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Heisler, Marcus; Jönsson, Henrik

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Modelling Auxin Transport and Plant development

M. G. Heisler[1] and H. Jönsson[2]

[1]Division of Biology, California Institute of Technology, Pasadena, CA, USA, mheisler@caltech.edu

[2]Corresponding author, Computational Biology and Biological Physics, Department of Physics, Lund University, Lund, Sweden, henrik@thep.lu.se, Ph.+46 46 222 0667, Fax +46 46 222 9686

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Summary

The plant hormone auxin plays a critical role in plant development. Central to its function is its distribution in plant tissues, which is in turn, largely shaped by intercellular polar transport processes. Auxin transport relies on diffusive uptake as well as carrier-mediated transport via influx and efflux carriers. Mathematical models have been used to both refine our theoretical understanding of these processes and to test new hypotheses regarding the localization of efflux carriers in order to understand auxin patterning at the tissue level. Here we review models for auxin transport and how they have been applied to patterning processes including the elaboration of plant vasculature and primordium positioning. Secondly we investigate the possible role of auxin influx carriers such as AUX1 in pattering auxin in the shoot meristem. We find that AUX1 and its relatives are likely to play a crucial role in maintaining high auxin levels in the meristem epidermis. We also show that auxin influx carriers may play an important role in stabilizing auxin distribution patterns generated by auxin-gradient type models for phyllotaxis.

Introduction

The hormone auxin plays a central role in plant development. It acts both as a global coordinator, dictating where and when developmental events occur (Benkova and others 2003), and as a mediator of morphogenesis, both in the context of whole tissues and individual cells . Critical to its role in plant development is its polar intercellular transport. Disruption to auxin transport, either by chemical means or through mutation results in many plant defects including the inhibition of cell elongation, a loss of asymmetric embryo morphogenesis (Friml and others 2003; Hadfi and others 1998), cell division defects (Campanoni and Nick 2005), defects in vascular patterning (Sieburth 1999), a loss of tropic responses to light and gravity and the absence of organ initiation (Okada and others 1991). The importance of auxin transport is further underscored by its ability to act as a buffer against changes in auxin homeostasis, particularly during embryo development (Weijers and others 2005). In this review we will briefly outline the current conception of how auxin transport occurs and how mathematical models and computer simulations have helped us test this conception against experimental data. Secondly we will outline how modelling efforts have also enabled us to explore various hypotheses for how transport routes are specified. Lastly we will present new simulations of auxin transport that explore the potential role of auxin influx carriers within the shoot apical meristem.

Our current understanding of auxin transport is based on the chemiosmotic model proposed by Rubery and Sheldrake (Rubery and Sheldrake 1974) and Raven (Raven 1975). This model is based on the fact that indole-3-acetic acid (IAA), which is the most predominant naturally occurring auxin, exists in two alternate states depending on pH. Outside the cell plasma membrane, where the pH is close to 5, a significant proportion of IAA exists in an uncharged form, which can enter cells passively. However inside cells the pH is around 7 and the vast majority of IAA present there is negatively charged. Thus in order to exit a cell, there has to be an efflux carrier present that enables negatively charged IAA to move through the plasma membrane down the electrochemical gradient

between the inside and outside of the cell. It is the polar subcellular localization of the efflux carrier that is thought to give auxin transport its overall directionality. In addition to entering cells passively, auxin is taken up by a saturable influx carrier which symports two protons with one IAA molecule (Bennett and others 1996; Marchant and others 1999; Rubery and Sheldrake 1974; Yang and others 2006).

Early transport models

Perhaps the earliest attempt to model polar auxin transport is that of Leopold and Hall in 1966. In this and an accompanying study, a simple polarized transport model (basal "secretion") is compared with experiments in which an auxin source is applied to a file of auxin transporting cells (de la Fuente and Leopold 1966; Leopold and Hall 1966). These studies reveal that a small basally biased asymmetry in transport polarity at the cellular level should be sufficient to generate a large asymmetry in auxin transport at the tissue level. Their model also predicts the observation that the ratio of auxin that accumulates in basal vs. apical cells grows exponentially with the distance from an apical source (de la Fuente and Leopold 1966; Leopold and Hall 1966).

