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Modelling the emergence of polarity patterns for the intercellular transport of auxin in plants

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The hormone auxin is actively transported throughout plants via protein machineries including the dedicated transporter known as PIN. The associated transport is ordered with nearby cells driving auxin flux in similar directions. Here, we provide a model of both the auxin transport and of the dynamics of cellular polarization based on flux sensing. Our main findings are: (i) spontaneous intracellular PIN polarization arises if PIN recycling dynamics are sufficiently nonlinear, (ii) there is no need for an auxin concentration gradient and (iii) ordered multi-cellular patterns of PIN polarization are favoured by molecular noise.

1. Introduction

In plants, the initiation of different organs such as roots, leaves or flowers depends on the cues received by cells, be they from the environment or signals produced by the plant itself [1]. Among these signals, the hormone auxin plays a central role. Auxin was discovered over a half century ago along with some of its macroscopic effects on leaf and root growth [2]. It is actively transported throughout the whole plant and it is a major driver of the plant's architecture [3,4].

In the past decade, much has been learned about the molecular actors controlling auxin movement. First, cell-to-cell auxin fluxes depend on two classes of transporters [5–7]: (i) PIN (for 'PIN-FORMED'), that pumps auxin from inside to outside cells [8] and (ii) AUX1 (for 'AUXIN RESISTANT 1'), which pumps auxin from outside to inside cells. Second, auxin accumulation drives cell proliferation and differentiation. Third, cells are polarized in terms of their PIN content, that is PIN transporters localize mainly to one side of cells [9]. In addition, these polarizations are similar from cell to cell so that auxin is systematically transported along the direction of this polarization. That ordering has major consequences for the growth and morphogenesis of the plant because it affects the distribution of auxin, and auxin drives both organ growth and the initiation of new organs [10,11]. Much work has focused on how PIN polarization patterns lead to auxin distributions, but two major questions remain unanswered concerning the emergence of PIN polarization patterns: (i) how can PIN become polarized in cells in the absence of auxin gradients? (ii) Can PIN polarization patterns be coherent on the scale of many cells?

To address these questions, we take a modelling approach here, incorporating the main ingredients of what is currently known about (i) intercellular auxin transport and (ii) intracellular PIN dynamics. We will first provide a deterministic framework using differential equations for modelling the dynamics of auxin and of PIN cellular polarization. Our model exhibits multiple steady states that we characterize, the simplest ones being translation-invariant with all cells having the same PIN polarization. We find that the emergence of polarization depends on the degree of nonlinearity within the PIN recycling dynamics. We then include molecular noise in this system coming from the stochastic dynamics of PIN intracellular localization. Interestingly, for biologically

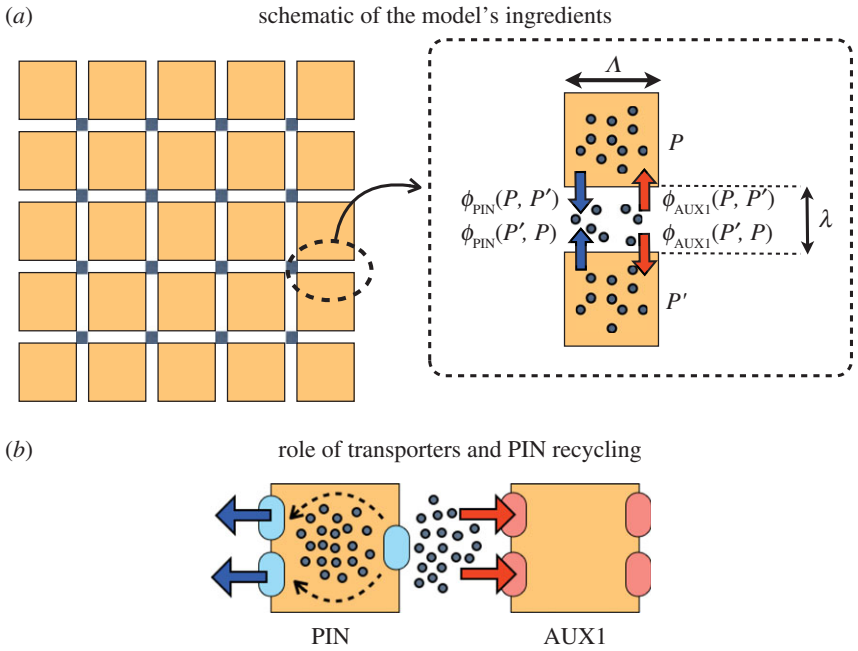

Figure 1. (*a*) Schematic two-dimensional view of the system consisting of a single layer of cells. Cubic cells of size Λ are in orange, apoplasts of width λ are in white. On the right: a zoom on two neighbouring cells. Grey circles stand for auxin and red arrows represent the incoming AUX1-mediated fluxes in cells, while light blue arrows represent the out-going PIN-mediated fluxes. In these views from above, the thickness (Λ) of the cells is not shown. (*b*) Role of transmembrane transporters. PINs pump auxin from the inside of the cell to an adjacent apoplast. AUX1 plays the reverse role, pumping auxin from apoplasts to the inside of the cell. Dashed arrows within a cell illustrate PIN recycling. (Online version in colour.)

realistic values of the parameters of the model, the system is driven into a state where cells coherently polarize in the same direction. In effect, the noise selects a robust self-organized state having homogeneous PIN polarization, corresponding to a noise-induced ordering scenario.

2. The model of auxin dynamics and PIN recycling

Auxin transport in plants is typically organized in sheets, each sheet consisting of a single layer of cells. For instance, in the case of the tips of shoots, almost all the transport arises in a single-cell sheet which is referred to as the L1 layer [12–16]. For our model, we shall therefore work with one layer of cells. We start with a lattice of cubic cells having edges of length Λ separated by apoplasts—the space between two adjacent cells—of width λ (cf. figure 1 which represents a view from above of this system). Tissues consist of closely packed cells so $\lambda \ll \Lambda$; typical values are $\lambda \approx 1\ \mu$m and $\Lambda \approx 20\ \mu$m. The hormone auxin [3] is subject to different processes:

— production and degradation inside cells, with rate constants β and ρ;
— passive diffusion within cells, within apoplasts, and also between cells and apoplasts; and
— active transport across cell membranes via transporters.

As cell membranes form barriers to exchanges of molecules, taking a molecular species from one side to the other often requires dedicated transporters. In the case of auxin, the cell membrane does allow some amount of diffusion of the hormone but much less than the inside of the cell or of the apoplast where diffusion is very rapid. We call D the associated diffusion constant (measured in $\mu m^2\ s^{-1}$) *within* the membrane of thickness ϵ, whereas formally we consider diffusion inside cells and inside apoplasts to arise infinitely quickly; as a consequence, intracellular variations of auxin concentration are negligible and so are those within an apoplast. In addition, auxin is subject to active processes that transport it across the cell membrane. Experimental evidence has shown that there are different molecular transporters for the in-going and out-going fluxes, transporters called, respectively, AUX1 and PIN [4,17]. These transporters are normally localized on the cell membrane where they can play their role to actively transport auxin between the inside and the outside of the cell. The out-going transporters belong to a large family whose members specialize to different organs and tissues of the plant [18]: in our context, we will refer to these transporters simply as PIN [19].

