Reviewing models of auxin canalisation in the context of vein pattern formation in *Arabidopsis* leaves

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Introduction

Several hypotheses have been formed to explain vein pattern formation. Sachs proposed that veins develop as a result of the gradual canalisation of auxin: the auxin transport capacity of some cell files increases, draining auxin from neighbouring cell files. Mitchison implemented models of auxin canalisation by postulating that the transport of auxin between cells is controlled by a feedback mechanism, in which the parameters of the transport process vary as a function of auxin flux. He considered two mechanisms: facilitated diffusion, in which transport is diffusive; and polar transport, in which transport has a non−diffusive character. Many experimental studies fit well within the canalisation hypothesis: auxin has been shown to be necessary for vein formation; it is transported in a polar way; and the PIN1 protein, involved in auxin efflux, localises progressively to one end of the cell. The growing amount of molecular data on auxin transport mechanisms and leaf venation patterns in *Arabidopsis* suggests that enough information may now be available to model these patterns in a realistic way. In that context, we examine Mitchison's models and show that models employing either facilitated diffusion or polar transport mechanisms can reproduce elements of *Arabidopsis* leaf vein pattern formation, such as the acropetal development of the primary vein, and the formation of the first two secondary veins. In the latter case, generating realistic loop patterns may depend on incorporating growth into the model. Finally, we use Mitchison's models to qualitatively reproduce some patterns observed in *Arabidopsis* mutants, such as the patterns that occur when the auxin transport is inhibited, and venation patterns with discontinuities.

Reference

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Introduction

Several hypotheses have been formed to explain vein pattern formation. Sachs (1981) proposed that veins develop as a result of the gradual canalisation of auxin: the auxin transport capacity of some cell files increases, draining auxin from neighbouring cell files. Mitchison (1980,1981) implemented models of auxin canalisation by postulating that the transport of auxin between cells is controlled by a feedback mechanism, in which the parameters of the transport process vary as a function of auxin flux. He considered two mechanisms: facilitated diffusion, in which transport is diffusive; and polar transport, in which transport has a non-diffusive character. Many experimental studies fit well within the canalisation hypothesis: auxin has been shown to be necessary for vein formation (Sieburth, 1999); it is transported in a polar way (Sachs, 1981); and the PIN1 protein, involved in auxin efflux, localises progressively to one end of the cell (Steinmann et al., 1999). The growing amount of molecular data on auxin transport mechanisms and leaf venation patterns in *Arabidopsis* suggests that enough information may now be available to model these patterns in a realistic way. In that context, we examine Mitchison's models and show that models employing either facilitated diffusion or polar transport mechanisms can reproduce elements of *Arabidopsis* leaf vein pattern formation, such as the acropetal development of the primary vein, and the formation of the first two secondary veins. In the latter case, generating realistic loop patterns may depend on incorporating growth into the model. Finally, we use Mitchison's models to qualitatively reproduce some patterns observed in *Arabidopsis* mutants, such as the patterns that occur when the auxin transport is inhibited, and venation patterns with discontinuities.

Models

Leaf tissue was modelled as a 2D grid of square cells of unit area. Each internal cell (i.e. those not lying on the border of the tissue) is assumed to have four neighbours. The concentration c of auxin in the cell varies according to the equation

$$dc/dt = \sigma + \sum_{i=1}^{n} \phi_i \,, \qquad (1)$$

where σ is the auxin production of the cell (i.e. source activity), n the number of neighbours, and values ϕ_i capture auxin transport between neighbouring cells.

We distinguished two transport mechanisms: facilitated diffusion and polar transport. The facilitated diffusion model was implemented as proposed by Mitchison (1980). The flux of auxin from cell 1 to cell 2 in unit time is described by the equation

$$\phi = \widetilde{D}(c_1 - c_2) \,, \qquad (2)$$

where $\widetilde{D}$ is the diffusion coefficient between the two cells, and c_1 and c_2 are the concentrations of auxin in cells 1 and 2. This is similar to a normal diffusion equation, except that $\widetilde{D}$ is dependent on the flux and varies according to the equation

$$d\widetilde{D}/dt = \alpha\phi^2 + \beta - \gamma\widetilde{D} \,, \qquad (3)$$

where α, β and γ are constants. Furthermore, $\widetilde{D}$ is bounded between values D_{min} and D_{max}.