Later mathematical models were developed to establish a firm theoretical basis for the chemiosmotic model. Both Mitchison (1980) and Goldsmith and others (1981) showed that the model predicted constant velocity for an auxin pulse travelling through tissue characterized by an asymmetric distribution of anion carrier, as is observed experimentally (Goldsmith, 1977). Mitchison (1980) also showed that the polarity or asymmetry in permeability required for polar transport depended on the route of auxin within cells, i.e. whether transport was mainly restricted to a thin cytoplasmic sheath surrounding a large vacuole or whether the tonoplast was considered permeable to the anion. Differences in the mathematical description of the two studies were later reconciled (Martin and others 1990). Interestingly both Mitchison and Goldsmith and others noted that to achieve the known auxin transport rate of 1 cm/h in their simulations their ratio between basipetal and acropetal transport had to be much higher than what had been suggested by Leopold and Hall. However Mitchison found that this contradiction could be reconciled assuming that efflux was saturable (Mitchison 1980a). Since these early theoretical studies considerable experimental progress has been made in identifying

and characterizing the molecular components involved in auxin transport. Members of the PIN family of membrane proteins (Galweiler and others 1998) have been shown to mediate auxin efflux (Petrasek and others 2006) and their polar localization has been shown to be required for polar auxin transport (Wisniewska and others 2006). PIN protein localization has been documented in many plant tissues and the consequences of disruption to PIN function have also been characterized (Benkova and others 2003; Friml and others 2002a; Friml and others 2003; Friml and others 2002b; Galweiler and others 1998; Okada and others 1991; Reinhardt and others 2003). The characterization of auxin influx carriers has also progressed with the identification of *AUX1* and its relatives. *AUX1* encodes an amino acid permease like protein that has been shown to mediate auxin influx (Bennett and others 1996; Yang and others 2006). However so far only AUX1 has been extensively investigated at the level of function, localization and expression while its three close relatives remain relatively uncharacterised.

Models for generating auxin transport patterns associated with vascular patterning

One of the most intriguing aspects of plant development linked to auxin transport is the patterning of plant vasculature. From studying the influence of wounding and exogenously applied auxin on vasculature patterning, Sachs proposed that plant veins form along the paths of auxin flow. Sachs also suggested that these preferred flow paths form because of positive feedback between auxin flux and auxin transport capacity. Cells with an increased capacity for transport are suggested to act as auxin sinks thereby depleting auxin from neighbouring cells. A critical aspect of this model not explicitly defined by Sachs is the way in which a transporting cell becomes a “sink”. Addressing this issue, Mitchison showed that if the concentration of auxin within a transport route decreased with increasing flux then auxin would move preferentially into this transport route simply by diffusion (Mitchison 1980b; Mitchison 1981). Mitchison further showed that such a scenario could be achieved if transport capacity is increased faster than linear as a function of flux. Small differences in auxin flux were found in simulations of this model to be sufficient to destabilize a uniform initial flow pattern into preferred pathways, as postulated by Sachs. By also changing the location of auxin sources and sinks Mitchison was able to generate circular transport channels which are suggested to

correspond to the loops of vascular elements induced by experimentally manipulating exogenous auxin sources (Sachs 1981). One criticism of this model has been that experimental evidence indicates that auxin levels in developing vascular tissues are higher than the surrounding cells rather than lower. For instance Sachs and others had shown that high auxin concentrations are required for vascular cell differentiation and cell elongation. More recently it has been found that many auxin-regulated genes are specifically expressed in developing vasculature (Mattsson and others 2003; Scarpella and others 2004; Scarpella and others 2006). However Mitchison proposed a solution to this criticism in his 1981 paper in the supplementary discussion section. He suggests that cells that become incorporated into a preferred transport route need only contain relatively low auxin concentrations at the beginning of the canalisation process when they are acting as sinks. During the later differentiation stages, if the auxin anion channel becomes localized to one end of the cell and depleted from the other sides, auxin can flow into this cell even if it has a relatively high auxin concentration. This proposal has now been modelled explicitly and simulations confirm that auxin could specifically accumulate in transporting cells under these conditions (Feugier and others 2005; Fujita and Mochizuki 2006).

Recent detailed observations of PIN1 localization in developing veins has revealed some aspects of localization predicted by the flux model as well as other patterns that are more puzzling (Scarpella and others 2006). For instance the initiation of tertiary veins is marked by the expression of PIN1 in a cell adjacent to an existing secondary vein. Later PIN1 expression then appears in a new cell adjacent to the first cell but located further away from the pre-existing vein. PIN1 polarity in all the cells is also directed towards the pre-existing vein. This pattern of localization and expression resembles efflux carrier distributions in simulations of the flux model in which a new vein forms from a local pre-existing auxin sink. In such cases a new channel forms from the sink towards the source. However a critical question here is in what sense do pre-existing veins represent auxin “sinks”? For instance, do they contain relatively low concentrations of auxin as Mitchison originally proposed? In fact expression of the DR5 auxin marker in many provascular cells suggests that the majority of these cells contain high levels of auxin in contradiction to Mitchison’s predictions (Mattsson and others

2003; Scarpella and others 2004; Scarpella and others 2006). However it may be that cells that contain high levels of auxin can still act as sinks because they express high levels of auxin influx carriers. In fact specific influx carrier expression has been suggested to be a precondition for auxin accumulation (Kramer 2004). Another interesting observation made by Scarpella and others (2006) is that within tertiary vein loops, there is a cell in which PIN1 is localized to two opposite sides. According to the flux model, such a bi-polar cell would be expected to act as an auxin source. Intriguingly a recent modelling study that incorporates local auxin sources together with a simplified version of the flux model is capable of generating realistic patterns of leaf vein formation (Runions and others 2005). It will be interesting to examine this model further and test whether local auxin sources exist in developing leaves at the locations of these bipolar cells.

