristem. Thus, this simple model, based on local auxin perception, provides a plausible explanation for the formation of auxin maxima.

How can PIN1 polarize toward the highest auxin concentration in adjacent cells? In the phyllotaxis modeling papers [19,28], it was suggested that auxin-inhibited endocytosis of PIN1 [29] might be the responsible mechanism.

Because cells pump auxin into and from the apoplast, extracellular concentrations probably correlate to the intracellular concentrations in the adjacent cells; therefore, PIN1 endocytosis during constitutive recycling might be inhibited directly by extracellular auxin [29] or via an extracellular receptor, that is, ABP1. Another possibility is that mesophyll cells undergo a polarization switch in response to local auxin gradients, in a mechanism related to that of eukaryotic chemotaxis [30,31]. Indeed, RHO-of-plants proteins (ROPs), whose animal homologs play a crucial role in cell polarization [32], are involved in polar targeting of PINs [33]. Thus, auxin-responsive localization of PIN1 could occur either through a direct, local inhibition of PIN1 endocytosis or through an indirect overall polarization switch.

A traveling-wave auxin-channeling hypothesis

Considering that auxin channeling during venation patterning and auxin accumulation during organ initiation might be different outcomes of the same, underlying developmental mechanism [34], we derived our model from the Jönsson *et al.* model of phyllotaxis [19]. We assume that auxin is transported actively using auxin transporter proteins, and possibly passively, at a very low rate, by leaking away from auxin maxima [35]. Of the auxin transporters, we currently only consider PIN1. Auxin enters the leaf primordium at its margin with a constant flux, whereas PIN1 molecules localize preferentially toward the shoot apical meristem to pump the auxin away (flat bottom row of cell walls). Other boundaries of the simulation domain are impermeable to auxin. The model currently neglects the apoplast, so auxin moves directly from one cell into the next.

PIN1 dynamics are crucial in vascular patterning. We assume that vesicles containing PIN1 receptors shuttle between the endosome and the membrane with fast rates k_3 and k_{-3}, respectively (Figure 2). We also assume that auxin inhibits PIN1 endocytosis in neighboring cells [29], possibly mediated by a cell surface auxin receptor, such as ABP1, or by a secondary, auxin-dependent signal. Upon receptor activation by auxin or the second messenger, the vesicles internalize into the cell membrane with rate k_1. From here, PIN1 returns to the endosome at a slow rate k_2. Because it is unclear to what extent auxin diffuses passively [35], we can alternatively interpret it as the flux due to a residual pool of membrane-localized auxin transporters, exocytosed independently of auxin signaling.

In agreement with the Jönsson *et al.* model, with constitutive PIN1 expression (i.e. a constant level of PIN1, and no PIN1 production or decay) our model generates discrete auxin maxima (Figure 3a and Movie S2 in the supplementary material online) practically fixed at one position. However, auxin induces expression of PIN1 in the root [36] and in the leaf [15,16]. With such auxin-induced PIN1 expression the position of the auxin maxima formed in the phyllotaxis models by Jönsson *et al.* [19] and Smith *et al.* [28] become unstable; the auxin peaks move around in the plant tissue [37]. Could such moving auxin peaks travel in a specific direction and induce polarized auxin transport channels? If we include auxin-induced PIN1 expression as