The dynamics of auxin concentration in each region (cell or apoplast) is specified by the transmembrane flux densities of auxin (ϕ_{AUX1} and ϕ_{PIN} for the active transport and a diffusion contribution proportional to the diffusion constant D) along with production and degradation terms. In the case of cells, we have

$$\frac{dA_c(P,t)}{dt} = \beta - \rho A_c(P,t) + \Lambda^{-1} \sum_{P'} \left[\frac{\phi_{AUX1}(P,P',t) - \phi_{PIN}(P,P',t) + D(A_a(P,P',t) - A_c(P,t))}{\epsilon} \right]. \tag{2.1}$$

In this equation, $A_c(P, t)$ is the auxin intracellular concentration of the cell centred at position $P = (x, y)$ at time t, and $A_a(P, P', t)$ is the auxin concentration in the apoplast separating nearest neighbour cells P and P'. Both concentrations will be

specified in micromoles per litre (μM for micromolar). Furthermore, only the diffusion constant of auxin *within* the cell membrane appears because it is far smaller than that within a cell or apoplast; note that the flux is proportional to the gradient, thus the factor D/ϵ, where ϵ is the thickness of the membrane. $\phi_{\mathrm{AUX1}}(P, P', t)$ and $\phi_{\mathrm{PIN}}(P, P', t)$ are the auxin flux *densities* carried by the transporters through the corresponding 'face' of cell P, i.e. the area of the membrane of cell P that faces cell P'. By convention, the sign of each flux is positive, the one for PIN going from the inside to the outside of the cell, and the one for AUX1 going from outside to inside. These flux densities have units of micromoles per second per surface area (μm^2). The sum over cells P' is restricted to the neighbours of P so in effect one sums over all sides of the cell P under consideration that connect it to the rest of the system. The parameters β and ρ are the rates of auxin production and degradation. In addition, the division by the factor Λ (the width of a cell) appears because one goes from flux densities to effects on the concentrations inside cells. Lastly, in our framework as depicted in figure 1, apoplasts connect only to cells and vice versa, so there are neither cell-to-cell nor apoplast-to-apoplast contacts.

In a similar fashion, the concentration $A_{\mathrm{a}}(P, P', t)$ of auxin in the apoplast (of thickness λ) separating cells P and P' obeys the following differential equation:

$$\frac{\mathrm{d}A_{\mathrm{a}}(P, P', t)}{\mathrm{d}t} = \lambda^{-1}\left[\frac{\phi_{\mathrm{PIN}}(P, P', t) - \phi_{\mathrm{AUX1}}(P, P', t) + \phi_{\mathrm{PIN}}(P', P, t) - \phi_{\mathrm{AUX1}}(P', P, t) + +D(A_{\mathrm{c}}(P, t) + A_{\mathrm{c}}(P', t) - 2A_{\mathrm{a}}(P, P', t))}{\epsilon}\right]. \quad (2.2)$$

Note that there is neither production nor degradation of auxin in the apoplast (it is a passive medium and auxin has a long lifetime in the absence of the active degradation processes present in cells).

In *Arabidopsis*, which currently is the most studied plant, the propensity of AUX1 influx transporters seems to be several times higher than that of passive diffusion [20,21], thus active processes are probably the main drivers of auxin distribution. Furthermore, the transporters AUX1 and PIN are believed to be completely unidirectional; the associated molecular mechanisms are unclear but involve first the binding of auxin and then conformational changes. Because these processes are analogous to enzymatic reactions, we model the associated auxin fluxes via irreversible Michaelis–Menten kinetics:

$$\phi_{\mathrm{AUX1}}(P, P', t) = \frac{N^{\mathrm{AUX1}}}{\Lambda^2}\cdot\alpha \cdot\frac{A_{\mathrm{a}}(P, P', t)}{1 + (A_{\mathrm{a}}(P, P', t)/A^{*}) + (A_{\mathrm{c}}(P, t)/A^{**})} \quad (2.3)$$

and

$$\phi_{\mathrm{PIN}}(P, P', t) = \frac{N^{\mathrm{PIN}}}{\Lambda^2}\cdot\gamma \cdot\frac{A_{\mathrm{c}}(P, t)}{1 + (A_{\mathrm{a}}(P, P', t)/A^{*}) + (A_{\mathrm{c}}(P, t)/A^{**})}, \quad (2.4)$$

where α and γ are kinetic constants analogous to catalysis rates. The factor Λ^2 on the right-hand side of these equations corresponds to the surface of the face of each cell and connects the flux *density* to the (absolute) flux. At a molecular level, N^{AUX1} (respectively, N^{PIN}) refers to the *number* of AUX1 (respectively, PIN) transporters on the area of P's membrane which faces cell P'. Finally, A^{*} and A^{**} play the role of Michaelis–Menten constants associated with saturation effects; these could have been taken to be different in equations (2.3) and (2.4) without any qualitative consequences for the behaviour of the model.

We are not aware of any experimental evidence that the distribution of AUX1 transporters changes with time or that these transporters contribute to cell polarity. Thus, we shall assume that their numbers are constant on each face of the cell. By contrast, PIN transporters are particularly important for driving morphogenesis through the formation of polarity patterns. Often they define clearly polarized fields in tissues [12,22] where cells see their PINs predominantly localized to one of their faces, with the specific face being the same for many cells. That polarity leads to coherent auxin transport, even on the scale of the whole plant, allowing in particular auxin to be transported from shoots to roots [23,24]. To take into account this possibility of intracellular polarization of PIN, we introduce the four faces of a cell as N for north, S for south, E for east and W for west. (The two faces parallel to the sheet play no role in our simplified model involving a single layer of cells.) Then each face of a cell has a potentially variable number of PIN transporters:

$$N_f^{\mathrm{PIN}}, \quad f = N, S, E, W.$$

Furthermore, we impose the constraint $\sum_f N_f^{\mathrm{PIN}} \equiv \sigma$, a cell-independent constant so each cell has the same number of PIN transporters at all times.

The dynamics of PIN seem complex: it is known that PINs are subject to 'recycling' within a cell through different mechanisms including transport from the membrane to the Golgi apparatus and back to the membrane [7,25–27]. Most modelling takes PIN dynamics to be driven by surrounding auxin concentrations [15,16,20,21,28,29]. For instance, it has been postulated that PIN might accumulate to the membrane facing the neighbouring cell with the highest concentration of auxin [15,16]. As a consequence, the presence of an auxin gradient becomes a necessary condition for PIN polarization. Here, we consider instead dynamics based on *flux sensing* where PIN recycling rates are modulated by the amount of auxin flux transported by those same PIN transporters [30,31]. Mathematically, we take the PIN dynamics on a face f ($f = N, S, E, W$) of a cell to be specified by a Hill equation of exponent h:

$$\frac{\mathrm{d}N_f^{\mathrm{PIN}}}{\mathrm{d}t} = -\frac{3}{4\tau}N_f^{\mathrm{PIN}}\frac{1}{1+(\phi_f^{\mathrm{PIN}}/\phi^{*})^{h}} + \frac{1}{4\tau}\sum_{f'}N_{f'}^{\mathrm{PIN}}\frac{1}{1+(\phi_{f'}^{\mathrm{PIN}}/\phi^{*})^{h}}. \quad (2.5)$$

Note that PINs are treated here as continuous variables because in the following the number of molecules is high so such an approximation is appropriate. Nevertheless, later we shall treat the actual numbers via our stochastic model. The above differential equations model the competitive recycling of the PINs among the faces of a given cell in a flux-dependent manner. Note that τ is the characteristic timescale of the recycling process and that the dynamics enforce the constraint of conservation of the total number of PIN transporters inside

each cell. Also, in equation (2.5), ϕ_f^{PIN} is the flux density through face f, while ϕ^* is a Michaelis–Menten-like constant.