Auxin transport and phyllotaxis

Due to the striking geometrical arrangements of plant organs, the study of phyllotaxis, or arrangement of plant organs, has a long history (Adler and others 1997). Phyllotactic patterning can be understood as the superimposition of several processes. Firstly there is a mechanism that restricts primordial emergence to a narrow region called the peripheral zone, which is located a certain radial distance from the centre of the shoot apical meristem (Steeves and Sussex 1989). Secondly there is the continuous generation of new tissue within the meristem that results in the steady displacement of cells away from the peripheral zone (Reddy and others 2004). Thirdly there is a positioning process that imposes a regular spacing between the locations of new primordia (Mitchison 1977). Lastly, for many plant species, the shoot apical meristem can change size gradually during seedling growth. Most theoretical studies have been concerned with hypotheses for how spacing mechanisms may work. Generally these can be divided into four categories. One general type of mechanism involves primordia producing a diffusible substance that inhibits primordia from initiating nearby (Schoute 1913). A second type of model involves primordia depleting a positive activator of primordium development with the activator only reaching the critical initiation concentration threshold a certain distance away from pre-existing primordia (Reinhardt and others 2003). A recent variant on this

theme, discussed further below, is a polarized transport model based on intercellular auxin gradients (Jönsson and others 2006; Smith and others 2006). A fourth type of mechanism involves both positive and negative regulators in a reaction-diffusion network (Meinhardt and others 1998). Lastly mechanical buckling with positive feedback has also been proposed (Green and others 1996; Shipman and Newell 2004; Shipman and Newell 2005).

Experimental data support the second two hypotheses listed above in which auxin acts as a limiting positive regulator. As early as the 1930s, Snow and Snow discovered that by applying lanolin paste containing auxin to shoot meristems of Lupin, ectopic growth of leaf or floral tissue could be induced (Snow and Snow 1937). More recently it was found that plants deficient in auxin transport activity developed pin-like apices devoid of lateral organs (Okada and others 1991). By applying auxin to such apices organ growth could be restored at the site of application (Reinhardt and others 2000). These experiments suggested that auxin acts as a positive regulator of organ initiation and that its transport-dependant distribution might determine where organs arise. By immuno-localization of the PIN1 putative auxin efflux carrier, it was found that polar transport was indeed likely to transport auxin directly to sites of organ initiation since the PIN1 protein was found to be localized on the sides of meristem cells located closest to where a primordium was predicted to form (Reinhardt and others 2003). PIN1 protein was also localized basally within primordia and on the apical sides of epidermal cells located below the meristem. Lastly, the AUX1 protein was found predominantly localized to the epidermis (Reinhardt and others 2003). These findings suggested a scenario in which auxin is transported apically to the meristem where it then becomes localized to the epidermis and sites of primordium emergence. As primordia develop auxin would then get transported basally down the developing vasculature. These data and previous findings also prompted Reinhardt and co-workers to propose that it is the transport of auxin into the vasculature and resulting depletion of auxin in surrounding cells that inhibits primordial development nearby. The next primordium would therefore be placed furthest away where auxin levels first reach a certain threshold (Reinhardt and others 2003).

More recently a series of modelling papers have been published in an effort to bring our theoretical understanding up to date with experimental results and to propose mechanisms for determining PIN1 polarity patterns. Two of these papers propose positive feedback between cellular auxin concentrations and auxin transport direction as a mechanism for positioning primordia (Jönsson and others 2006; Smith and others 2006). The third paper quantifies PIN1 localization patterns within the meristem to gain a more accurate picture of probable auxin accumulation patterns (de Reuille and others 2006). Unlike the flux model, the proposed feedback models suggest cell-cell signalling as a means of coordinating PIN1 polarity by which cells polarize PIN1 towards neighbouring cells containing the most auxin. One problem with a model based solely on this mechanism however is that there is no means by which auxin maxima become fixed to the underlying cells. Hence in response to the changing geometry of a plant apex and the formation of new auxin maxima, auxin accumulation patterns are not stable resulting in variable phyllotactic patterns. This problem is exacerbated by incorporating the experimental result that in the apical meristem PIN1 expression is auxin induced (Heisler and others 2005) (as discussed below) since when PIN1 expression intensifies, auxin is more easily able to move out of cells within the maxima and into neighbouring cells (unpublished observations). Smith and others incorporate a number of additional assumptions in their model to try and prevent this phenomenon from occurring. One such assumption is that auxin synthesis occurs in primordial cells after these cells are first specified. Another assumption is that PIN1 localization towards the centre of primordia becomes fixed after they first form (Smith and others 2006).