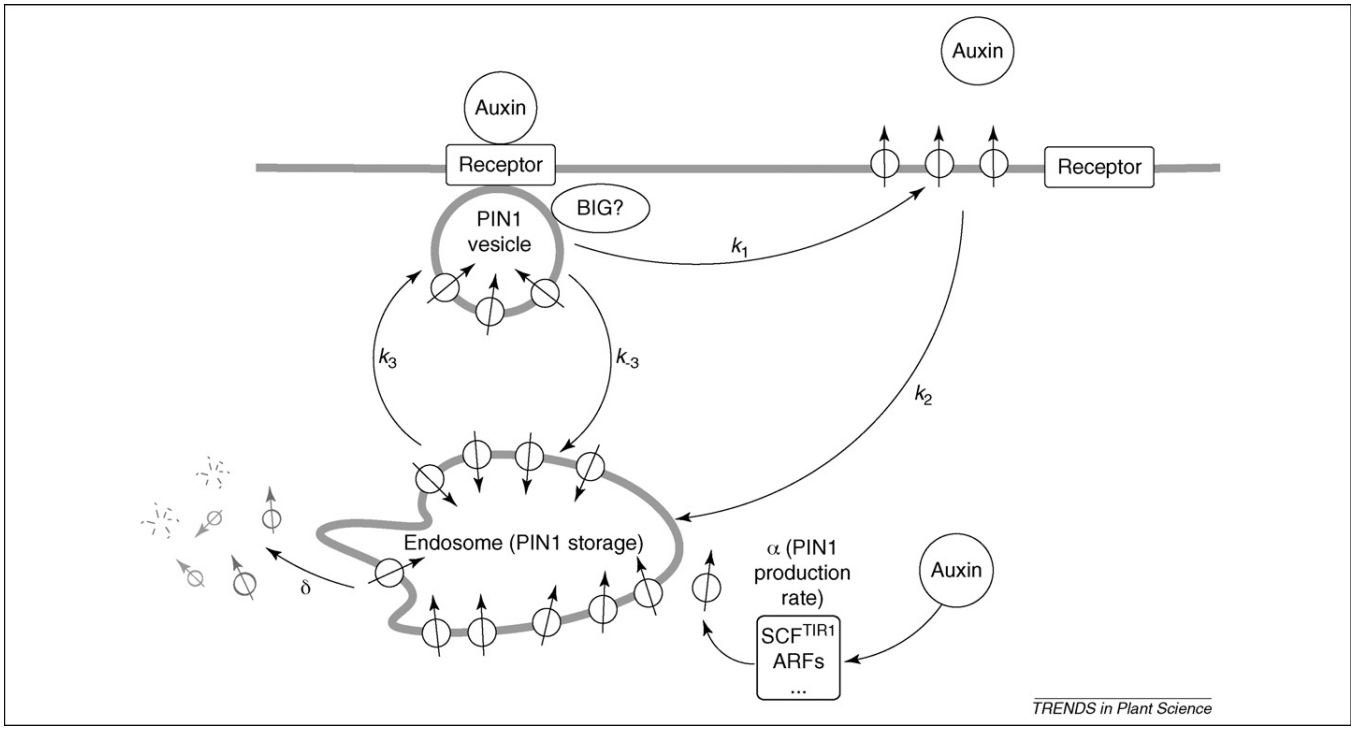

Figure 2. Modeling auxin-driven PIN1 polarization. Vesicles containing PIN1 receptors recycle between the endosome and the membrane with fast rates k_3 and k_{-3}. We assume that auxin inhibits PIN1 endocytosis in neighboring cells [29], possibly mediated by a cell surface auxin receptor (e.g. ABP1), or a secondary, auxin-dependent signal. Upon receptor activation by auxin or the second messenger, the vesicles internalize into the cell membrane with rate k_1; the PIN1 molecules return to the endosome at a rate k_2. We assume that PIN1 recycles quickly between the endosome and the auxin receptor, and we assume fast formation and break-down of the auxin/receptor complex. This allows us to use a quasi steady-state, Michaelis-Menten approximation for the receptor PIN1 and receptor/auxin complexes. We hypothesize that BIG, a membrane-bound protein involved in NPA-mediated inhibition of auxin transport, acts as a 'docking station' for PIN1 vesicles [41]. PIN1 is produced at an auxin-dependent rate α and is broken down at a constant rate δ.