The polar transport model was also proposed by Mitchison (1981), but we implemented it with some modifications intended to better capture current understanding of molecular transport. We suppose

that there is some level of background diffusion, and that an efflux through a cell wall promotes the production of efflux carriers near that wall, which further increases the efflux. The flux from cell 1 to cell 2 in unit time is thus described by the equation

$$\phi = D(c_1 - c_2) + p_1 c_1 - p_2 c_2 , \qquad (4)$$

where D is the fixed diffusion constant between the two cells, p_1 controls the efflux out of cell 1 at the interface between the two cells, and p_2 controls the efflux out of cell 2 at the interface between the two cells. Parameters p_1 and p_2 indirectly represent numbers of carrier molecules. These parameters change according to the equations:

$$dp_1 / dt = \alpha\phi^2 - \gamma p_1 \qquad (5)$$

if the efflux from cell 1 to cell 2 is positive, and

$$dp_1 / dt = -\gamma p_1 \qquad (6)$$

if it is negative. The constant α represents how much p_1 increases with the flux, whereas the constant γ represents the rate of decay of the efflux molecules. Symmetric equations govern production and decay of carrier p_2 in cell 2. The values p_1 and p_2 are bounded between 0 and p_{max}.

In all of Mitchison's original models, initial concentrations formed a linear gradient from high concentrations at the top of the tissue to zero concentrations at the bottom edge. Boundary conditions were a constant influx of auxin at the top edge of the tissue, and a sink line at the bottom edge. In the models presented here, we also experimented with different starting conditions, such as no initial gradient of concentrations, absence of influx at the top edge of the tissue, and a single sink cell instead of a line. In the following descriptions, we assume that cells are indexed by row number (starting with 1 at the bottom) and by column number (starting with 1 on the left).

Results

We reproduced Mitchison's results and confirmed that the initially uniform linear gradient of concentrations is unstable, and as soon as it is destabilised, strands emerge. Using both facilitated diffusion models (Figure 1a-c) and polar transport models (Figure 1d-f), destabilisation could be achieved by inducing auxin production ($\sigma > 0$) in one or several cells. By comparison with the facilitated diffusion model, polar transport gave more robust patterns, as weak and strong sources generated similar strands (Figure 1e-f, compare with Figure 1b-c).

Having confirmed basic properties of Mitchison's canalisation models, we applied them to capture specific features of venation formation in young *Arabidopsis* leaves. To do so, we assumed that increased auxin flux leads to vein differentiation.

1) Observations show that the primary vein differentiates acropetally (from leaf base to leaf tip), in spite of auxin being transported basipetally from source to sink (see Dengler (2001) for a review). This scenario can be reproduced assuming a single sink at the bottom of the tissue, and an incoming flux of auxin along the entire top edge of the tissue (Figure 2a). Both facilitated diffusion and polar transport models produce similar results.

2) After the primary vein has been formed, secondary veins appear as loops connecting to the primary vein at different points (Figure 2b). This can be modelled using facilitated diffusion, in a model that includes a simple growth simulation. In this case, the sink is progressively moved further away from the initial source, and at different time points new sources are added in lateral positions with respect to the initial source (Figure 2c, compare with 2b). The timing of each lateral source defines where the corresponding secondary loop connects to the midvein: a source added later induces a strand that will connect closer to the sink. Including growth in models using the polar transport model did not produce loops, but loops could be formed without growth by setting an extended source of auxin (3 cells wide) at the top of the grid (figure 2d). However, we did not succeed in producing loops that reconnect to the midvein at different points.

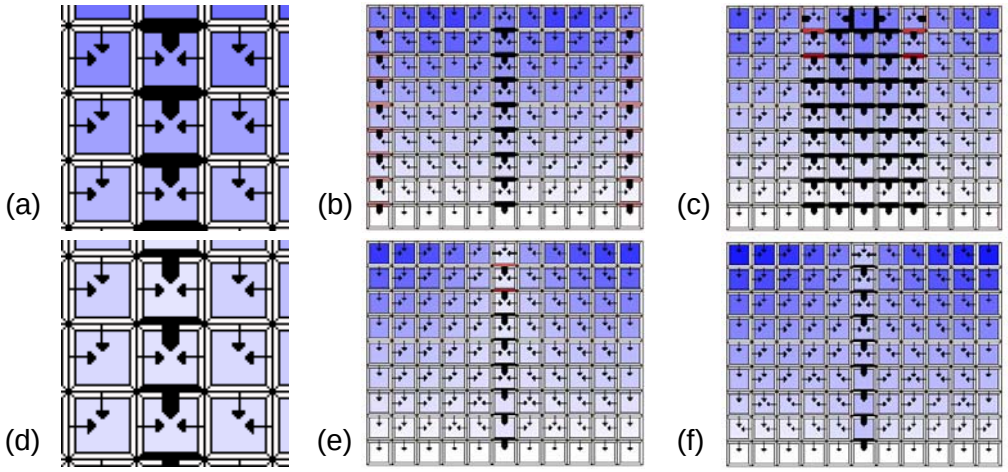

Figure 1: Examples of strand formation. Each diagram represents a grid of square cells with their walls, after 10000 steps of simulation. Auxin concentration is color-coded pale to bright blue for lower to higher concentrations. We postulate that veins differentiate in zones of high flux. (a-c) Facilitated diffusion model. Initially, a linear gradient of concentrations was set up with high concentrations at the top, and zero concentrations at the bottom line of cells. For boundary conditions we used a constant flux of auxin coming from the top edge of the tissue, and a sink for auxin (c=0) in the bottom line of cells. This created a uniform vertical flow, which was then disrupted in different ways. (a): Iconic representation of the facilitated diffusion model. Arrows represent the direction of flux between cells. Larger fluxes are represented with larger arrows, up to a chosen maximum. Values for $\widetilde{D}$ are color-coded in the walls from pale to bright red, and eventually are black when the maximum value is reached. (b): A weak source of auxin (σ=50) was placed in cell (9,6) (row nine, column six; i.e., top row, middle column). This produced a strand in the middle column of cells. Strands also formed on the sides of the tissue because the middle strand was not strong enough to attract flux from the whole tissue. (c): same as (b) with a strong source (σ=300): the middle strand extended into neighbouring strands, and the resulting fat strand was strong enough to attract flux from the whole tissue. (d-f) Polar transport model, same initial conditions as (a-c). (d): Iconic representation of the polar transport model. Similar to (a) except that values p_i for efflux carriers are shown in the four individual walls of each cell. When the number of transport molecules has reached its maximum, the wall is shown in black (thick horizontal bars). (e): A weak source (σ=50) was placed in cell (9,6). (f): A strong source (σ=300) was placed in cell (9,6). In both cases, a single strand was formed. Key simulation parameters were an incoming flux of 15 at the top of the tissue, and α= 0.00005, β= 0.005 and γ= 0.05 for the facilitated diffusion models, or α=0.00001, γ=0.05 for the polar transport models.

3) When plants are treated with transport inhibitors such as NPA during early leaf development, the venation pattern is altered (Mattsson et al., 1999). In particular, the midvein tends to be wider than normal or to separate in several strands, and the veins along the leaf edge are thicker. Severe transport inhibition impairs the midvein connection between the top and the base of the leaf (Figure 3a-c). We could model these effects by reducing either parameter D_{max} or α (equations 3 and 5). We obtained similar results with both facilitated diffusion and polar transport models, although in the latter case D_{max} or α had to be reduced more to see an impact on the venation pattern. Moderate transport inhibition resulted in a thicker midvein, whereas a severe inhibition prevented the connection between source and sink (Figure 3d-f).

4) Some mutants have been described in which the venation pattern seems close to normal except that veins are discontinuous (Figure 3g). Consistent with these descriptions, we observed that in the models the zones of high fluxes sometimes extend from both the source and the sink at the same time, with a gap in between (Figure 3h). If the canalisation process is stopped before completion of the high flux strand, this generates discontinuous veins.

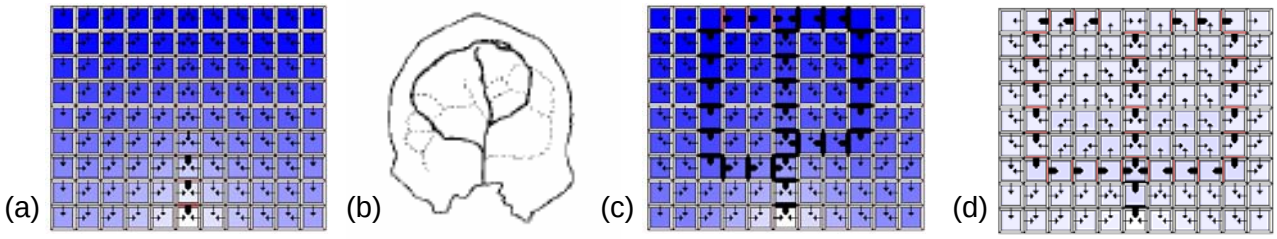

Figure 2. Models of vein formation. (a): Model of primary vein formation, using the facilitated diffusion model. Initially, a linear gradient of concentrations was set up with high concentrations at the top, and zero concentrations at the bottom line of cells. For boundary conditions we used a constant flux of auxin coming from the top edge of the tissue, and a sink for auxin (c=0) in cell (1,6). Simulation is shown at step 500: a strand started forming acropetally. (b-c) Formation of secondary veins. (b): Early stage of vein formation in an Arabidopsis leaf, showing the primary vein and the first two loops of secondary veins as full black lines. *Reproduced from Candela et al., (1999)*. (c): Model of loops using facilitated diffusion. Initial concentrations were zero everywhere, and there was no incoming flux of auxin. A first auxin source was set in cell (9,6) until step 50, a second auxin source was set in cell (9,9) from step 1600 onwards, a third source was set in cell (9,3) from step 2000 onwards. Initially, cell (8,6) was a sink, then every 500 steps the sink was moved down one cell, until the final stage shown: step 4000. (d): Model of loops using polar transport. Initial concentrations were zero everywhere and there was no incoming flux of auxin. A source was set in cells (9,5), (9,6) and (9,7). A sink (c=0) was set in cell (1,6). Simulation is shown at step 10000.

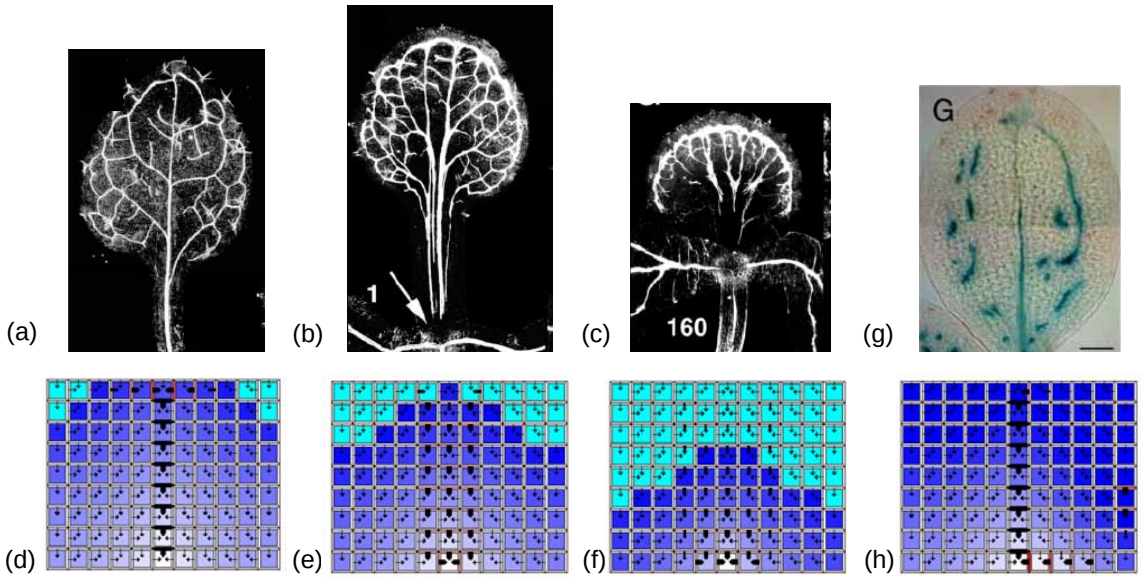

Figure 3. Modelling of altered venation patterns. (a-c) *Arabidopsis* leaf venation patterns resulting from a treatment with transport inhibitor (NPA). (a): control. (b): mild inhibition. (c): severe inhibition. *Reproduced from Mattsson et al., 1999*. (d-f) Modelling the effects of reduced transport. Initial conditions were a linear gradient of concentrations with high concentrations at the top, and zero concentrations at the bottom line of cells. Boundary conditions were a constant flux of auxin coming from the top edge of the tissue, and a sink for auxin (c=0) in cell (1,6). The highest concentrations of auxin are shown in cyan. (d): Same as Fig. 2a after 10000 steps (normal transport: D_{max}=4). (e): D_{max}=1. (f): D_{max}=0.5. (g): Early stages of vascular tissue formation in a cotyledon of a *van3 Arabidopsis* mutant, shown by the expression pattern of the *pAthb8::GUS* gene. *Reproduced from Koizumi et al., 2000*. (h): Illustration of discontinuities during strand formation. Initial concentrations were zero and there was an influx of auxin from the top of the grid. Two sources were set at step 1 and maintained throughout the simulation. The first source (σ=100) was set in cell (9,6), and the second source (σ =50) was set in cell (5,11). Simulation is shown at step 1800: the strand between the second source and the sink has started to form both from the sink and from the source. If at that stage canalisation is interrupted by fixing $\tilde{D}$, the two strands of high flux will not join, leading to discontinuous veins when differentiation occurs.

Discussion

The auxin canalisation models proposed by Mitchison (1980, 1981) can be used to model features of venation patterns in Arabidopsis. We show that under specific conditions, the strand of increased flux resulting from auxin canalisation can proceed from the sink towards the source, which provides a solution to the apparent contradiction between the acropetal development of the midvein and the basipetal direction of auxin flow. Another interesting result is that simulating growth by moving the sink location allows realistic loop patterns to be produced. Although in the simulation these patterns did not persist after the cessation of growth, in reality they may be fixed by cell differentiation.

Although it was shown that tissues exposed to auxin can differentiate veins (Sachs, 1981), it remains unclear whether vein formation responds to high auxin concentrations or high fluxes. The only mathematical models of canalisation so far, proposed by Mitchison (1980, 1981), postulated that veins would form as a result of high fluxes. However, in the case of auxin transport inhibition, we need to postulate that vein differentiation at the margins is caused by high auxin concentrations rather than fluxes. This apparent contradiction may perhaps be solved by assuming that all that matters is the number of auxin molecules a cell is exposed to over a period of time: this can be achieved by a long exposure to a large number of molecules (high concentration, low flux), or a rapid turnover of exposure to a small number of molecules (high flux, low concentration). In that case veins may form both under prolonged high auxin concentrations, and high auxin fluxes with low concentrations. Specifically, in Figure 1g-h, zones of increased concentrations (cyan) would form thick veins at the margin whereas zones of increased flux would form veins by the process described by the models.

The discovery of mutants with discontinuous venation patterns has put the canalisation hypothesis in doubt (Koizumi, 2000). However, our simulations show that during canalisation strands of high flux may arise in a discontinuous manner. If successive sources of auxin are produced in different parts of the leaf during its early development, as current evidence suggests (Aloni, 2003), a mutation impairing the completion of canalisation could then result in discontinuous vein patterns. Therefore it seems that mutants with discontinuous veins do not invalidate the canalisation hypothesis.

Canalisation of auxin can be modelled both with facilitated diffusion or polar transport, and at this stage it is not clear which process is more likely to be responsible for vein pattern formation. Both classes of models are capable of generating a large variety of patterns, of which only a fraction were presented here. In both types of models, robust patterns seem to emerge more easily if there is some background diffusion. This suggests that "ordinary" diffusion may be responsible for the onset of facilitated diffusion or polar transport.

In reaction-diffusion, morphogens act by controlling their production rates or those of other morphogens through feedback mechanisms. By contrast, in the models presented here, morphogens act by modifying the parameters of the transport process. Therefore these models can be viewed as a class of pattern formation mechanisms on their own, perhaps complementary to reaction-diffusion.

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