Here we attempt to explore the possible role of auxin influx carriers in this type of feedback model. Our aim is to test the previously proposed hypothesis that AUX1 may help to concentrate auxin in the meristem epidermis (Reinhardt and others 2003) and secondly, to test whether auxin influx carriers including AUX1 may help stabilize the concentration feed-back model by maintaining high auxin concentrations in cells in which auxin first accumulates.

Including influx mediators when modelling auxin patterning in the SAM

Our modelling approach is based on basic biochemical descriptions of molecular reactions including the chemiosmotic transport theory for auxin where model parameters are mainly based on experimental estimates. All interactions are described using ordinary differential equations and we obtain a numerical solution describing molecular concentrations in a tissue of cell and wall compartments over time. Compared to our previous study (Jönsson and others 2006), here we add AUX1 as an explicit auxin influx mediator. We use a symmetric distribution of AUX1 in the membranes surrounding a cell, which in contrast to the previously used passive influx, allows individual cells to regulate their auxin influx from surrounding walls. We then discuss the consequences that the explicit inclusion of an influx mediator has for phyllotaxis models based on auxin transport since influx mediators have not been included in recent models. More explicitly, we show that in a model taking into account the whole structure of the SAM, including epidermal and sub-epidermal cells, and in a model including auxin-induced PIN1, influx carriers are important to maintain a stable auxin pattern.

By examining the expression pattern of AUX1 transcripts as well as a functional AUX1-YFP fusion protein (Swarup and others 2004) we find that AUX1 is expressed predominantly in the epidermal layer (L1) of cells of the *Arabidopsis* inflorescence meristem (as also found for the vegetative meristem in (Reinhardt and others 2003)) with higher expression levels in developing primordia (Figure 1A-B). Using real-time PCR we also find that AUX1 mRNA is upregulated 2.2 (2.0-2.3) fold in *pin1-1* meristems in response to treatment with 5 mM IAA after 30 min. The regulation of auxin influx carrier transcription by auxin has also been found in hybrid aspen (Schrader and others 2003) and thus it may be a widely conserved mechanism for regulating cellular auxin levels. The way in which AUX1 is included in our models is based on these experimental data. We incorporate specific AUX1 expression in the L1 layer of cells within the SAM and we also assume auxin-induced expression of AUX1, which leads to a phyllotactic expression pattern of AUX1 (Fig. 1B). Since PIN1 transcription in the apical meristem is also known to be auxin responsive (Heisler and others 2005) we also include similar behaviour for PIN1, with auxin-induced expression and preferential expression in the L1 layer of cells (Fig. 1C). The resulting auxin-transport regulatory model is illustrated in

Figure 1D. The protonated auxin passively crosses membranes between cells and walls, where the influx and efflux rates differ depending on the fraction of the auxin, which is in the protonated form. Active transport of the auxin anion is dependent on saturable influx and efflux mediator proteins, which cycle between the cytoplasm and cell membranes. The influx is mediated by AUX1 and the efflux is mediated by PIN1. Where applicable, the epidermal layer of cells is allowed to have higher levels of transport mediators. In some simulations auxin induced production of the transport mediators is taken into account, and as a pattern generating mechanism a positive feedback from auxin to PIN1 localization in neighbouring cells is used ($f(a_j)$ in Fig. 1D, (Jönsson and others 2006)). The model parameters are mainly based on experimental estimates (Jönsson and others 2006; Swarup and others 2005), which together with the model are described in more detail in Appendix 1. Lastly, we also allow for auxin diffusion between neighbouring cell wall compartments.