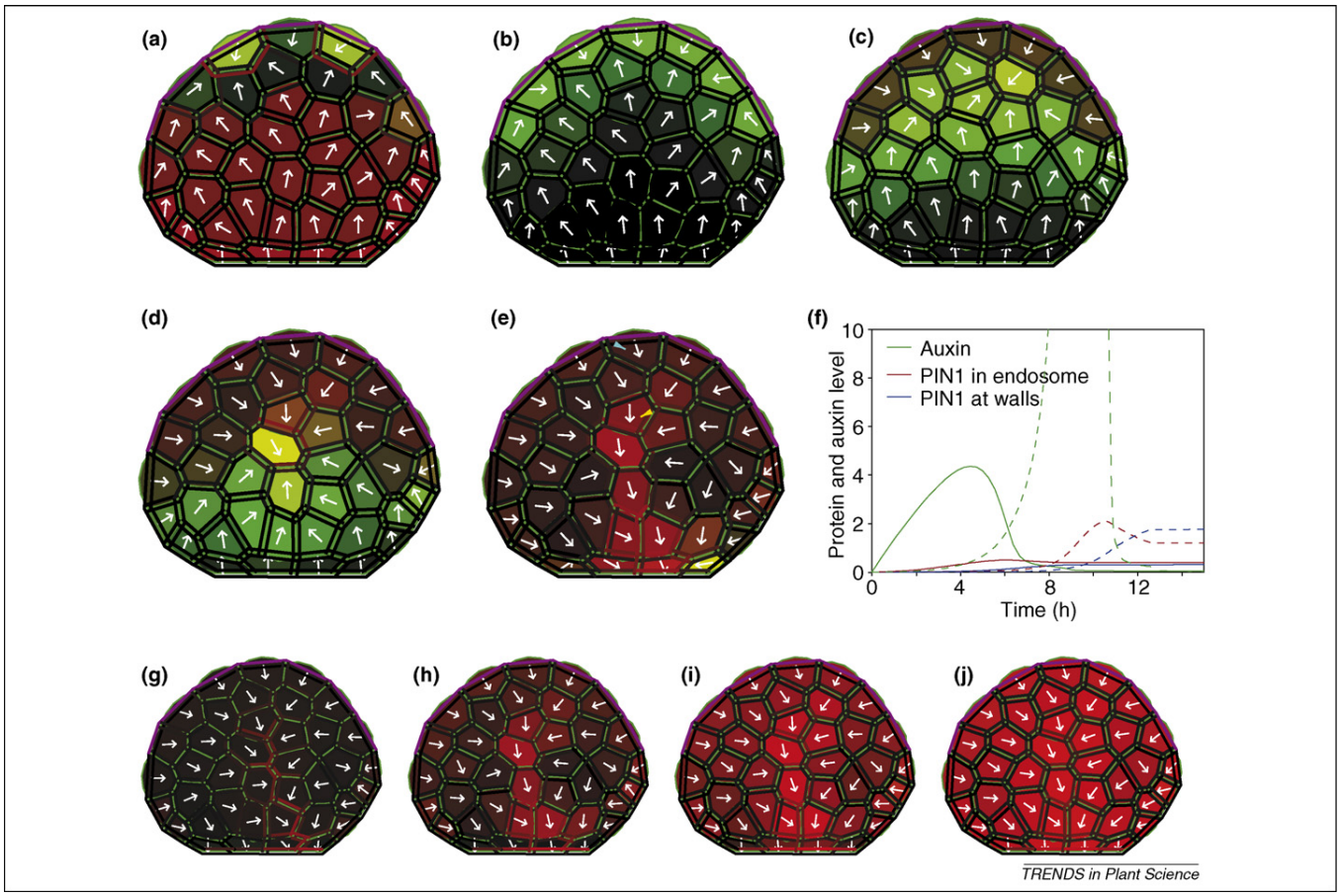

Figure 3. Traveling-wave auxin-channeling mechanism. **(a)** Formation of PIN1 convergence points in a model equivalent to the phyllotaxis model by Henrik Jönsson and coworkers [19], with constant level of PIN1 and zero PIN1 production and decay. PIN proteins (red) localize preferentially toward cells with high auxin concentration (bright green); as a result, cells pump auxin upstream leading to auxin maxima (bright green). Auxin flows in from the leaf tip (purple cell walls). White arrows indicate the average polarization of PIN1 proteins. See also Movie S2 in supplementary material online. **(b–d)** If we take into account that PIN expression is auxin-dependent, a traveling auxin wave forms a polar auxin channel. See also Movie S3 in supplementary material online. Auxin (shown in green) flows in from the leaf tip (at the purple cell walls). PIN (red) localizes preferentially at cell walls adjacent to cells with the highest auxin concentration as in recent phyllotaxis models [19,28]. **(b)** t = 3 h; auxin accumulates at the leaf edge, some auxin leaks into the lower cell layers. PIN1 production rises. **(c)** t = 8 h; some PIN1 localizes to the lower cell layers and actively pumps auxin away; the deeper cell layers pump auxin back into the top layer, but at a much slower rate because of their lower PIN1 levels. **(d)** t = 13 h; auxin converges into a small peak, toward which the adjacent cells polarize. **(e)** t = 20 h; completed auxin channel, traced out by the traveling wave of auxin. **(f)** Simulated auxin and PIN1 levels in endosome and membrane compartments for two cells (magenta arrow: solid curves; yellow arrow; dashed curves). **(g–i)** Simulated effect of NPA treatment, where we hypothesize that NPA reduces the number of membrane 'docking' sites for PIN1-carrying vesicles. For lower values of R the PIN1 expression domain widens, reproducing PIN1::GFP observations in leaf primordia [15]. Our current model does not reproduce supernumerary PIN convergence points as observed experimentally [15]. **(g)** $R = 1000$; **(h)** $R = 100$ (default); **(i)** $R = 10$; **(j)** $R = 1$.

an assumption in our model, the auxin maximum travels from the leaf margin toward the leaf base. It converges onto a motile auxin peak that leaves behind a trail of polarized PIN1 expression (Figure 3b–e and Movie S3 in the supplementary material online). We can understand this mechanism by considering transport along the apical–basal (vertical) and medio–lateral (horizontal) axes separately. Initially diffusion dominates auxin transport, and steep apical–basal auxin gradients originate from the leaf margin. Provided that auxin induces PIN1 expression in a concentration-dependent manner and sufficiently quickly, a PIN1 expression gradient follows the auxin expression (Figure 3f). The top row of cells localizes relatively large amounts of PIN1 toward their neighbors, and to a lesser extent to the second layer of cells that also contains some auxin. Although the second layer also polarizes toward the top cell row, they have fewer PIN1 molecules, because of their lower levels of auxin. In this way, the apical–basal PIN1 gradient drives a net apical–basal auxin flux. Along the medio–lateral axis, the Jönsson mechanism converges the auxin maxima and corresponding PIN1 maxima onto a narrow file of PIN1-expressing cells. Indeed, progressive narrowing of PIN1 expression domains is observed in leaves [15]. Thus, including auxin-induced PIN1 expression into the Jönsson auxin-convergence model [19], reproduces a polar auxin-channeling mechanism consistent with experimental observations.