To understand the consequences of equation (2.5), consider first the low flux limit, ϕ_f^{PIN} being small for all faces, so that all denominators can be ignored. At a molecular level, each transporter leaves the cell membrane at a rate proportional to τ^{-1}; transporters are then brought into the cytoplasm or Golgi apparatus; finally, they get reallocated randomly to any of the four faces. In such a low flux regime, cells will show no PIN polarization. Second, consider instead the case where for at least one face f, $\phi_f^{\mathrm{PIN}}/\phi^*$ is large. That face will *benefit* from the recycling, recruiting more transporters from the other faces than it loses. (ϕ^* is just the scale at which flux sensing in this system becomes important.) At an individual transporter level, the competitive recycling means that transporters which are actively shuttling auxin see their rate of recycling go down. How this happens depends on unknown molecular details, nevertheless, the rate of detachment of a transporter from the membrane probably depends on what fraction of the time it is binding auxin and thus the rate of detachment will show a dependence on ϕ_f^{PIN}. One may attempt to model this via a simple hyperbolic law to describe saturation effects. To be more general, we have introduced a Hill exponent, h, into the dynamics as given in equation (2.5). Such a functional form is often used in the kinetic modelling of binding processes; in that framework, h is an integer related to the number of molecules that must co-localize, and as such, it reflects cooperative effects. In the absence of detailed knowledge of the molecular mechanisms controlling PIN recycling, we use this phenomenological form where h is associated with the nonlinearity of the PIN recycling dynamics and we will see whether or not h plays an important role. In the electronic supplementary material, we will see that our conclusions are insensitive to the precise form of the equations describing PIN recycling by replacing the Hill form with a stretched exponential.

The model is now completely specified and involves the 15 parameters Λ, λ, ϵ, α, β, γ, ρ, A^*, A^{**}, ϕ^*, τ, σ, N^{AUX1}, D and h. Some of these parameters can be absorbed in scale changes. Nevertheless, to keep the physical interpretation as transparent as possible, we stay with the dimensional form of the equations. Whenever possible, we assign values to the parameters using published estimates or compilations thereof [20,21,32]. For instance, mass-spectrometry measurements [33] in very young leaves quantify the concentration of auxin to be about 250 pg mg^{-1} of tissue. As the molecular mass of auxin is about 175 Da, A_c is of the order of 1 µM. As we shall see, in the steady-state regime, $\beta/\rho = A_c$, a relation providing a constraint on those two parameters. Furthermore, a direct estimate of β follows from isotopic labelling measurements [33] which show that biosynthesis replenishes auxin within about 1 day; we have thus set $\beta = 1/\mathrm{day}$. Radioactive labelling has also provided estimates for mean displacement velocities of auxin [20,21] which we have used to constrain the parameters α and γ. Unfortunately, for other parameters (and in particular the Michaelis–Menten constants), no direct or indirect estimations from experimental data are available. For most such cases, we use ballpark estimations that seem reasonable, for instance 100 PIN molecules seems too low, while 10^4 is perhaps on the high side. However, for h, which provides a phenomenological parametrization of nonlinear effects in PIN recycling, we have little choice but to study the behaviour of the model as a function of its value. We use the same strategy for D. Thus, both D and h will be used as control parameters, allowing us to map out a two-dimensional phase diagram. For instance, when increasing D, passive diffusion will overcome the effects of active transport, allowing one to probe the importance of active versus passive transport in the establishment of PIN polarization. Unless specified otherwise, all other parameter values are set as provided in table 1.

Table 1. Parameters used in the model. M, molarity (i.e. number of moles per litre); l, litre.

β	1 µM d^{-1}
ρ	1 d^{-1}
α	0.1 l s^{-1}
Γ	10^{-4} l s^{-1}
A^*	2×10^{-3} µM
A^{**}	0.8 µM
ϕ^*	4×10^{-6} moles µm^{-2} s^{-1}
N^{AUX1}	200 per face
σ	1000
Λ	20 µm
λ	1 µm
ϵ	10 nm
τ, τ_{1D}	30 min

As our aim is to understand how ordered polarity patterns arise in a system described by this model, it is appropriate to define an order parameter to quantify the ordering of flux directions or PIN intracellular localization. We thus introduce the two-dimensional *polarization* vector $\boldsymbol{\delta}$ for a cell at position $P = (x, y)$; its components depend on the face-to-face difference of the number of PINs along each direction (say horizontal or vertical) in the following way:

$$\boldsymbol{\delta}(x,y) \equiv \begin{cases} \delta_1(P) = \dfrac{N_E^{\mathrm{PIN}}(P) - N_W^{\mathrm{PIN}}(P)}{\sigma} \\ \delta_2(P) = \dfrac{N_N^{\mathrm{PIN}}(P) - N_S^{\mathrm{PIN}}(P)}{\sigma}. \end{cases} \tag{2.6}$$

The vector in equation (2.6) has a length $|\boldsymbol{\delta}(P)| \in [0, 1]$: the two extreme values represent, respectively, the unpolarized case, i.e. $N_f^{\mathrm{PIN}} = \sigma/4$ for all f, and the fully polarized case, i.e. $N_f^{\mathrm{PIN}} = \sigma$ for one face while $N_f^{\mathrm{PIN}} = 0$ for all other faces. Individual components can vary in $[-1, 1]$ and the extreme points give the maximum polarization in one direction or the other.

A first step will consist of understanding the behaviour of this system in a one-dimensional framework.

3. Analysis of the one-dimensional model

3.1. Dynamical equations

Let us replace the square lattice represented in figure 1 by a *row* of cubic cells forming a one-dimensional lattice. As before, between two adjacent cells, there is exactly one apoplast. In this one-dimensional model, all diffusion and transport is horizontal and PIN is defined only on the left (West) and right (East) face of each cell. The dynamics of A_c and A_a in each

cell are obtained from equations (2.1) and (2.2) by setting the vertical fluxes to 0. Given the constraint of conservation of total PIN transporters in each cell and the fact that only two faces contribute, the dynamics of PIN numbers are completely determined via the dynamics of N_E^{PIN} and there is just one independent equation for each cell:

$$\frac{\mathrm{d}N_E^{\mathrm{PIN}}}{\mathrm{d}t} = -\frac{1}{\tau_{1D}} N_E^{\mathrm{PIN}} \frac{1}{1+\left(\phi_E^{\mathrm{PIN}}/\phi^*\right)^h} + \frac{1}{\tau_{1D}} N_W^{\mathrm{PIN}} \frac{1}{1+\left(\phi_W^{\mathrm{PIN}}/\phi^*\right)^h}, \tag{3.1}$$

where $\tau_{1D} = 4\tau/3$ and $N_W^{\mathrm{PIN}} = \sigma - N_E^{\mathrm{PIN}}$. Furthermore, polarization is no longer a vector but a scalar, given by the first component of equation (2.6). It varies in $[-1, 1]$: when $\delta(x) \approx -1$, almost all the PINs are on the left-hand side of the cell, while when $\delta(x) \approx 1$ they are almost all on the right-hand side.

3.2. Steady-state auxin concentrations given translation-invariant PIN configurations

Assuming periodic boundary conditions, the row of cells becomes a ring; this idealization is convenient for the mathematical and phase diagram analysis. Consider the steady-state solutions of the differential equations. With periodic boundary conditions, one expects some steady states to be translationally invariant. In that situation, all quantities are identical from cell to cell and from apoplast to apoplast. We can then drop all time and spatial dependence in the variables, e.g. $A_c(P, t) = A_c$ for all P and t.