Auxin in epidermal and sub-epidermal tissue of the SAM

In the SAM, influx and efflux mediator proteins are predominantly expressed in the L1 layer of cells (Fig. 1). Previously it has been suggested that the epidermal expression of AUX1 may facilitate the retention of auxin in the epidermis (Reinhardt and others 2003). To investigate this hypothesis further we simulated a non-polarized transport model on a two-dimensional cellular template representing a longitudinal section of the SAM where auxin is supplied uniformly to all cells (Figure 2). A model that explicitly includes L1 localized efflux mediators without any influx mediators leads to a depletion of auxin from the epidermal layer of cells (Figure 2A). A similar result is achieved with a uniformly expressed influx mediator. In contrast, the explicit inclusion of an influx mediator expressed predominantly in the L1 leads to higher auxin concentrations in the epidermis (Figure 2B). For this simulation, an estimate of the relative PIN1 and AUX1 expression levels in the epidermal layer compared to the sub-epidermal cells were extracted from measuring average and maximal GFP intensities from confocal images in a number of rectangular areas within and below the epidermal cells. This somewhat crude measure indicates that AUX1 is more locally expressed compared to PIN1 as the subepidermal cells showed an AUX1 intensity of about 5-15%

of the intensities measured in the epidermal cells, while the corresponding measure for PIN1 was 35-45% (compare Fig. 1A and C). To further investigate the expected sensitivity of the auxin distribution to the expression patterns of transport mediators we measured the auxin levels in simulations with varying AUX1 and PIN1 expression patterns. AUX1 and PIN1 were varied from being uniformly expressed (same amount of protein in the epidermal and sub-epidermal cells) to being completely L1 localized (protein expressed only in the epidermal layer). The relative average auxin concentration in the epidermal layer of cells compared to the sub-epidermal cells is presented in Figure 2C. It can be seen that increasing the relative expression of AUX1 in the epidermis leads to increased auxin in the epidermal layer, while the opposite is true for PIN1. Thus our results suggest that in order for auxin to accumulate specifically in the epidermis AUX1 needs to also be specifically expressed in the epidermis while PIN1 expression must be less localized. This prediction is similar to that of a previous study (Kramer 2004), where it is shown that to maintain high auxin concentrations within a transport channel in which PIN1 is expressed, it is beneficial to have PIN1 expressed also in surrounding cells. Our final conclusion is that the observed highly localized L1 expression pattern of influx carrier is required for retention of auxin in the epidermis, as previously postulated (Reinhardt and others 2003). However we also predict that such an accumulation of auxin in the epidermis also requires the expression pattern of PIN1to be relatively less L1 localized, as is observed.

Auxin patterning in models with auxin-induced transport mediators

In the previous section we could conclude that if PIN1 expression was localized specifically in the epidermis, this would lead to auxin being depleted from these epidermal cells. A similar behaviour then, may also be expected in a tissue in which auxin concentration maxima form and where auxin induces PIN1 expression. The cells within the auxin peak will increase their PIN1 content leading to increased efflux from these cells to the surrounding cells.

To investigate the consequences of auxin-induced transcription of transport mediators on patterning phyllotaxis we simulated a pattern-generating model on a static two-dimensional cellular template representing the epidermal layer of a plant apex, where

we include auxin-induced PIN1 and AUX1 production. Figure 3 shows the time evolution of peak positions in simulations of different auxin induction scenarios. In a model without any auxin-induced transport mediator production, peaks form and stay at constant positions except for some minor slow rearrangements (Fig. 3A), as expected from our previous work (Jönsson and others 2006). If auxin-induced PIN1 is added to the model, the stability of the peak positions is lost (Fig. 3B). Here we have used a maximal PIN1 expression level approximately three times the minimal in accordance with the variation in cellular PIN1 intensity levels in an epidermal layer template (Fig. 1C in (Jönsson and others 2006)). Finally, if auxin-induced AUX1 expression is introduced, the resulting pattern becomes stable (Fig. 3C). These results suggest that the ability of auxin to modulate PIN1 transcription may be a destabilizing influence on auxin distribution patterns while auxin-induced influx carrier activity has the converse effect. Although *aux1* single mutants do not exhibit disrupted phyllotaxis, we suggest AUX1 may act redundantly together with other members of the AUX1 family of proteins to help stabilize plant organ positioning.

Discussion

Modelling of auxin transport has long been used to complement experimental results and to formalize and evaluate hypotheses. It is indeed interesting to note that the chemiosmotic formulation of auxin transport, first introduced over 25 years ago, is still applicable to new problem settings. However although there is now a consensus on how auxin transport is modelled in detail, it is important to realise that the data used for defining parameters in current models come from studies using many different plants and an assortment of tissue types. Although it should be possible to derive these parameters through the reverse engineering and optimisation of models, such a strategy requires us to have a reliable estimate of auxin concentration at subcellular resolution, which, up until recently, has been beyond our capabilities. However with the identification of ABP1 (Lobler and Klambt 1985) and TIR1 (Dharmasiri and others 2005; Kepinski and Leyser 2005) as two distinct auxin-binding proteins, the development of a fluorescence resonance energy transfer (FRET) based auxin biosensor such as has become a real possibility.