Reproducing experimental treatments: a model validation

A plausible model should also reproduce the leaf primordium's response to experimental manipulations. Leaves treated during their development with the auxin transport inhibitor NPA have fewer, wider veins and thicker veins along the leaf edge than wild-type leaves [12]. NPA interferes with actin-mediated protein trafficking in general, but does not directly affect PIN1 localization [38]. Although the precise molecular effects of NPA are unknown, several high- and low-affinity NPA-binding proteins (NBP) have been identified [39]. The membrane-bound protein BIG

might mediate the effect of NPA on auxin transport [40], either as a direct target of NPA or as a regulator of an NBP. BIG is required for auxin-mediated inhibition of endocytosis, and *big (tir3/doc1)* knockouts have fewer NPA-binding sites [29]. Thus, one hypothesis is that BIG might act as a 'docking element' required for proper PIN1 localization [41]. NPA, through the direct or indirect inhibition of BIG, might reduce the number of membrane-docking sites for PIN1-carrying vesicles, which we simulate by reducing the level of active auxin receptors (R). For lower values of R, the PIN1 expression domain widens (Figure 3g–j), thus reproducing PIN1::GFP observations in leaf primordia [15]. NPA might also directly inhibit protein cycling [38]; indeed the PIN expression domains also widens when we reduce both the exocytosis and endocytosis rates k_1 and k_2 (not shown).

Enrico Scarpella and coworkers [15] have demonstrated that PIN1 expression domains form normally in *van-3* discontinuous vein mutants, breaking up into disconnected patches later on. A similar phenomenon occurs in our model if we increase the endocytosis rate k_2 (Figure S1 and Movie S4 in the supplementary material online), but the VAN-3 protein is probably not involved in vesicle endocytosis because it is localized in the *trans*-Golgi network [42]. VAN-3 is an ARF-GAP protein, which indicates that it might be involved in the transport of proteins, including PIN1, to and from the membrane [42]. VAN-3 is auxin-induced and is possibly self-regulated [42], suggesting that it might function to sustain PIN1 exocytosis after the adjacent auxin signal has faded. Thus, reproducing the effects of experimental treatments, knockout and overexpression phenotypes helps validate the mechanism, and provides mechanistic insights into the sequence of events leading to patterning defects.

Conclusions and future directions

We have argued that an essential part of the molecular machinery needed for Sachs' canalization hypothesis, an auxin flux sensor, has not yet been identified in plants. Although we cannot exclude the possibility that plant cells sense auxin flux, experimental observations seem more consistent with mesophyll cells responding to auxin concentrations.

Using computer simulations, we have presented a traveling-wave hypothesis for the formation of polar, auxin transport channels. The proposed mechanism is a variation on the recently proposed mechanisms for phyllotaxis [19,28]. In its present form, the model should be considered a proof-of-principle. It illustrates that besides Sachs' seminal, auxin-flux-dependent canalization hypothesis, we can construct alternative auxin-channeling mechanisms that are fully consistent with published experimental observations. Thus, our model provides a basis for more detailed and accurate models and hypotheses.

Although the phenomena discussed here are stable across large ranges of parameter values, we will need to determine their precise values experimentally. Obtaining rate constants for protein recycling and auxin transport is one of the major challenges for creating a more quantitative model of venation patterning [35]. Like the model by Jönsson *et al*. [19], our model lacks apoplastic (intercellular) compartments. We assume that the intercellular auxin concentrations are proportional to those in the two neighboring cells, and that the auxin concentration in the neighboring cell is a sufficiently accurate proxy for that. This assumption does not necessarily hold: take two adjacent cells with different auxin concentration, the two cells would sense a different concentration, although, in reality, both should 'see' the same intercellular level of auxin. Including the apoplast in our model will probably not affect the auxin-channeling mechanism we propose here, because differential PIN1 expression, rather than differential PIN1 localization, drives the auxin maxima forward.

Cell division and leaf expansion are major regulators of leaf venation patterning: experimentally, new PIN1 convergence points appear as the leaf primordium expands [15]. Using the biomechanical model we briefly introduced in Figure 1, we can study the traveling-wave, auxin-channeling hypothesis in a dividing and expanding tissue. Integration between computer simulation studies and experimental results is required and instrumental for efficient progress in unraveling the dynamic mechanisms of leaf venation patterning.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tplants.2007.08.004.

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