We consider here an arbitrary translation-invariant configuration of PIN transporters (steady-state or not), which implies that the auxin equations will depend only on the total number of transporters per cell. One then has the following equations for steady-state auxin concentrations:

$$\begin{cases} 0 = \beta - \rho A_c + \dfrac{2D}{\Lambda\epsilon}(A_a - A_c) + \dfrac{2\alpha}{\Lambda^3} N^{\mathrm{AUX1}} \dfrac{A_a}{1+(A_a/A^*)+(A_c/A^{**})} - \dfrac{\gamma}{\Lambda^3}\sigma \dfrac{A_c}{1+(A_a/A^*)+(A_c/A^{**})} \\ 0 = \dfrac{2D}{\lambda\epsilon}(A_c - A_a) - \dfrac{2\alpha}{\lambda\Lambda^2} N^{\mathrm{AUX1}} \dfrac{A_a}{1+(A_a/A^*)+(A_c/A^{**})} + \dfrac{\gamma}{\lambda\Lambda^2}\sigma \dfrac{A_c}{1+(A_a/A^*)+(A_c/A^{**})}. \end{cases} \tag{3.2}$$

These two equations determine A_c and A_a. One can first solve for A_c by noting that in apoplasts there is no source or degradation of auxin, thus in the steady state the total flux (transport and diffusion counted algebraically) through an apoplast vanishes and so the same holds for cells. Therefore, within cells auxin degradation must compensate exactly auxin production, leading to $\beta - \rho A_c = 0$. This result, namely $A_c = \beta/\rho$, is independent of all other parameters and in particular of the PIN polarization and of D as illustrated in figure 2. Furthermore, with A_c determined in this way, the two equations become equivalent and can be solved for A_a.

Experimental evidence [20,21] suggests that active transport dominates passive (diffusive) transport in *Arabidopsis*. Thus the biologically relevant regime probably corresponds to D small. In the low-diffusion limit ($D \to 0$), the last equation shows that A_a goes to a limiting value that is strictly positive. (A zoom of figure 2 would also show this is the case.) Solving this equation using the values of the parameters in table 1, one finds that the concentration of auxin in cells is much greater than that in apoplasts because $2N^{\mathrm{AUX1}}\alpha \gg \sigma\gamma$, i.e. auxin molecules are more easily transported by AUX1 than by PIN (cf. the left limit in figure 2). One may also consider what happens when diffusion is important; clearly as $D \to \infty$, the transporters become irrelevant and the equations immediately show that A_c and A_a become equal. The overall behaviour is displayed in figure 2.

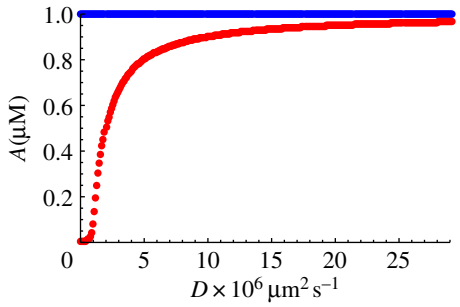

Figure 2. Auxin steady-state concentrations (red for apoplasts and blue for cells) in an arbitrary PIN translation-invariant configuration as a function of the diffusion constant D in $\mu\mathrm{m}^2\,\mathrm{s}^{-1}$. The other relevant model parameters are given in table 1. (Online version in colour.)

3.3. Translation-invariant dynamics of PIN in the quasi-equilibrium limit for auxin

Microscopic molecular events associated with auxin transport (be they active or passive) arise on very short timescales, whereas PIN recycling requires major cellular machinery and so arises on much longer timescales. Let us therefore take the *quasi-equilibrium* limit where auxin concentrations take on their steady-state values A_c and A_a. Consider now the dynamical equation for δ, the PIN polarization. As it involves a single variable, it can always be written as gradient descent relaxational dynamics, i.e. there exists a function $\mathcal{F}(\delta)$ such that

$$\frac{\mathrm{d}\delta}{\mathrm{d}t} = -\frac{1}{\tau_{1D}} \frac{\mathrm{d}\mathcal{F}(\delta)}{\mathrm{d}\delta}. \tag{3.3}$$

$\mathcal{F}(\delta)$ plays the role of an effective potential. $\mathcal{F}(\delta)$ is minus the integral of a known function of δ; this integral can be obtained in closed form in terms of hypergeometric functions (see the electronic supplementary material).

The extrema of $\mathcal{F}$ correspond to steady states for the PIN dynamics, i.e. $\mathrm{d}\delta/\mathrm{d}t = 0$. Maxima are unstable and minima are stable. Thus it is of interest to map out the form of $\mathcal{F}$ as a function of the parameter values. Take for instance $h = 2$. Starting with a large value for D, use of Mathematica shows that $\mathcal{F}$ has a single global minimum, corresponding to the unpolarized steady state, $\delta = 0$. Then as D is lowered (the

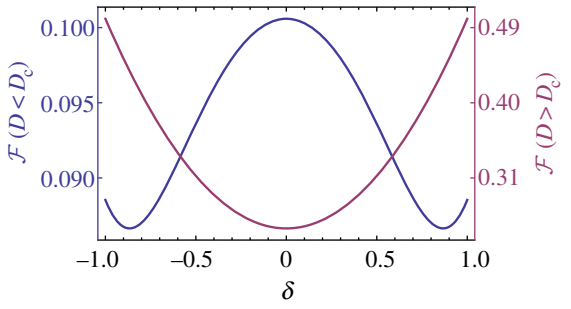

Figure 3. Effective potential as a function of the polarization δ for $D > D_c$ (purple) and $D < D_c$ (blue) rescaled with σ^2. $h = 2$, other parameter values are given in table 1. (Online version in colour.)

Mathematica code of the electronic supplementary material provides the user with a knob to change D), $\mathcal{F}$ takes on a double-well shape, symmetric about the zero polarization abscissa where the curvature is now negative. At the same time, two new local minima appear at $\pm\delta^*$. Thus as D is lowered, the unpolarized state becomes unstable while two new stable steady states of polarization $\pm\delta^*$ appear; this situation is illustrated in figure 3. If we had used instead $h = 0.5$, we would have found no regime in D where $\mathcal{F}$ has the double-well structure: there, the only steady state is the unpolarized one.

Within this framework, it is possible to determine a critical point D_c, i.e. a threshold value of the diffusion constant, below which spontaneous symmetry breaking sets in. The value of D_c is obtained from the following condition (see also the electronic supplementary material):

$$\frac{\partial^2 \mathcal{F}}{\partial \delta^2}|_{\delta=0} = 0. \tag{3.4}$$

In particular, for $h = 2$, this leads to a critical value $D_c \approx 9.4 \times 10^{-7}\ \mu m^2\ s^{-1}$. This overall framework provides a convenient intuitive picture for PIN dynamics.

3.4. Spontaneous symmetry breaking and phase diagram for translation-invariant steady states

The previous formalism is complicated because of the form of $\mathcal{F}$. However, if one is only interested in the steady states and one does not care about relaxational dynamics, the steady-state equation to solve is relatively simple (cf. equation (3.1) where the left-hand side must be set to 0). We assume as before that N_E^{PIN} is translation-invariant but also that it is subject to PIN recycling. As A_c and A_a in steady states have been previously calculated, all quantities in equation (3.1) are known except for N_E^{PIN}; it is enough then to solve the associated nonlinear equation (we have used Mathematica for this purpose). At given h that is not too small, we find a transition from polarized to unpolarized states as D crosses the threshold D_c (see figure 4*a* which illustrates the case $h = 2$). The position of the threshold depends on h. However, if h is too small, the transition point disappears, and there are no longer any polarized steady states. To illustrate the situation, consider fixing D to a small value, say $D = 10^{-7}\ \mu m^2\ s^{-1}$, and then solve for N_E^{PIN} as a function of the Hill exponent h. For h less than a critical threshold $h_c \approx 1.09$, there is a unique solution and it corresponds to the unpolarized state, $N_E^{PIN} = N_W^{PIN} = \sigma/2$. For $h > h_c$, two new steady states appear which are polarized. These two states are related by the left–right symmetry, so there is a spontaneous symmetry breaking transition at h_c (figure 5). As $h \to \infty$, these states tend towards full polarization, $\delta = \pm 1$. To represent simultaneously the behaviour as a function of the diffusion constant D and of the Hill exponent h, figure 6*a* provides the overall phase diagram via a heat map. Note that when h is too low *or* D is too high, the only steady state is the unpolarized one.

The origin of this spontaneous symmetry breaking is the change of stability of the unpolarized state. To quantitatively understand that phenomenon, set $N_E^{PIN} = \sigma/2 + \delta/2$ and then linearize equation (3.1) in δ. Defining $F = \gamma A_c \sigma/[2(1 + A_a/A^* + A_c/A^{**})\Lambda^2 \phi^*]$ (this is independent of polarization but varies with D because of its dependence on A_a), one has $\phi_E^{PIN}/\phi^* = ((1+\delta)/\sigma)F$ and $\phi_W^{PIN}/\phi^* = ((1-\delta)/\sigma)F$. Then the linearization in δ leads to

$$\tau \frac{d\delta}{dt} = -2\left[\frac{1-(h-1)F^h}{(1+F^h)^2}\right]\delta. \tag{3.5}$$

Instability arises if and only if $(h-1)F^h > 1$. Note that the case of Michaelis–Menten-type dynamics ($h = 1$) therefore does *not* lead to PIN polarization. To have spontaneous polarization inside cells, the nonlinearity must be strong enough. The mathematical condition is $h > h_c = 1 + F^{-h_c}$, where h_c is the critical Hill exponent where the instability sets in. This demonstrates the essential role of the nonlinearity parametrized here by h. Of course other forms of nonlinearity can be expected to lead to similar conclusions. In particular, we have found that the same qualitative behaviour arises when using stretched exponentials rather than Hill functions (see the electronic supplementary material). We thus conclude that, in general, the spontaneous polarization of PIN is driven by the strength of the nonlinearity parametrizing PIN recycling dynamics.

One may also investigate the stability of the polarized steady state. First, within the space of translation-invariant configurations, a linear stability analysis using Mathematica shows that the polarized state is always linearly stable. This is exactly what the adiabatic approximation predicts (cf. figure 3). Second, one can ask whether our translation-invariant steady states are *global* attractors when they are linearly stable. We have addressed this heuristically by simulating the dynamical equations starting from random initial conditions. When $h \leq h_c$ (or $D \geq D_c$ if one considers h as fixed), it seems that the unpolarized state is the *only* steady state and that all initial conditions converge to it. When $h > h_c$, the system always seems to go to a steady state: we have never observed any oscillatory or chaotic behaviour. Sometimes the steady states are the previously found translation-invariant polarized states but sometimes they are not, and contain cells with opposite signs for the PIN polarization. This situation is much like what happens when quenching the Ising model where there is a proliferation of such disordered states. In the electronic supplementary material, we characterize some of these non-translation-invariant steady states. The main conclusion to draw from the arguments gathered there is that as one approaches D_c the number of steady states diminishes. Furthermore, one expects that this effect is accompanied by a reduction in both stability and size of basin of attraction of steady states having defects, leading to an increase in the coherence length (or domain sizes where a domain is a block of cells having the same sign of polarization) as one approaches D_c. Such properties naturally lead one to ask whether noise might enhance the coherence of

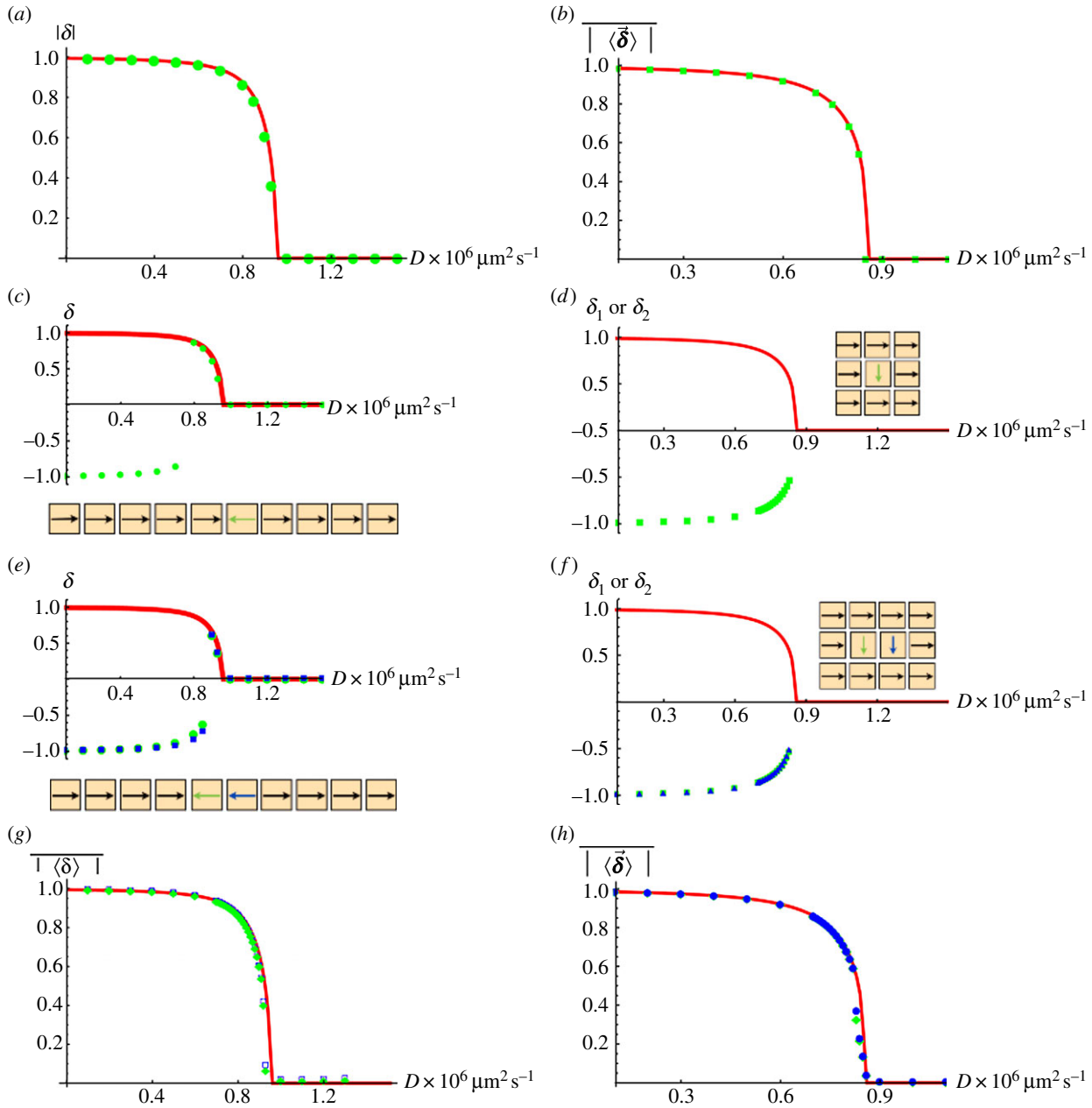

Figure 4. (*a*,*b*) Absolute value of the (translation-invariant) PIN polarization as a function of the diffusion constant D in $\mu m^2\ s^{-1}$ in steady states, respectively, for the one- and the two-dimensional models. Red line: analytical result obtained using Mathematica. Green circles: results of simulating the dynamics of the model containing, respectively, 20 cells on a ring and 20 × 20 cells on a lattice until a steady state was reached; a fourth-order Runge–Kutta algorithm [34] was used and starting configurations were randomized but had positive local PIN polarizations. (*c*–*f*) PIN polarization at a defect (green and blue) and in the absence of a defect (red) for a ring of 20 cells as a function of D for the one-dimensional case (*c*,*e*) and a lattice of 20 × 20 cells for the two-dimensional case (*d*,*f*). Drawings (below and insets): initial orientation of PIN polarizations; the green and blue arrows represent the defects. (*g*,*h*) Absolute value of the mean PIN polarization per site, averaged over time, as a function of the diffusion constant for the stochastic model for three different ring/lattice sizes (in (*g*), $N_{cells} = 20$ green diamonds, $N_{cells} = 10$ blue squares, while in (*h*) $N_{cells} = 5$ blue circles, $N_{cells} = 10$ green diamonds) and for the deterministic model (red line). Simulations were performed using cells on a ring/lattice. D_c is slightly lower when using stochastic dynamics. In all the plots, $h = 2$ while other parameter values are given in table 1. In (*g*), $\tau = 1$ s. (Online version in colour.)

polarization patterns, driving the emergence of order from disorder [35].

3.5. Properties of the stochastic model

As the number of PIN transporter molecules in our system is modest, noise in the associated dynamics may be important. Thus in this section, we reconsider the system by using a stochastic framework where each individual PIN transporter can move from one face to another according to probabilistic laws. The parameters of those laws are known via the fluxes in the deterministic model: these fluxes give the *mean* number of such PIN recycling events per unit time. To study the stochastic model, we simulate these random events from which we can extract the average properties arising in the presence of such molecular noise. (See the electronic supplementary material for implementation details.)

The stochastic dynamics are ergodic, so given enough time the system will thermalize, there being a unique 'thermodynamic equilibrium state'. Although in principle, this state depends on the value of τ_{1D}, if auxin concentrations are close to their steady-state values which is the case here, τ_{1D} just introduces a timescale and has no effect on the equilibrium state. We use simulations to study the equilibrium, with a particular focus on the behaviour of PIN polarization. Observables must be averaged over time. Just as in other thermodynamical

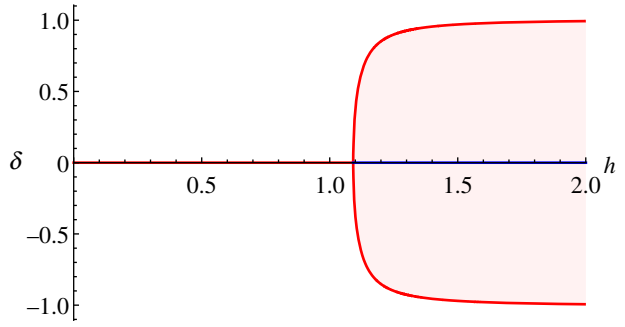

Figure 5. Bifurcation diagram for translation-invariant states in the one-dimensional model. δ is the PIN polarization. The unpolarized state is stable for $h < h_c \approx 1.09$ (orange). Beyond that threshold, two symmetric polarized states appear. These are stable (in red), whereas the unpolarized state becomes unstable (in blue). Here, $D = 10^{-7}\ \mu m^2\ s^{-1}$ while other parameter values are given in table 1. (Online version in colour.)

systems having spontaneous symmetry breaking, care then has to be used when extracting the order parameter. We thus measure the mean PIN polarization defined by first averaging δ over all cells to obtain $\langle\delta\rangle$, then taking the absolute value, $|\langle\delta\rangle|$, and then averaging over simulation time: $\overline{|\langle\delta\rangle|}$.

In figure 4*g*, we show the mean polarization thus defined as a function of D for systems having 10 and 20 cells. At low D, the analysis of the model in the absence of noise suggested that the system will not polarize coherently because the typical noiseless steady state had random polarizations (cf. §3.4). Nevertheless, here we see that, in the presence of noise, the system seems to have a global polarization, in agreement with the order from disorder scenario [35]. If one refers to the special translation-invariant steady state in the absence of noise, it seems at low D that the presence of noise leads to almost exactly the same value of the order parameter, so noise can be thought of as 'selecting' that particular ordered state. As D grows, polarization intensity decreases and noise effects are amplified. As might have been expected, polarization is lost earlier in the presence of noise than in its absence.

Figure 4*g* could be interpreted as suggesting that the equilibrium state in the stochastic model has a real transition between a polarized phase and an unpolarized one. However, one has to bear in mind that for a system containing a large enough numbers of cells the equilibrium state will in fact contain multiple domains of polarization, some being oriented in one direction and others in the opposite direction. This is inevitable in any one-dimensional system having short-range interactions [36,37], and so no true long-range order arises in this system if the number of cells is allowed to be arbitrarily large. To add credence to this claim, note that the polarization curves are slightly different for the different lattice sizes, the polarization *decreasing* as the number of cells increases. It is thus plausible that in the limit of an infinite number of cells, the polarization vanishes for all D.

4. Analysis of the two-dimensional model

4.1. Steady-state auxin concentrations given translation-invariant PIN configurations

In two dimensions, we again begin by considering auxin steady-state concentrations in the presence of translation-invariant PIN configurations. Auxin concentrations are then also translation-invariant, but compared to the one-dimensional case, vertical and horizontal apoplasts need not have the same concentrations of auxin. We denote these concentrations as A_a^N and A_a^W.

In all steady states, the total rate of auxin production must be compensated by the total rate of auxin degradation. This immediately gives $A_c = \beta/\rho$ just like in the one-dimensional model. In addition, A_a^W is determined by the equation

$$0 = 2D(A_c - A_a^W) - 2\alpha N^{\mathrm{AUX1}} \frac{A_a^W}{1 + (A_a^W/A^*) + (A_c/A^{**})} + \gamma\sigma^E \frac{A_c}{1 + (A_a^W/A^*) + (A_c/A^{**})}, \tag{3.6}$$

where $\sigma^W = N_E^{\mathrm{PIN}} + N_W^{\mathrm{PIN}}$. A^N is determined by the analogous equation in which the index W is replaced by N and $\sigma^N = N_N^{\mathrm{PIN}} + N_S^{\mathrm{PIN}}$. Thus, in contrast to the one-dimensional case, the concentration of auxin in apoplasts depends not only on model parameters like D but also on PIN polarization. Unpolarized configurations lead to $\sigma^W = \sigma^N = \sigma/2$ and then $A_a^N = A_a^W$, in which case the equations take the same form as in one dimension.

The lowest and highest possible values of A_a^W arise when $\sigma^W = 0$ and $\sigma^W = \sigma$, respectively. These lower and upper bounds are represented in figure 7 along with the value of A_c as a function of D. Clearly, auxin concentrations are hardly affected at all by PIN polarization. Furthermore, both qualitatively and quantitatively, the situation is very close to that in the one-dimensional model.

4.2. Translation-invariant dynamics of PIN in the quasi-equilibrium limit for auxin

In the one-dimensional model, we saw that translation-invariant dynamics of PIN polarization followed from a potential energy function when auxin was assumed to be in the quasi-equilibrium state. In two dimensions, there are four dynamical variables which satisfy the conservation law $N_E^{\mathrm{PIN}} + N_W^{\mathrm{PIN}} + N_N^{\mathrm{PIN}} + N_S^{\mathrm{PIN}} = \sigma$. Each N_f^{PIN} obeys a first-order differential equation; the question now is whether these follow from a potential energy function $\mathcal{F}$:

$$\frac{\tau \mathrm{d}N_f^{\mathrm{PIN}}}{\mathrm{d}t} = -\frac{\partial \mathcal{F}}{\partial N_f^{\mathrm{PIN}}}. \tag{3.7}$$

The answer is negative: no potential exists because the velocity field has a non-zero curl. Nevertheless, if in the initial conditions, the PINs obey the symmetry $N_N^{\mathrm{PIN}} = N_S^{\mathrm{PIN}}$ (or the symmetry $N_E^{\mathrm{PIN}} = N_W^{\mathrm{PIN}}$), then this symmetry is preserved by the dynamics. (Note that the symmetry is associated with reflecting the system of cells about an axis.) Then one sees that the differential equations for the two other PIN numbers are nearly identical to those in the one-dimensional model. For instance, if $N_N^{\mathrm{PIN}} = N_S^{\mathrm{PIN}}$, the equation for N_E^{PIN} is that of the one-dimensional model if one substitutes σ by σ^W. The difficulty is that σ^W itself follows from solving the differential equations and thus can depend on time. Although one does not have a true potential energy function, the important property is that the instantaneous rate of change of N_W^{PIN} can be mapped to its value in the one-dimensional model via the aforementioned substitution. We thus expect to have the same kind of spontaneous symmetry breaking where the unpolarized steady state goes from being stable at low

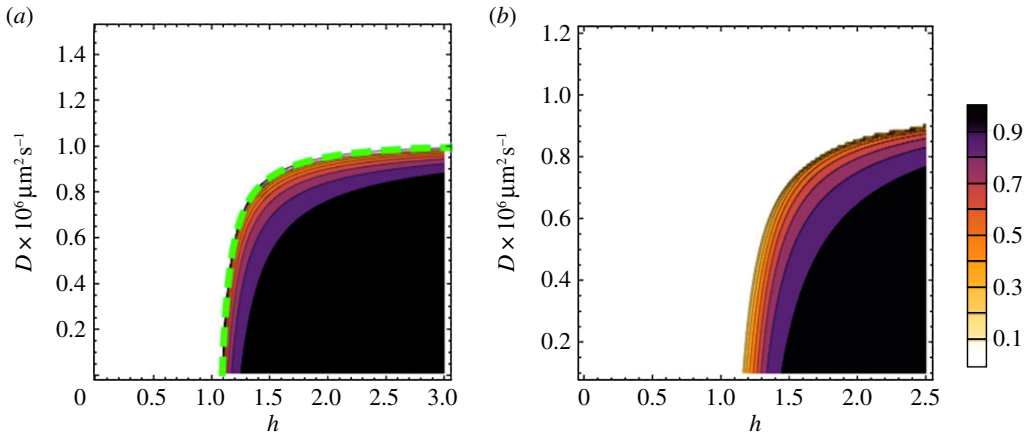

Figure 6. Heat map of PIN polarization in translation-invariant steady states as a function of h (dimensionless) and D in $\mu m^2\ s^{-1}$ scaled by a factor 10^6 for better readability. Other parameter values are given in table 1. (*a*) One-dimensional model. The green dashed line refers to the theoretically derived critical line. (*b*) Two-dimensional model. (Online version in colour.)

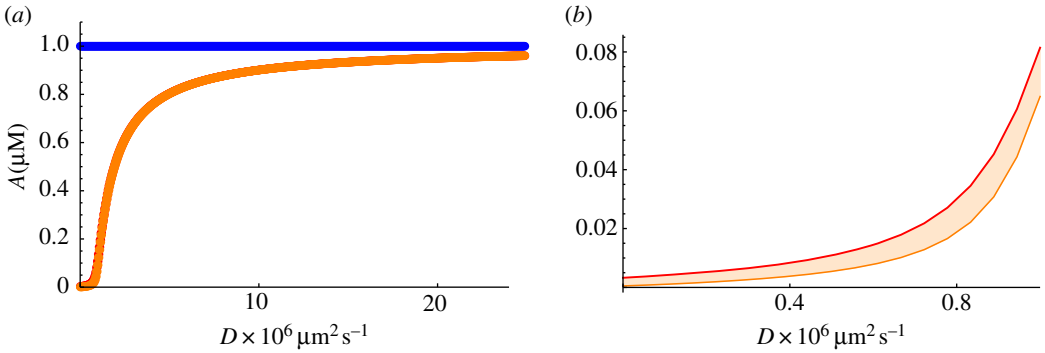

Figure 7. (*a*,*b*) Steady-state auxin concentrations in translation-invariant PIN configurations as a function of the diffusion constant D. On the left: in blue, the concentration within cells. On the left and via a zoom on the right: in red and orange, the minimum and maximum values of auxin concentration within apoplasts, arising when the number of PIN transporters on the faces of the apoplast takes on its minimum and maximum value. $h = 2$, other parameter values as in table 1. (Online version in colour.)

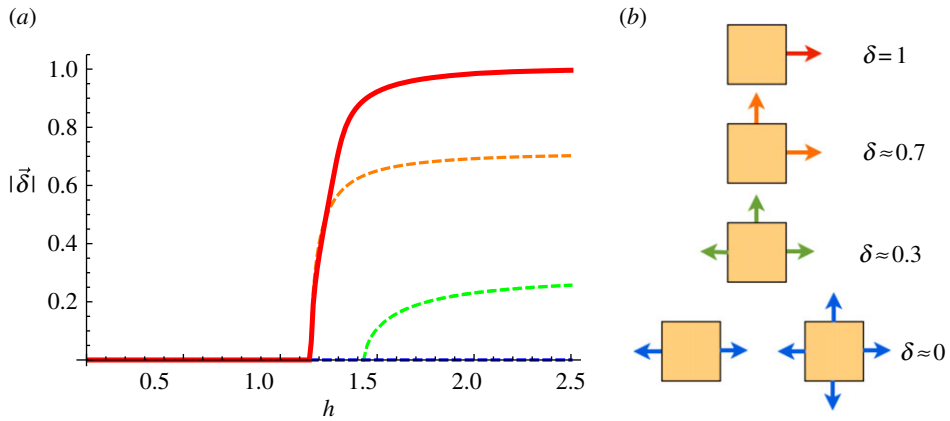

Figure 8. (*a*) $|\vec{\boldsymbol{\delta}}|$ in translation-invariant steady states as a function of the Hill exponent h in the low-diffusion regime ($D = 10^{-7}\ \mu m^2\ s^{-1}$). Stable steady states are shown in red, the others are linearly unstable. (*b*) For each type of steady state, we show the corresponding PIN configurations along with the norm of δ in the limit of large h. (Online version in colour.)

h to being unstable at high h, with an associated appearance of stable polarized steady states.

4.3. Spontaneous symmetry breaking and phase diagram for translation-invariant steady states

To determine the translation-invariant steady states, one must solve six simultaneous nonlinear equations, two of which give A_a^W and A_a^N in terms of the N_f^{PIN}, the other four being associated with PIN recycling. We tackle this task using Mathematica.

Qualitatively, one obtains the same behaviour as in the one-dimensional model. As displayed in figure 4*b*, there is a continuous transition between a polarized state at low D and an unpolarized state at large D.

Equivalently, for low values of h there is only one steady state and it is unpolarized (cf. figure 8). Increasing h, there is spontaneous symmetry breaking at a first threshold where

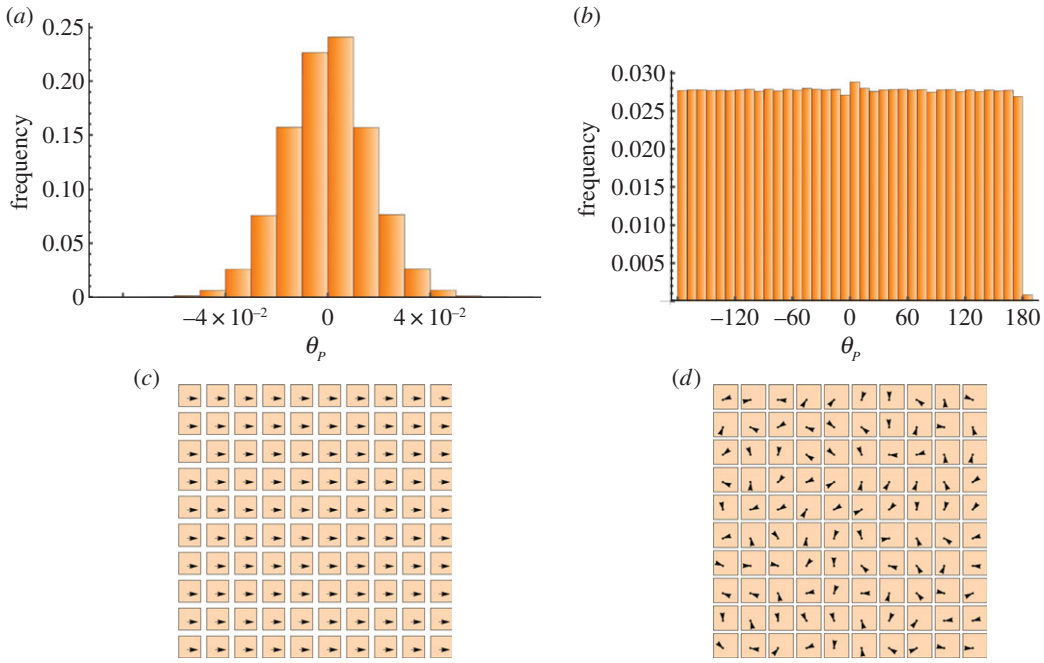

Figure 9. (*a*,*b*) Histograms of the lattice-wide polarization angle, θ_P, in degrees, for the regimes of low (*a*) and high (*b*) *D*, accumulated during a simulation of the model using a 10 × 10 lattice. (*c*,*d*) Typical equilibrium configurations in those same two regimes. (*c*) $D = 10^{-7}$ μm^2 s^{-1}. (*d*) $D = 10^{-6}$ μm^2 s^{-1}. (Online version in colour.)

the unpolarized state becomes unstable and a new polarized steady state appears. Cells in that polarized state have a large number of PIN transporters on one face and no polarization in the perpendicular direction. Because of this last property, the system effectively behaves as a stack of rows which do not exchange auxin, each row being like the polarized one-dimensional system. Surprisingly, a second spontaneous symmetry breaking transition arises at a very slightly larger value of *h* and even a third still beyond that. The associated translation-invariant steady states behave as illustrated in figure 8. However, these spurious states are always linearly unstable and so will not be considered further.

To get a global view of the behaviour as a function of both *D* and *h*, we present via a heat map the complete phase diagram in figure 6*b* where the norm of the polarization vector is given only for the (unique) stable (and translation-invariant) steady state.

4.4. Properties of the stochastic model

The method of introducing molecular noise into the dynamical equations is oblivious to the dimensionality of the model. Thus each dynamical equation can be rendered stochastic for the two-dimensional model without any further thought by following the procedure outlined above for the one-dimensional case. We can then use this to study the thermodynamic equilibrium state. Once equilibration was observed, we measured the average polarization vector $\langle \boldsymbol{\delta} \rangle$, the average $\langle \cdot \rangle$ being taken over the whole lattice at one specific time. We also define θ_P as the angle of that averaged vector, $\tan(\theta_P) = \delta_2/\delta_1$. In the low *D* regime, the cells stay highly polarized and are oriented close to a common direction along one of the axes of the lattice. This situation illustrated in figure 9 where we also show the distribution of θ_P over the time of the simulation. On the contrary, for 'high' *D*, PINs tend to distribute quite evenly among the faces of a cell and this leads to a relatively flat histogram for the values of θ_P (figure 9). However, this histogram is slightly misleading because the polarization vectors $\langle \boldsymbol{\delta} \rangle$ have a very small magnitude and in effect each cell is essentially depolarized.

Just as in the one-dimensional case, one may ask whether there is a true transition from a globally polarized state to an unpolarized state when *D* goes from low to high values. A naive way to do so would be to average $\langle \boldsymbol{\delta} \rangle$ over the length of the simulation. However, because the dynamics is ergodic, this average should vanish in the limit of a long run. The same difficulty arises in all systems that undergo spontaneous symmetry breaking. It is necessary to first take the norm of $\langle \boldsymbol{\delta} \rangle$, then average over time and finally check for trends with the size of the lattice. In figure 4*h*, we show this time average, $\overline{|\langle \boldsymbol{\delta} \rangle|}$, as a function of *D* for lattices of different sizes. For comparison, we also show the corresponding curve in the absence of noise.

The behaviour displayed is compatible with a true ordering transition as might be expected from the analogy with the behaviour of the Ising model. Such a behaviour is also in agreement with the noise-induced ordering scenario [38] and related phenomena [35].

5. Conclusion

Although auxin transport in meristematic tissues (roots, shoots and cambium) has been actively studied in the past decade while associated molecular actors have been identified, the questions of how intracellular PIN polarization arises and how globally coherent polarization patterns emerge have not been sufficiently addressed. Our work is based on modelling both auxin transport across cells and PIN recycling within individual cells. The dynamics we use for PIN recycling is modulated by an auxin flux-sensing system. Such recycling allows PIN transporters to move within a cell from one face to another. The PINs can accumulate on one face if there is a feedback which allows such a polarized state to maintain

itself. Given this framework and estimates for a number of model parameter values, we mapped out a phase diagram giving the behaviour of the system in terms of specific parameters. The one-dimensional model, describing a row of cells in a plant tissue, allowed for large-scale PIN polarization in the absence of any auxin gradient. Furthermore that toy limit was analytically tractable and correctly described all the features arising in the two-dimensional model. The detailed analysis revealed a particularly essential ingredient: PIN polarization requires a sufficient level of *nonlinearity* in the PIN recycling rates. In terms of our mathematical equations, this nonlinearity was parametrized by the Hill exponent h appearing in equation (2.5), which is associated with cooperativity in the field of enzyme kinetics. If Michaelis–Menten dynamics is used (corresponding to $h = 1$ and thus no cooperativity), the system always goes to the unpolarized state. On the other hand, when h rises above a threshold h_c, the homogeneous unpolarized state becomes unstable and polarized PIN patterns spontaneously emerge. We showed that the same qualitative behaviour occurs when using nonlinearities based on stretched exponentials rather than Hill equations (cf. electronic supplementary material). That result shows that our model's predictions are robust to changes in assumptions about the dynamical equations.

In addition, by studying the feedback between auxin concentrations and PIN recycling, we showed that nearby cells tend to polarize in the same direction. Another particularly striking result found was that the molecular noise in the PIN recycling dynamics seems to impose long-range order on the PIN polarization patterns. This 'noise-induced ordering' could be the mechanism driving the ordering found for instance in the cambium, ordering that can span tens of metres in the case of trees.

Given that these conclusions follow from our hypothesis that PIN recycling is based on flux sensing, experimental investigations should be performed to provide stringent comparisons with the predictions of our model. The most direct test of our hypothesis would be to determine whether cells depolarize when the auxin flux carried by PINs is suppressed. In *Arabidopsis*, the polarization of PIN can be observed thanks to fluorescent PIN transporters so what needs to be done is to apply a perturbation affecting auxin flux. One simple way to achieve this is to inject auxin into an apoplast; the associated increase in auxin concentration will likely inhibit PIN transport into that apoplast. If such an injection cannot be performed without mechanically disrupting the cell membranes, a less invasive manipulation could be obtained if the AUX1 transporters can be modified so that they may be locally photo-inhibited. Exposure to a laser beam would then prevent the auxin from leaving a given apoplast, followed by a rapid increase in auxin concentration just as in the simpler experiment previously proposed. In both cases, our model predicts that the PIN recycling dynamics would lead to depolarization of the cell polarized *towards* the apoplast, while the neighbouring cell, polarized away from the apoplast, would hardly be affected.

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