Apart from modelling the dynamics of auxin efflux and influx, a future challenge will be to incorporate other modulators of auxin transport that mediate pattering at the tissue level. For understanding phyllotaxis for example, a detailed polarized auxin transport model can only provide part of the explanation. Important issues such as the demarcation and definition of the peripheral zone, understanding the role of genes such as *PINOID* (Benjamins and others 2001; Christensen and others 2000; Friml and others 2004) and *MONOPTEROS* (Aida and others 2002; Hardtke and Berleth 1998; Przemeck and others 1996) and incorporating the down stream events leading to primordial growth are important goals for future experimental and theoretical studies.

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Appendix 1: Detailed model description

In the model tissue is built up by cytoplasmic and wall compartments separated by plasma membranes. The auxin transport model is based on the detailed chemiosmotic description for transport across membranes partly dependent on saturable efflux and influx mediators. In addition to cross-membrane transport also apoplastic diffusion is modelled. Transport mediators cycle between cytoplasm and plasma membrane compartments. In the present study it is assumed that the cycling is fast such that transport mediators are positioned in equilibrium. In each compartment auxin is divided into pH-dependent fractions of the protonated and anion forms (assuming fast dynamics and equilibrium for the reversible reaction $a^H \leftrightarrow a^- + H$). The auxin flux J_a from a cytoplasm compartment into a wall compartment is described by

$$J_a = p_{a^H}\left(f_{a^H}^{cell} a_i - f_{a^H}^{wall} a_{ij}\right) + p_{PIN} P_{ij}\left(N(\Phi)\frac{f_{a^-}^{cell} a_i}{K_P + f_{a^-}^{cell} a_i} - N(-\Phi)\frac{f_{a^-}^{wall} a_{ij}}{K_P + f_{a^-}^{wall} a_{ij}}\right)$$
$$+ p_{AUX} A_{ij}\left(N(-\Phi)\frac{f_{a^-}^{cell} a_i}{K_A + f_{a^-}^{cell} a_i} - N(\Phi)\frac{f_{a^-}^{wall} a_{ij}}{K_A + f_{a^-}^{wall} a_{ij}}\right)$$

where a_i, a_{ij} are the auxin concentrations in the cytoplasm and wall compartment respectively. P_{ij} and A_{ij} are the PIN1 and AUX1 concentrations in the membrane, p_a, p_{PIN}, and p_{AUX} are the permeabilities, f_a^{cell}, f_a^{wall}, $f_{a^-}^{cell}$, and $f_{a^-}^{wall}$ are the fractions of protonated and anion forms of auxin within the cell and wall compartments. $N(\Phi)$, and $N(-\Phi)$ are factors coming from the carrier mediated transport across the membrane potential given by

$$N(\Phi) = \frac{\Phi e^{\Phi}}{e^{\Phi} - 1}$$
$$\Phi = \frac{z V_m F}{RT}$$

where $\Phi=\pm 4.65$ has been used assuming a membrane potential V_m=-120 mV (negative inside). z is the valence, F is the Faraday constant, R is the gas constant, and T is the absolute temperature.

In addition to the membrane transport there is diffusion between neighbouring walls with a diffusion constant D_a. In the equations describing the transport also spatial factors are included (Jönsson and others 2006), where we have simplified by using constant values for these assuming in the two-dimensional simulations a cell "volume" of 25 μm^2, a cell-wall crossing "area" of 5 μm, and a wall thickness of 50 nm.

The PIN1 and AUX1 cycling determines how much of the proteins are within the cell membranes towards different neighbours. While we use a symmetric AUX1 polarization, the PIN1 cycling is also dependent on the auxin in the neighbouring cells. In the simulations we use the equilibrium calculated from the cycling rate (from cytosol to membrane compartment)

$$J_A = k_{A1} A_i - k_{A2} A_{ij}$$

$$J_P = k_{P1}\left((1 - c_P) + c_P \frac{a_j^n}{K_H + a_j^n} \right) P_i - k_{P2} P_{ij}$$

where a_j is the auxin in the neighbouring cell, A_i, (P_i) is the AUX1 (PIN1) in the cytoplasm compartment, and A_{ij} (P_{ij}) is the AUX1 (PIN1) in the membrane. The cytoplasm concentrations are measured as molecules per volume while the membrane concentrations are measured as molecules per area. For a more thorough description we refer to Jönsson and others 2006, where a minor difference is that we here also include a symmetric term for PIN1 endocytosis (the $k_{P1}(1-c)$ term in J_P).

