# Supporting Text

## Description of the Model

A model was designed for simulating auxin transport in the meristem. The aim of this model is to study possible stationary regimes of auxin fluxes inferred from the observed distribution of auxin efflux facilitator PIN1.

**Representation of the Meristem** The organization of the cells in the L1-layer is represented as an undirected graph, called the "topological graph", in which vertices represent the cells and edges between vertices represent the cell walls. For each cell $c$ in the topological graph, $\mathcal{N}(c)$ defines the set of cells adjacent to $c$. Similarly, the presence of the efflux facilitator protein PIN1 on cell walls is represented by a second graph, called the "pump graph", taking into account the orientation of the auxin transport. As in the case of the topological graph, vertices of the pump graph represent the meristem cells. However, edges are now oriented and denote the presence of PIN1 on cell membranes: there exists an oriented edge from cell $c$ to cell $n$ if PIN1 protein is present in $c$ on the membrane next to $n$. We denote $\mathcal{P}_o(c)$ the set of neighbors of $c$ reached by an outgoing edge from $c$ and $\mathcal{P}_i(c)$ the set of neighbors of $c$ that are the origin of an incoming edge in $c$.

The topological and pump graphs are constructed by hand using a dedicated software, called MERRYSIM, developed to ease the operation.

In some cases, the presence or orientation of PIN1 protein on cell walls is difficult to assert without ambiguity. For this reason, we considered four levels of confidence in the identification of the auxin pumps:

1. PIN1 is clearly seen with its polarity ($89\%$ of the links in all the meristems);

2. PIN1 is clearly seen but its polarity cannot be determined ($4\%$ of the links);

3. PIN1 presence and orientation are discerned with difficulty ($6\%$ of the links);

4. PIN1 presence is discerned with difficulty and its polarity cannot be determined ($1\%$ of the links).

Edges in the pump graph are generated depending on this level of confidence. For each pump at confidence level 1 or 3, a single oriented edge is created in the pump graph. For each pump at level 2 or 4, two oriented edges (in both directions) are created. These levels are recorded for each edge.

Each cell $c$ is associated with a state defining its properties. This state includes the volume of the cell ($V_c$), the concentration of auxin in the cell ($a_c$), the presence of PIN1 proteins oriented toward the provascular system of the meristem ($E_c$ where $E_c = 1$ if there is such PIN1 proteins, otherwise $E_c = 0$) and the relative efficiency of these pumps ($\beta_c$, see below). Of these properties, only the concentration of auxin is variable throughout time.

**Transport Process.** Auxin distribution is assumed to depend on a combination of four mechanisms: (*i*) passive diffusion of auxin through cell walls, (*ii*) active transport of auxin facilitated by PIN1 protein in the surface of the meristem, (*iii*) active transport of auxin toward the deeper layers of the meristem, and (*iv*) natural turn-over of auxin.

(*i*) Diffusion is a passive process that tends toward the equilibrium of concentrations. We assume that the quantity of auxin diffusing through a cell wall is proportional to the difference of concentrations between the cells sharing the wall. Considering a cell $c$ and one of its neighbors $n$, then

$$\gamma_{c \leftarrow n}(t) = \Gamma \left[ a_n(t) - a_c(t) \right], \qquad \textbf{(1)}$$

where $\gamma_{c \leftarrow n}$ is the quantity of auxin per time unit diffused from $n$ to $c$, and $\Gamma$ is the diffusion coefficient. Note that $\gamma_{c \leftarrow n}$ is positive if auxin enters cell $c$.

(*ii*) According to our immunolabeling images, we considered that a quantification of the actual amount of PIN1 proteins based on pixel intensity would be unreliable. For this reason, we assumed that active transport is either present (in the case a marked zone of PIN pixels on the image) or absent. If present, the efflux of auxin due to this active transport is assumed to be constant

$$\psi_{c \leftarrow n} = \Psi, \tag{2}$$

where $\psi_{c \leftarrow n}$ is the quantity of auxin per time unit pumped from $n$ to $c$ by the PIN1 protein in $n$ on the wall toward $c$, $\Psi$ is the constant strength of the pumps.

This constant pump strength corresponds in the model to a default behavior. However, in order to take into account physical aspects of this pumping mechanism, we slightly modified this equation to correct the default behavior of the system in two extreme situations.

First, we consider that if auxin is rare in a cell, it may be more difficult for PIN1 proteins to recruit auxin molecules. Hence, the efficiency of the pump cannot remain constant when auxin is rare. Rather, we assume its optimal efficiency decreases proportionally with the lack of auxin in this cell. This is carried out by correcting the constant efflux with a saturating term depending on $a_n(t)$

$$\psi_{c \leftarrow n} = \Psi(1 - e^{-\alpha a_n(t)}), \tag{3}$$

where $\alpha$ is the slope of $\psi_{c \leftarrow n}(t)$ when the auxin in $n$ is depleted.

On the other hand, with the default system behavior, auxin can accumulate at very high levels within a cell. Diffusion would contribute to balance this phenomenon, at least partially. However, we also considered the physical possibility that, due to an excess of auxin in the destination cell, pump efficiency decreases when the destination cell reaches a concentration at a certain threshold. This led us to introduce a saturation term that is intended to correct the pump efficiency for high auxin concentrations, which reaches 0 when the cell is completely

saturated. The product of the two saturation terms denotes independent handling of these two extreme cases

$$\psi_{c\leftarrow n}(t) = \Psi\left(1 - e^{-\alpha a_n(t)}\right)\left(1 - e^{-\alpha(\sigma - a_c(t))}\right). \quad \textbf{(4)}$$

We made simulations with and without this last assumption about the saturation of the destination cell (see Fig. **13**).

(*iii*) In primordia cells, auxin is evacuated toward deeper layers by PIN1 proteins pumping auxin to the provascular internal cells. This pumping is similar to the pumping inside the L1-layer except that there is no saturation threshold in the inner layers

$$\psi_{c\downarrow}(t) = \beta_c \Psi\left(1 - e^{-\alpha a_c(t)}\right), \quad \textbf{(5)}$$

where $\psi_{c\downarrow}$ is the quantity of auxin per time unit pumped from $c$ toward the inner layers of the meristem by the PIN1 protein in $c$, $\Psi$ is the maximum efficiency of the downward pumps and $\beta_c$ is a coefficient decreasing with the distance to the primordium center. Pumping is assumed to be maximum in the primordium center and to exist also in the other primordium cells, with a decreasing strength as the primordia cells are positioned farther from the central cell. This is to take into account the observed orientations and quantities of PIN1 labeling in the immunolabelings (ref. 1 and J.T., unpublished results).

(*iv*) The turn-over of auxin is modeled as auxin degradation

$$\tau_c(t) = \delta a_c(t), \quad \textbf{(6)}$$

where $\tau_c$ is the variation of auxin concentration and $\delta$ the degradation factor.

The global variation of auxin within a cell $c$ is thus defined by

$$\frac{da_c}{dt}(t) = \frac{1}{V_c}\left(\sum_{n\in\mathcal{N}(c)} \gamma_{c\leftarrow n}(t) + \sum_{n\in\mathcal{P}_i(c)} \psi_{c\leftarrow n}(t) - \sum_{n\in\mathcal{P}_o(c)} \psi_{n\leftarrow c}(t) - E_c\psi_{c\downarrow}(t)\right) - \tau_c(t). \quad \textbf{(7)}$$

At each time step, the auxin content $a_c$ in each cell $c$ is updated according to Eq. **7**. This equation characterizes the exchange between a cell and its neighbors.

First, during a time period $dt$, the auxin concentration can augment in a cell in two ways. Auxin can diffuse from any neighbor cell $n$ to the current cell $c$ if $a_n$ is greater than $a_c$. Auxin can also be actively transported from a neighbor cell $n$ toward the current cell $c$ if PIN1 protein is localized in $n$ on the walls toward the cell $c$.

Reciprocally, during the same time period, the auxin concentration can decrease in four ways. Symetrically to the previous case, auxin can leave a given cell by diffusion (if $a_n$ is less than $a_c$) or active transport (if PIN1 protein is localized in $c$ on the walls toward the cell $n$). In addition, in cells that belong to primordia, auxin can be actively transported toward the inner layers. At last, the auxin content of a cell is decremented by a quantity corresponding to the natural auxin turn-over (modeled by a constant degradation rate throughout the meristem).

Eq. **7** can be expanded into

$$\begin{aligned}\frac{da_c}{dt}(t) &= \sum_{n\in\mathcal{N}(c)} \frac{\Gamma}{V_c}\left(a_n(t)-a_c(t)\right)\\ &+ \sum_{n\in\mathcal{P}_i(c)} \frac{\Psi}{V_c}\left(1-e^{-\alpha a_n(t)}\right)\left(1-e^{-\alpha(\sigma-a_c(t))}\right)\\ &- \sum_{n\in\mathcal{P}_o(c)} \frac{\Psi}{V_c}\left(1-e^{-\alpha a_c(t)}\right)\left(1-e^{-\alpha(\sigma-a_n(t))}\right)\\ &- E_c\frac{\Psi}{V_c}\left(1-e^{-\alpha a_c(t)}\right)-\delta a_c(t).\end{aligned} \tag{8}$$

**Boundary Conditions.** As stated in the main text, auxin is supposed to arrive in the meristem from the subapical parts of the plant via the L1-layer.

To simulate this behavior, a ring of auxin injection points representing "the lower parts of the plant" is added during meristem digitizing. Each of these points is linked to all of its neighboring cells in the meristem by a pump. These pumps are ruled by an equation similar to Eq. **4**. In particular, the external injection points always provide enough auxin to guarantee maximal pumping. If the injection points do not provide enough auxin, no auxin accumulations are observed in the meristem. If they provide too much, the meristem is almost completed

saturated by auxin (see Fig. **9** and text below). Based on tests, we chose a value for auxin injection inbetween these two extremes. Thus, the equation ruling the quantity of auxin exported by the external cell $n$ into the internal cell $c$ is

$$\psi_{c \leftarrow n}(t) = \Psi' \left(1 - e^{-\alpha(\sigma - a_c(t))}\right). \quad \textbf{(9)}$$

As an alternative hypothesis, auxin is produced/injected by every cell in the meristem.

To simulate this behavior, two processes are involved. First, a constant quantity of auxin is produced/injected at each time point in each simulated cell

$$\rho_c(t) = P. \quad \textbf{(10)}$$

Then, to represent the production of the non-simulated sub-apical parts of the plant, we use the pumping described in Eq. **9**.

## Implementation

A dedicated software, MERRYSIM, was developed to create the topological and the pump graphs, to carry out numerical simulations and to analyze the results. It is released under the terms of the GPL license*.

Data acquisition and analysis tools were implemented in C++ and PYTHON using the QT3 toolkit for the design of user interaction (`www.trolltech.com`).

A simulation engine was developed in MGS, a language dedicated to simulation of dynamic systems with dynamic structures (*2, 3*). To solve the system of ordinary differential equations (ODEs) we used an explicit Euler integration scheme with constant time step $dt$ (*4*). To avoid stability problems, we used a first-order approximation, when $a_c(t)$ vanishes to 0. This provides a condition for $a_c(t)$ not to become negative

$$a_c(t) \geq -(N_{max} + 1)\frac{\Psi}{V_{min}}\alpha a_c(t)dt, \quad \textbf{(11)}$$

*The GPL licence can be downloaded at `www.gnu.org/licenses/gpl.html`.

where $N_{max}$ is the maximum number of neighbors of a cell in the meristem, and $V_{min}$ is the volume of the smallest cell. This condition enables us to define an upper bound for $dt$

$$dt \leq \frac{V_{min}}{(N_{max}+1)\alpha\Psi}. \quad \textbf{(12)}$$

Visualizations were made using the PYTHON module SVGDRAW (`www2.sfk.nl/svg`) to transform the simulation results into SVG files.

The different parts of the software system are coordinated using the PYTHON language. MERRYSIM is a free software that can be downloaded freely at: `ftp://ftp.cirad.fr/pub/amap/VirtualPlants/merrysim/`.

## Sensitivity Analysis

To express the biological knowledge of the processes involved in the above model, we introduced parameters (e.g., the saturation threshold, the degradation rate) whose actual values cannot be estimated directly from experiments or cannot be exactly determined because of limitations in the original images (e.g., presence or orientation of certain pumps).

In order to test the validity of the conclusions, we made a number of changes in the values of these parameters to study the effect of these changes on the overall results. The set of parameters involved in the model is reflected in Eq. **8**. These parameters can be separated into two groups: (*i*) *the structural parameters* whose values are set during meristem digitization and have ordinal or symbolic values. This group includes: $\mathcal{N}(c)$, $\mathcal{P}_i(c)$ ($\mathcal{P}_o(c)$) and $E_c$. (*ii*) *The functional parameters*: $\Gamma$, $\alpha$, $\sigma$, $\delta$, $\Psi$ and $\Psi'$ that have real values.

To test the sensitivity of the model to the different parameters, two different procedures were used depending on the group of the parameter tested.

For the first group of parameters, different configurations of $\mathcal{P}_i(c)$ and $\mathcal{P}_o(c)$ were tested, according to the confidence level of the identified PIN1 pumps. As these pumps were classified into four categories with decreasing confidence level, simulations were carried out using the $n$

first categories with $n$ varying from 1 to 4. This allowed us to perform simulations using first only pumps whose presence was certain (i.e., $90\%$ of the pumps) and integrating progressively pumps with lower confidence level.

For the second group, we tested the variation of each parameter independently. Since auxin concentrations are entirely determined by the relative values of the parameters at a scale factor close, we were able to fix the value of one parameter: in all the simulations, the saturation parameter was set to 255 (auxin concentration thus ranging from 0 to 255 in each cell). For the other parameters, we used a range of nine different values (depicted in Table 1) and fixed the other parameters to the following reference values: $\Gamma = 0.03$, $\alpha = 0.025$, $\delta = 0.006$, $\Psi = 20.0$, $\Psi' = 3.5$.

**Results**

For all meristems, we observed the same general pattern of auxin accumulation (see main text). Auxin was accumulating in the primordia and at the meristem summit. In this latter zone, a protrusion could always be seen in the direction of the initium I-1. We subsequently carried out the complete set of robustness tests on 5 meristems.

The auxin distribution patterns obtained using reference values were used as a starting point for these tests.

**Auxin Injection Rate ($\Psi'$) (Fig. 9).** As the auxin flux from the injection points to the meristem increases, the total amount of auxin in the meristem augments. With increasing hormone quantities, auxin starts to accumulate first at the meristem summit, followed by the primordia and the initium. When the injection rate increases, almost the entire meristem is filled with auxin. However, some of its parts remain at a low auxin concentration. The patterns are relatively sensitive to this parameter as they do not support more than a twofold variation.

The alternative hypothesis where auxin was injected into every cell provided no significant difference in the global pattern of auxin accumulations (data not shown).

**Degradation Factor ($\delta$) (Fig. 10).** The degradation factor also affects the total quantity of auxin in the meristem. In this case the patterns are relatively insensitive as auxin accumulation can still be observed at the primordia sites at low total auxin quantities (i.e. high degradation rates). The patterns support a 16-fold variation.

**Pumping Strength ($\Psi$) (Fig. 11).** Without pumping, the meristem is filled by the periphery and auxin diffuses toward the center. For a nonnull pumping, typical auxin accumulation patterns are readily observed, even for the weakest pumping forces tested. With increasing pumping forces, a better separation of the center and the primordia can be observed, mainly due to a stronger counter-effect on diffusion. In this case, the patterns support a fivefold variation.

As indicated in the main text, simulations showed that even an increase of transport rates by a factor of 10 in the young primordia (where PIN1 labeling was the strongest) did not significantly change the final outcome of the simulations (Fig. 4).

**Diffusion Rate ($\Gamma$) (Fig. 12).** Without diffusion, most of the cells are either saturated or empty and accumulation sites cannot always be clearly identified. With the increase of the diffusion rate, the accumulation zones are better formed and enlarge until auxin is almost regularly distributed in the meristem. In this case, the patterns support a 13-fold variation.

**Saturation ($\sigma$) (Fig. 13).** Without any saturation threshold, the auxin accumulation patterns are not significantly changed. However, very high auxin concentration in the meristem summit are observed (much higher than in the primordia).

**Simulations Using Pumps With Varying Confidence Level (Fig. 14).** No significant differences between simulations using all the pumps or only pumps with highest confidence level could be observed.

This sensitivity analysis showed that the auxin accumulation pattern predicted by the simulations was particularly robust to changes in almost all the model parameters. The actual ranges of parameter values for which the auxin accumulation pattern of each meristem could be identified are summarized in Table 2.

## Tracking Auxin Paths in the Meristem

We also identified the paths followed by auxin molecules to reach different zones of the meristem. Let us denote $q_c(t)$ the total amount of auxin molecules contained in cell $c$ at time $t$. We consider a cell $c'$ different from $c$ and we define $q_{c\leftarrow c'}(t)$ the quantity of auxin contained in $c$ at time $t$ that went through cell $c'$ at some anterior date. This number of molecules divided by the cell volume $V_c$ defines a concentration of auxin molecules in $c$ at time $t$ that went through $c'$, denoted by $a_{c\leftarrow c'}(t)$. By extension, we define $a_{c\leftarrow c}(t) = a_c(t)$.

At each time step $t$, a cell $c$ loses globally a quantity $f_c^-(t)$ of auxin (via pumping, diffusion, and degradation) and receives from each of its neighbor $n$ a quantity $f_{c\leftarrow n}^+(t)$ of auxin. To compute $q_{c\leftarrow c'}(t)$ when $c \neq c'$ we add the hypothesis that the auxin arriving in a cell merges instantaneously with the auxin already present in this cell. This implies that when a quantity $f_{c\leftarrow n}^+(t)$ of auxin goes from cell $n$ to cell $c$, the variation of $q_{c\leftarrow c'}$ is determined by the product between the proportion of auxin in $n$ that went through $c'$ and the total quantity of auxin going from $n$ to $c$

$$d^+ q_{c\leftarrow c'}(t) = \sum_{n\in\mathcal{N}(c)} \frac{q_{n\leftarrow c'}(t)}{q_n(t)} f_{c\leftarrow n}^+(t)dt. \quad \textbf{(13)}$$

Similarly, when a quantity $f_c^-(t)$ of auxin leaves the cell $c$, the variation of $q_{c\leftarrow c'}$ is determined by the product between the proportion of auxin in $c$ that went through $c'$ and the quantity

of auxin going out of $c$

$$d^- q_{c \leftarrow c'}(t) = \frac{q_{c \leftarrow c'}(t)}{q_c(t)} f_c^-(t) dt. \tag{14}$$

The total variation of $q_{c \leftarrow c'}, c \neq c'$ is thus

$$dq_{c \leftarrow c'}(t) = d^+ q_{c \leftarrow c'}(t) + d^- q_{c \leftarrow c'}(t), \tag{15}$$

$$\frac{dq_{c \leftarrow c'}}{dt}(t) = \sum_{n \in \mathcal{N}(c)} \frac{q_{n \leftarrow c'}(t)}{q_n(t)} f_{c \leftarrow n}^+(t) - \frac{q_{c \leftarrow c'}(t)}{q_c(t)} f_c^-(t), \tag{16}$$

which leads to the following relationships on concentrations

$$\frac{da_{c \leftarrow c'}}{dt}(t) = \begin{cases} \displaystyle\sum_{n \in \mathcal{N}(c)} \frac{a_{n \leftarrow c'}(t)}{a_n(t) V_c} f_{c \leftarrow n}^+(t) - \frac{a_{c \leftarrow c'}(t)}{a_c(t) V_c} f_c^-(t) & \text{if } c \neq c' \\ \displaystyle\frac{da_c}{dt}(t) & \text{otherwise,} \end{cases} \tag{17}$$

where the two fluxes, $f_{c \leftarrow n}^+(t)$ and $f_c^-(t)$ are defined by

$$f_{c \leftarrow n}^+(t) = \psi_{c \leftarrow n}^+(t) + \gamma_{c \leftarrow n}^+(t), \tag{18}$$

$$f_c^-(t) = V_c \tau_c(t) - \sum_{n \in \mathcal{N}(c)} \left( \gamma_{c \leftarrow n}^-(t) + \psi_{c \leftarrow n}^-(t) \right) + E_c \psi_{c\downarrow}(t), \tag{19}$$

in which $\psi_{c \leftarrow n}^+(t)$ and $\gamma_{c \leftarrow n}^+(t)$ correspond respectively to the terms $\psi_{c \leftarrow n}(t)$ and $\gamma_{c \leftarrow n}(t)$ when they are positive and to 0 otherwise. Similarly, $\psi_{c \leftarrow n}^-(t)$ and $\gamma_{c \leftarrow n}^-(t)$ correspond to the terms $\psi_{c \leftarrow n}(t)$ and $\gamma_{c \leftarrow n}(t)$ when they are negative and to 0 otherwise.

This system of $N^2$ differential equations enables us to compute at each time point $t$, and for each cell $c$, the concentration $a_{c \leftarrow c'}(t)$ of auxin molecules in $c$ that went at an anterior date through a given cell $c'$, for any $c'$. This system of differential equations is solved using the finite difference method described above.

**Contribution of a Cell to the Auxin Concentration in Particular Zones.** Using the functions $a_{c \leftarrow c'}(t)$, it is possible to estimate the contribution of a particular cell to the overall auxin

concentration of a group of cells (primordia or summit cells) in the meristem at time $t$. For each cell $c'$, its contribution to the auxin in a group $G$ of cells, $\tau_{G \leftarrow c'}(t)$, is defined as

$$\tau_{G \leftarrow c'}(t) = \frac{1}{\sum_{c \in G} q_c(t)} \sum_{c \in G} q_{c \leftarrow c'}(t), \qquad \textbf{(20)}$$

where $q_{c \leftarrow c'}(t) = V_c a_{c \leftarrow c'}(t)$.

For a given group of cells $G$, e.g. the cells of a primordium, it is then possible to compute color maps showing a color intensity in each cell $c'$ in the meristem proportional to the contribution $\tau_{G \leftarrow c'}(t)$ of this cell to the group. These maps give an impression of the main paths followed by auxin to reach particular zones in the meristem (see Fig. 7).


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