Finally we include production and degradation for the molecules, which for auxin also could be interpreted as transport in and out of the simulated tissue at the boundary. These processes are described by

$$\frac{da_i}{dt} = c_a - d_a a_i$$

$$\frac{dA_i}{dt} = \left(c_{A1} + c_{A2} L_1\right)\left(\left(1 - k_A\right) + k_A \frac{a_i}{K_M + a_i}\right) - d_A A_i$$

$$\frac{dP_i}{dt} = \left(c_{P1} + c_{P2} L_1\right)\left(\left(1 - k_P\right) + k_P \frac{a_i}{K_M + a_i}\right) - d_P P_i$$

where a_i, A_i, and P_i are the concentrations of auxin, AUX1, and PIN1. All molecules have a simple degradation proportional to its concentration. Production is allowed to be different in the epidermis (L_1=1) compared to inside the tissue (L_1=0). The proteins are produced only within the cytoplasmic compartments and have one constant term and one Michaelis-Menten term for the auxin-induced production.

The parameter values used in the simulations are presented in Table A1, and are mostly estimated from experiments. All simulations are done using a C++ software based on a 5th order Runge-Kutta solver with adaptive step length for the numerical integration.

Table A1: Model parameters

Parameter	Value 1 (Figure 2)	Value 2 (Figure 3)	Unit
Auxin transport parameters:			
p_{aH}	0.55	0.55	μm/s
$p_{PIN\_}$	0.27	0.27	μm/s
p_{AUX}	0.55	0.55	μm/s
f_{aH}^{cell}	0.004	0.004	-
f_{aH}^{wall}	0.24	0.24	-
f_{a-}^{cell}	0.996	0.996	-
f_{a-}^{wall}	0.76	0.76	-
N(Φ)	4.69	4.69	-
N(-Φ)	0.045	0.045	-
K_P	1.0	1.0	μM
K_A	1.0	1.0	μM
D_a	67	67	$\mu m^2/s$
Protein cycling parameters:			
k_{A2}/k_{A1}	0.1	0.1	1/μm
k_{P2}/k_{P1}	0.1	0.1	1/μm
c_P	0.0	0.9	-
n	1.0	1.0	-
K_H	1.0	1.0	μM
Production and degradation parameters:			
c_a	0.001	0.001	μM/s
d_a	0.001	0.001	1/s
c_{A1}	0.0, 0.00015, [0 ... 0.001]	0.001, 0.001, 0.0025	μM/s
c_{A2}	0.0, 0.00085, [0 ... 0.001]	0.0	μM/s
c_{P1}	0.0, 0.0004, [0 ... 0.001]	0.001, 0.0015, 0.0015	μM/s
c_{P2}	0.001, 0.0006, [0 ... 0.001]	0.0	μM/s
k_A	0.0	0.0, 0.0, 0.8	-
k_P	0.0	0.0, 0.67, 0.67	-
K_M	1.0	1.0	μM
d_A	0.001	0.001	1/s
d_P	0.001	0.001	1/s

Parameter values used in the simulations. Column labelled Value 1 contains the values used in the vertical section showed in Fig. 2, and column labelled Value 2 contains the values used in the epidermal layer simulations presented in Fig. 3. When a column includes more than one value, the values represent the changes between A, B, and C in the figures.

Appendix 2: Experimental methods

Auxin treatments were carried out by either applying auxin paste made from 5 mM IAA, 1 % DMSO in lanolin (Sigma) or a mock treatment of 1% DMSO in lanolin to *pin1-1* apices. The apical 2 mm of tissue was then collected for RNA extraction after 30 min. RNA extraction and quantification was carried out according to (Heisler and others 2005). For amplifying *AUX1* we used the primers 5' GTCCAATCAATTCCGCTGTC 3' and 5' GCATAAAGAACGGTGGCTTC 3'. We used both the *ACTIN2* and *ACTIN8* genes as internal controls as described in (Heisler and others 2005).

In situ hybridizations was carried out according to (Long and Barton 1998).

Preparation of tissue for confocal imaging was carried out as described in (Heisler and others 2005).

Figure legends

Figure 1. Experimental data and model illustration. A) Longitudinal section showing AUX1::YFP illustrating the preferential epidermal expression of AUX1. B) Transverse section of the SAM epidermal layer showing immunostaining of AUX1 mRNA. The AUX1 is expressed in a phyllotactic pattern similar to assumed auxin localization. C) Vertical section of PIN1::GFP. D) Illustration of the auxin transport and regulatory interactions used in the model. Auxin transport between the cell and its surrounding walls is indicated by solid arrows. The anion transport is mediated by PIN1 and AUX1 in the membranes. Protein cycling is illustrated by solid arrows where the $f(a_j)$ indicates the dependence on auxin in the neighbouring cell. Dashed arrows indicate the auxin-induced protein production.

Figure 2. Auxin localization dependence on asymmetrically expressed PIN1 and AUX1 in a longitudinal section simulation. A) and B) show the auxin concentration in the cells (where the walls in-between have been left out). A) In a model only explicitly including the efflux mediator PIN1, the equilibrium auxin concentration is high beneath the epidermal layer when the PIN1 is expressed in the epidermal layer. B) A model that also includes an asymmetric influx mediator, results in auxin mainly within the epidermal layer. C) Restrictions on the asymmetry of the influx/efflux mediator expressions apply to achieve high auxin concentrations in the epidermal cells. The color-coding represents the measure $(a_{epi} - a_{int})/(a_{epi} + a_{int})$ where a_{epi} (a_{int}) is the average auxin concentration in the epidermal (internal) cells. The axes show the asymmetry where 0 is uniform expression in all cells and 1 is expression in epidermal cells only.

Figure 3. Auxin peaks in simulations on a static square lattice of cells with walls in-between. The simulations are started from a close to homogeneous auxin distribution. Parameter values are described in the text. A) Model without auxin-induced PIN1 and AUX1. Some minor rearrangements occur, in a stable pattern. B) Model with auxin-induced PIN1. The pattern is unstable as the peaks move around in the cell tissue. C) Model with both PIN1 and AUX1 induced by auxin. This results in a stable pattern.

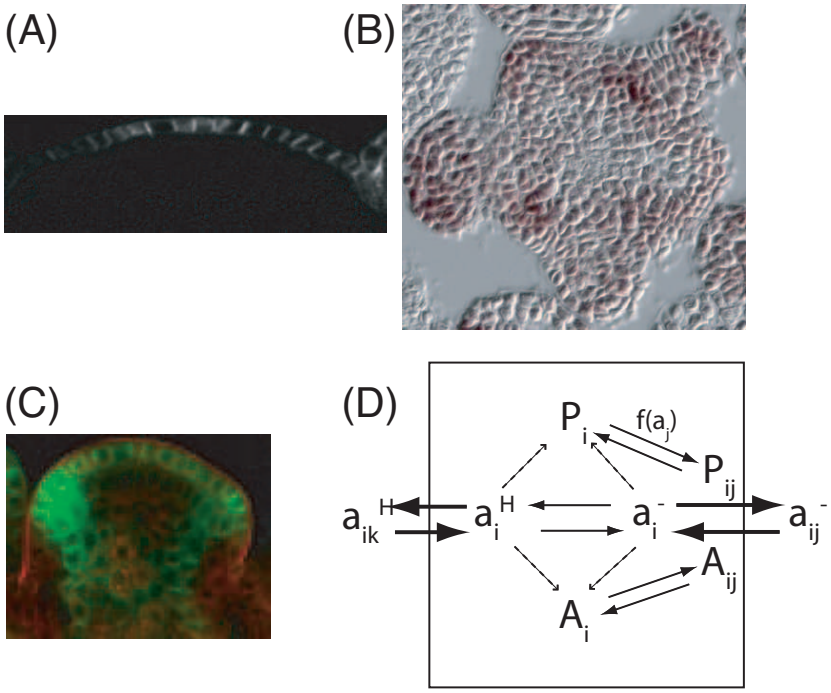

Figure 1:

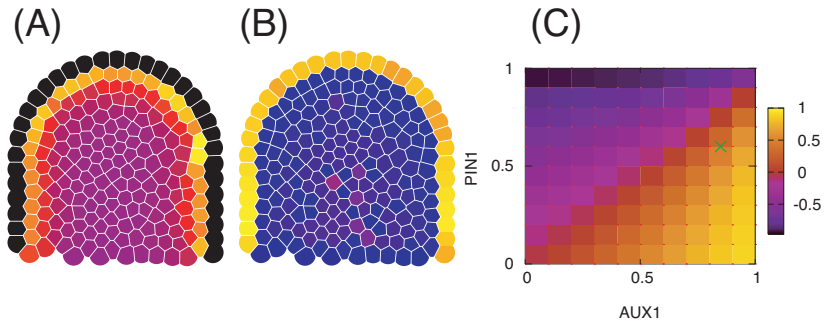

Figure 2:

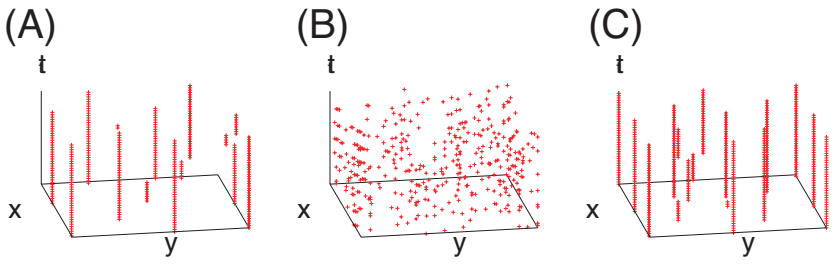

Figure 3: