Integration of hormonal signaling networks and mobile microRNAs is required for vascular patterning in Arabidopsis roots

Daniele Muraro\textsuperscript{a}, Nathanael Mello\textsuperscript{a}, Michael P. Pound\textsuperscript{a}, Hanna Help\textsuperscript{e}, Mikaël Lucas\textsuperscript{a,d}, Jérôme Chopard\textsuperscript{b}, Helen M. Byrne\textsuperscript{a,f}, Christophe Godin\textsuperscript{e}, T. Charlie Hodgman\textsuperscript{a}, John R. King\textsuperscript{a,f}, Tony P. Pridmore\textsuperscript{e}, Ykä Helariutta\textsuperscript{c}, Malcolm J. Bennett\textsuperscript{a}, and Anthony Bishopp\textsuperscript{a,c,1}

\textsuperscript{a}Centre for Plant Integrative Biology, School of Biosciences, University of Nottingham, Loughborough LE12 5RD, United Kingdom; \textsuperscript{b}The Weatherall Institute of Molecular Medicine, University of Oxford, John Radcliffe Hospital, Oxford OX3 9DS, United Kingdom; \textsuperscript{c}Institute of Biotechnology, University of Helsinki, FIN-00014, Helsinki, Finland; \textsuperscript{d}Equipe Rhizogenèse, Unité Mixte de Recherche Diversité Adaptation et Développement des plantes, Institut de Recherche pour le Développement, 34394 Montpellier, France; \textsuperscript{e}Institut National de Recherche en Informatique et en Automatique (INRIA), Virtual Plants Project Team, jointly with Centre de coopération internationale en recherche agronomique pour le développement (CIRAD) and INRIA, Unité Mixte de Recherche Amélioration Génétique des Plantes Méditerranéennes et Tropicales (AGAP), 34095 Montpellier, France; \textsuperscript{f}School of Mathematical Sciences, University of Nottingham, Nottingham NG7 2RD, United Kingdom; and \textsuperscript{1}Oxford Centre for Collaborative Applied Mathematics, Mathematical Institute, Oxford OX1 3LB, United Kingdom

Edited by Ben Scheres, Wageningen University, Wageningen, The Netherlands, and accepted by the Editorial Board December 5, 2013 (received for review December 13, 2012)

As multicellular organisms grow, positional information is continually needed to regulate the pattern in which cells are arranged. In the Arabidopsis root, most cell types are organized in a radially symmetric pattern; however, a symmetry-breaking event generates bisymmetric auxin and cytokinin signaling domains in the stele. Bidirectional cross-talk between the stele and the surrounding tissues involving a mobile transcription factor, SHORT ROOT (SHR), and mobile microRNA species also determines vascular pattern, but it is currently unclear how these signals integrate. We use a multicellular model to determine a minimal set of components necessary for maintaining a stable vascular pattern. Simulations perturbing the signaling network show that, in addition to the mutually inhibitory interaction between auxin and cytokinin, signaling through SHR, microRNA165/6, and PHABULOSA is required to maintain a stable bisymmetric pattern. We have verified this prediction by observing loss of bisymmetry in shr mutants. The model reveals the importance of several features of the network, namely the mutual degradation of microRNA165/6 and PHABULOSA and the existence of an additional negative regulator of cytokinin signaling. These components form a plausible mechanism capable of patterning vascular tissues in the absence of positional inputs provided by the transport of hormones from the shoot.

Significance

The vascular tissues form a continuous network providing the long-distance transport of water and nutrients in all higher plants (tracheophytes). To incorporate separate organs into this network, it is essential that the position of different vascular cell types is tightly regulated. Several factors required for root vascular patterning (including hormones and gene products) have previously been identified in the model plant Arabidopsis. We have now established a mathematical model formulating the interaction between these factors, allowing us to identify a minimal regulatory network capable of maintaining a stable vascular pattern in Arabidopsis roots. We envisage that this model will help future researchers understand how similar regulatory units can be applied to create alternative patterns in other species.


The authors declare no conflict of interest.

This article is a PNAS Direct Submission. B.S. is a guest editor invited by the Editorial Board.

www.pnas.org/cgi/doi/10.1073/pnas.1221766111


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This article is a PNAS Direct Submission. B.S. is a guest editor invited by the Editorial Board.

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\textsuperscript{1}To whom correspondence should be addressed. E-mail: anthony.bishopp@nottingham.ac.uk.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1221766111/-/DCSupplemental.

www.pnas.org/cgi/doi/10.1073/pnas.1221766111

PNAS | January 14, 2014 | vol. 111 | no. 2 | 857–862
expression of the ck signaling inhibitor AHP6. PHABULOSA (PHB) acts redundantly with other members of the class III homeodomain-leucine zipper (Hd-zip) transcription factor gene family to repress AHP6 expression in a dose-dependent manner (10). In turn, PHB levels are determined through the degradation of PHB mRNA by microRNA165/6 (miRNA165/6) (10, 11). The asymmetric input of hormones into the root meristem also affects root vascular patterning. During embryogenesis, there is a migration of auxin response from the cotyledons to the root pole (2), and in the growing root, both auxin and ck are transported through the phloem (12–14).

Here, we present a model for investigating auxin fluxes within the radial root cross-section (Fig. 1). We show that interplay between these elements is capable of generating realistic patterns and that both hormonal signaling and mobile miRNA are required to specify the correct domain of auxin response.

Results
Localization of PINs on the Lateral Membranes Is Sufficient to Generate High Auxin Response in the Xylem Axis. In addition to regulating the rootward flux of auxin in the root meristem (4, 15, 16), PIN1, PIN3, and PIN7 act in consort to channel a radial flow of auxin to the xylem axis (2). To test whether the radial transport of auxin alone offers a plausible mechanism to generate an auxin response maximum in the xylem axis, we developed a 2D computational model of the root vascular tissues based on realistic cell geometries taken from a cross-section through the root proximal meristem (SI Appendix, Fig. S1). Ordinary differential equations were embedded in each cell within this tissue geometry, and as in ref. 17, the temporal evolution of auxin concentration [Aux], in a given cell i is given by

$$\frac{d[Aux]}{dt} = -\frac{1}{V_i} \sum_{n \in N_i} S_{in} (j^{P}_{i-n} + j^{P}_{i-n}) + p_{aux} - d_{aux}[Aux]_i,$$

where $V_i$ is the cell volume, $N_i$ is the set of neighboring cells of cell i, $S_{in}$ is the exchange surface between cells i and n, $j^{P}_{i-n} = p_{aux} ([Aux]_i - [Aux]_n)$ represents the passive auxin diffusion at rate $p_{aux}$ using Fick’s First Law, and $p_{aux}$ and $d_{aux}$ are the respective intracellular biosynthesis and degradation rates of auxin. Active transport of auxin depends on the efficiency of PIN transporters, and therefore, $j^{P}_{i-n} = T_{aux} ([Aux]_i) [PIN]_{[PIN]_i} - [Aux]_n [PIN]_{[PIN]_i}$ is the flux of auxin caused by active transport at rate $T_{aux}$ proportional to PIN protein concentration $[PIN]_{[PIN]_i}$ on $S_{in}$. Individual parameters were selected based on the current literature or reasonable estimates. Specific parameters are discussed further in SI Appendix.

We used the CellSeT software (18) to determine the polarity of PIN-GFP from confocal images and examined 3D reconstructions of immunolocalizations for plants labeled with α-PIN1 or α-GFP antibodies (SI Appendix, Figs. S2–S5). PIN proteins were incorporated onto specific cell membranes in our multicellular geometry (Fig. 1B and SI Appendix, Fig. S6); the total concentration of PIN proteins in each cell was set to zero or one and proportionally divided on any given membrane, resulting in a concentration of $[PIN]_{[PIN]_i}$. We modeled auxin flux through the root and predicted the response by simulating the activity of the primary auxin response gene IAA2. Under these conditions, our model showed that the experimentally observed localization of the PINs on lateral membranes was capable of producing an auxin signaling maximum in the xylem axis closely resembling the observed pattern of IAA2 (Fig. 2 A and B and Movie S1). The positioning of this auxin signaling maximum persisted, regardless of whether auxin biosynthesis was assumed to be distributed uniformly across the root or localized in the phloem. We investigated the effect that PIN1, PIN3, and PIN7 exerted individually by running simulations with just one of three PINs (SI Appendix, Fig. S6). Simulations that considered only PIN1 or PIN7 did not show significant differences from the previous simulation, suggesting that these proteins play redundant roles in directing the auxin maximum (Fig. 2C and Movies S2 and S3). PIN3 activity alone was insufficient to reproduce the auxin signaling maximum seen in WT plants (Movie S4). To simplify our subsequent simulations and construct the minimum network required for vascular patterning, from this point, we only consider PIN7. Collectively, the simulations described above highlight that the observed localization of PINs on the lateral membranes is sufficient to direct auxin response to the xylem axis. We then applied our model to determine the minimum set of components that can operate alongside PIN7 during vascular patterning.

Integrating Signaling Networks into a Multicellular Model of Vascular Patterning. We have incorporated the regulatory pathways that are known to determine vascular patterning (2, 10) in the model by including the following processes (Fig. 1C): (i) The transcription of IAA2 and AHP6 is promoted by auxin; (ii) AHP6 transcription is negatively regulated by PHB; (iii) PHB mRNA...
and miRNA165/6 mutually degrade each other; (iv) miRNA165/6 is produced in the endodermis but diffuses throughout the vascular tissues; (v) ARRS5 transcription is activated by ck and repressed by AHP6; and (vi) PIN7 transcription is activated by ck response (ARR5).

We derived a system of ordinary differential equations governing the dynamics of the interaction network that was embedded in our multicellular geometry. Based on previous subcellular models (19, 20), the transcription of all genes was modeled using Hill Kinetics, with the mRNA $M_i$ produced through the transcription of gene $i$ in response to transcriptional regulator $P_j$ given by

$$
\frac{dM_i}{dt} = p_{Mi} F_{Mi} - d_{Mi} M_i,
$$

$$
F_{Mi} := b_i + \frac{\sum_{A_i \in Ai} (P_i/\theta_P)^{\nu_i}}{1 + \sum_{A_i \in Ai} (P_i/\theta_P)^{\nu_i} + \sum_{R_i \in Ri} (P_i/\theta_P)^{\nu_i}},
$$

where $A_i$ and $R_i$ are the sets of activators and repressors acting on gene $i$, respectively, $p_{Mi}$ and $d_{Mi}$ are production and degradation rates of $M_i$, respectively, $b_i$ is a dimensionless parameter regulating basal transcription, $\theta_P$ is a protein–DNA binding threshold, and $\nu_i$ is the Hill coefficient of protein $P_j$ when acting on gene $i$. Translation of the protein $P_j$ from $M_i$ is then governed by the equation

$$
\frac{dP_j}{dt} = p_{P_j} M_i - d_{P_j} P_j,
$$

where $p_{P_j}$ and $d_{P_j}$ are the production and degradation rates of $P_j$, respectively. Mutual degradation of PHB mRNA ($[PHB_{mRNA}]$) and miRNA ([miRNA165/166]) is governed by the reaction $[PHB_{mRNA}] + [miRNA165/166] \rightarrow \emptyset$ at rate $d_{mRNA/mRNA}$, which in the evolution equations of $[PHB_{mRNA}]$ and $[miRNA165/166]$, is described by the negative term $-d_{mRNA/mRNA}[PHB_{mRNA}] [miRNA165/166]$ (21). Passive diffusion of ck, miRNA165/6, and SHORT ROOT (SHR) are governed by the same rules as auxin. The full set of reactions constituting this signaling network is listed in SI Appendix, section 1.

**Mutual Degradation of miR165/6 and PHB Generates Sharp Boundaries of Gene Expression.** We first considered mobile miRNA165/6. The fate of miRNA after it has regulated its target is not well-understood. However, a previous study has shown that the degradation of miRNA223 is accelerated when its target is present (22). Additionally, a previous modeling study has postulated that the mutual degradation of target and miRNA can sharpen the boundary of its mRNA target’s spatial distribution (21). Levine et al. (21) developed a general model based on reaction–diffusion equations in one spatial dimension, in which small RNA molecules interact with a target gene and move from cell to cell through diffusion. Although diffusion generally smooths spatial expression patterns, Levine et al. (21) found that intercellular mobility of small RNAs sharpens the boundaries between target expression domains in a robust manner, and this sharpening was because of the codegradation of both small RNAs and their targets. The codegradation means that, when the small RNAs diffuse into areas of low gene expression, they can eliminate the expression of their targets in these cells, whereas they are less able to affect the levels of target genes in cells in which the target is expressed abundantly. Carlsbecker et al. (10) proposed that a similar mechanism may operate during vascular patterning as endodermally produced miRNA165/6 moves into the vascular cylinder and encounters its target mRNA (PHB); however, they did not test this prediction with a mathematical model and did not explore whether the mutual degradation of miRNA165/6 and PHB mRNA is required to produce the observed expression patterns.

We introduced the molecular circuitry into our model to compare scenarios in which the degradation of both PHB and miRNA occurs mutually. In the former scenario, we assume that binding of miRNA and PHB mRNA targets both molecules for degradation, and in the latter scenario, miRNA accelerates the degradation of PHB but is not itself consumed. In simulations without codegradation of miRNA and target, we observed a diffuse gradient of PHB; resulting in accumulation of AHP6 throughout the xylem axis and boundaries in the protoxylem (Movie S5). By including the mutual degradation, we observed a much sharper boundary of PHB with high expression in the central cells and low expression in the outer cell layers, resulting in a much greater repression of AHP6 in the central cells. These simulations suggest that, under our parameter set, the mutual degradation of these components provides a suitable mechanism to generate distinct domains of expression of both components, where the expression of PHB and other network components are remarkably similar to the experimentally observed data (Fig. 3 A and B and Movie S6). We investigated the effect that doubling or halving these parameters had on this gradient and observed that, in almost every scenario, incorporating a mutual degradation between these two components resulted in a sharper gradient of PHB expression (SI Appendix, section 2).

**Additional Component Is Required to Position the ck Signaling Maximum Correctly.** When PHB regulation by miRNA165/6 is incorporated into our model, our simulations recreate the experimentally observed responses of some but not all markers. The predicted outputs of IAA2 and AHP6 match the data; IAA2 expression has been observed throughout the xylem axis, and AHP6 has only been observed in the marginal positions and the protoxylem-associated pericycle cells (Fig. 3 E and F and Movie S6). However, whereas in our experimental observations, ARRS5, PIN7, and the synthetic cytokinin reporter TCSn (23) were always absent throughout the xylem axis (Fig. 3 D and SI Appendix, Fig. S7), our simulations predict that both ARRS5 and ck are present in the metaxylem (Fig. 3 C and D and Movie S6), suggesting that an additional factor may be required to restrict ck homeostasis/...
cells, and in subsequent simulations, we allowed synthesis of auxin or ck in the phloem at two times the rate in other cells. We found that both scenarios were able to produce robust domains of hormonal output (Movies S7 and S8). However, when we specified the phloem as the main source of ck, we saw a non-uniform gradient of ARRS response, with the highest response close to the phloem (Movie S8). We have never observed such a distribution in our experimental data. Together, our simulations suggest that phloem-mediated ck transport is unlikely to act as a source of positional information but rather, ensures that there is a sufficient supply of ck in the root meristem.

Auxin influx carriers also control auxin transport in the vascular tissues (12). As previously published (26), we also observed localization of AUX1::AUX1-YFP on the lateral membranes of protophloem cells (SI Appendix, Fig. S7). To test whether such a component would have a significant effect on vascular patterning, we incorporated a phloem-localized auxin importer into our model and observed only modest changes in the predicted patterns, with a very slight increase in auxin response in protophloem and no change in the expression of key components, such as AHP6 (Movie S9).

Evaluation of Model Sensitivity. By encapsulating the interaction network and the experimentally observed localization of PIN proteins, our model can reproduce the observed expression patterns of many network components in each cell. As in most models of signaling networks based on differential equations, the predicted outcome of the model is reliant on the choice of its parameters (production and degradation rates, protein–DNA binding thresholds, and Hill coefficients) (27, 28). We have based certain parameters, such as the rates of auxin transport and permeability, on parameters used in previous models (4, 5), whereas we have had to estimate others. To explore the degree to which our choice of parameters affects the outcome of our model, we performed local and global sensitivity analyses of a 1D subcellular network model (SI Appendix, section 3). These analyses identified the parameters to which the model is most sensitive. These parameters were mainly associated with auxin and ck levels and, specifically in the metaxylem, the cooperativity of CKIN. We investigated the effect of perturbing all of these parameters alongside transport and permeability rates in the subcellular model. Although there was some variation in intensities, the pattern of key network components was maintained in all these simulations (SI Appendix, section 3).

Maintenance of Steady State Vascular Pattern. We next investigated whether the model was robust to small changes in the multicellular geometry. We repeated the simulations in new templates based on root cross-sections taken at ~40 μm from the quiescent center (QC), representing smaller vascular cylinders in which the cellular pattern is less developed. Although these alterations in geometry inevitably brought some small variation in the output of individual markers, the key patterning events were maintained in all these simulations (SI Appendix, Fig. S8). Vascular pattern is maintained robustly in living roots; therefore, small changes in hormone input have negligible effects, and only extreme changes (such as treatments with very high levels of ck) have dramatic effects on vascular patterning (9).

We next tested whether our model conferred a similar robustness. To test robustness, we moved away from simulations where the expression of the PINs were fixed based on experimental observations and allowed every cell the potential to express PIN7, meaning that all vascular cells could potentially express any network component (with the exception of miRNA165/6 and CKIN). We used the output from our previous simulation (Movie S7) as a set of initial conditions that closely resembled WT. We then ran this simulation to steady state and observed that, despite the fact that each cell had

Fig. 3. Integration of hormonal signaling and Hd-zip activity is sufficient to create a bisymmetric auxin response. (A–F) Model predictions and experimental observations of key marker genes are shown side by side. (A and B) Simulated PHB mRNA levels are shown alongside the experimentally observed pattern of pPHB:PHB:GFP, (C and D) predicted ARRS output is shown alongside pARR5::GUS, and (E and F) predicted AHP6 output is shown alongside pAHP6::GFP activity. These simulations are based on the original regulatory network lacking an additional inhibitor of ck. Although the simulations recover the observed pattern of PHB and AHP6, they fail to recover the observed pattern of ARRS. (G) By including the repressive effect of CKIN in the metaxylem, (H) our model reproduces the experimental pattern of pARR5::GUS.

Phloem Transport Does Not Provide an Essential Source of Positional Information During Root Vascular Patterning. Transport of both auxin and ck through the phloem provides an important source of hormones in the root meristem (12, 13). Plants with impaired phloem transport show unstable patterns of auxin response in the root meristem (14). However, it is unclear whether these unstable patterns are purely the result of decreased levels of auxin and ck in the vascular tissues or whether the phloem is required to provide a positional bias in the input of these hormones. We ran simulations to investigate the effect that introducing a spatial bias in the input of hormones had on vascular patterning. In the first simulation, auxin and ck were produced uniformly in all

signaling in these cells. Likely candidates include type A ARRs, which are known to inhibit ck signaling (24), or a member of the CKX family of proteins, which is known to degrade ck (25). Inclusion of either a CKX gene or an inhibitor of ck signaling driven in the metaxylem (CKIN) into our model (Fig. 3G) alters the pattern of ck response, such that all network components become similar to experimental observations (Fig. 3H and Movie S7).
a similar potential to express both ck and auxin marker genes and the same auxin and cytokinin production rates, the initial vascular pattern was maintained robustly (Fig. 4 A, B, F, and G) and Movie S10). For patterning to be maintained, adjacent cells of similar size and shape must be able show significantly different levels of steady state gene expression in response to a uniform auxin and cytokinin signal.

Robust patterning under homogenous conditions can be explained by the existence of multiple steady states of gene expression within each cell in the model, conferring a switch-like property in which different subsets of vascular cells can have either high AHP6 expression (protoxylem) or high ARR5 and PIN7 expression (procambium). This possibility was tested by finding the steady states of a simplified version of the model in a tissue structure consisting of two cells of equal size with a single shared cell wall (SI Appendix, section 4). The analysis shows that, using the default parameter set, at very low and very high auxin production rates, there is a single stable steady state for the model system that is equal in both cells. However, for a broad range of intermediate values for auxin production, there are two possible steady states, in which one cell has high ARR5 and PIN7 and low AHP6 and auxin and vice versa in the other cell. This bistability is also seen for a range of intermediate values of ck and sufficiently low PHB expression, suggesting that, at least in the two-cell model, after an asymmetry between neighboring cells has been established, it should be robust to small changes in auxin, ck, and PHB; however, for sufficiently large changes, the asymmetry is lost, and gene expression is equalized in both cells.

**Maintenance of Stable Vascular Pattern Requires Integration of Mobile miRNA165/6 with Hormonal Signaling.** The apparent affect of ck and PHB levels on pattern maintenance was additionally tested using the full model in the realistic tissue structure. The effect of exogenous treatment of ck was simulated by repeating the simulation shown in Movie S10 but increasing the rate of ck synthesis in all vascular cells. In a similar manner to previous experimental observations (2, 9), we observed loss of AHP6 expression and restriction of auxin response to the metaxylem (Movie S11). We next considered whether miRNA165/6 is required to maintain a stable auxin response in the xylem axis. The total levels of miRNA165/6 are dramatically reduced in the shr mutant. SHR is transcribed in the stele, but the protein is actively transported into the endodermis (29, 30). SHR is sequestered to the nucleus by the transcription factor SCR, where it promotes the expression of miRNA165/6 (10, 11).

To allow us to compare our simulation results with subsequent experimental data, we introduced SHR into our model. Guided by a previous modeling study (31), we allowed SHR transcription and protein synthesis within the stele and for SHR protein (but not mRNA) to move throughout the stele and into the endodermis through passive diffusion. Our model does not explicitly include SCR, but we have simulated its effect by allowing the production of miRNA165/6 by SHR only in the endodermis and assigning a Hill coefficient that reflects this cooperative binding. This mechanism reproduced the WT expression of all marker genes and produced similar results to our previous model (Movie S12). We then investigated whether vascular pattern could be maintained without SHR using the same conditions as shown in Movie S10 but setting SHR transcription to zero. Under these conditions, we predicted that PHB mRNA is present throughout the vascular tissues, and AHP6 is repressed in all cells. Uniformly low levels of AHP6 expression causes up-regulation of ck response at the protoxylem position and approximately homogeneous levels of PIN7 in all tissues except for the metaxylem. As a consequence, auxin is transported from the cells with high PIN7 concentration to the central cells in the xylem axis (Fig. 4 C and H and Movie S13). We then imported our entire regulatory network into the geometry of an shr mutant and ran another simulation from zero initial conditions, which predicted a highly similar pattern to the previous simulation (Fig. 4 E and J and Movie S14).

We tested these predictions by analyzing the expression of both PIN7 and the auxin response marker DR5rev::GFP in the shr mutant. Consistent with model predictions, we observed that the domain of auxin response was confined to the central cells of the xylem axis, and PIN7 was present in a radially symmetric pattern in all but the central vascular cells (Fig. 4 D and I). Together our results show that, in addition to the documented roles of SHR in specifying cell identity, stem cell function, and lateral root growth (10, 29, 32), SHR also directs hormonal responses during vascular patterning.
Discussion

We have used a multicellular mathematical model to probe whether the mutually inhibitory interaction between the transport/signaling of two hormones can act as a plausible vascular patterning mechanism. Previous models based exclusively on auxin transport in roots have analyzed the flux of auxin in the longitudinal sections (4, 5, 33). We have extended this concept to account for the lateral transport of auxin and shown that it determines radial patterning.

Our multicellular model also incorporates auxin and cytokinin signaling networks together with PHB, miRNA165/6, and SHR. It has previously been suggested that small RNA species could repress their targets with a tunable threshold to create sharp boundaries of gene expression (21). We have modeled this interactive mechanism at the organ scale and showed that it is capable of creating the sharp gradient of PHB seen in WT roots. Previously, it has been shown that miRNA165/6 can regulate PHB through ARR1 to control root growth (34). However, because ARR1 is absent in the domain that we have modeled, it suggests that similar components can regulate the same targets in a developmentally specific context. Our study provides insights into how these species may be regulated in vascular tissues, and it will be interesting to see whether these concepts can be applied more widely to the role of PHB in controlling root growth as well as other developmental contexts.

Our simulations show that the restriction of PHB by miRNA165/6 is required in establishing a bisymmetric auxin response, and we have verified this prediction experimentally by showing that shrt mutants (which lack miRNA165/6) are unable to generate a bisymmetric auxin response throughout the xylem axis. Our model also revealed that a missing component is required to explain the spatial expression of ARR5. By including such a component, our model yields gene expression patterns that are consistent with the experimental data, illustrating the power of computational modeling as a predictive tool for determining minimum network requirements. We have identified a minimum framework necessary for establishing vascular pattern in Arabidopsis roots. We recognize that, in biological systems, the minimal network is rarely used, and there is often a high degree of genetic redundancy. We have addressed this redundancy for the PINs and investigated the role of each PIN protein.

In conclusion, we have constructed a multicellular computational model that embodies the concepts that mobile miRNAs and mutually inhibitory domains of hormonal signaling can act as mechanisms for generating pattern. This regulatory network for vascular patterning differs conceptually from other mechanisms that have been shown to generate pattern in plants based on either reaction-diffusion dynamics or reflux patterns (4, 35). Our model shows that the integration of movement by diffusion (miRNA165/6), the targeted transport of auxin, and the mutual degradation of PHB and miRNA165/6 offer a plausible way to generate vascular pattern.

Materials and Methods

The mathematical model is described in detail in the SI Appendix, section 1. Plant lines were all in the Columbia background and are described in SI Appendix, section 6. Confocal microscopy was performed on an inverted Leica SPS confocal with propidium iodide used to counterstain the roots. Visualization of the GUS marker was performed as previously described (2).

ACKNOWLEDGMENTS. We thank Leah Band for helpful comments. We acknowledge the Biotechnology and Biological Sciences Research Council and the Engineering and Physical Sciences Research Council for funding. H.M.B. was funded, in part, by King Abdullah University of Science and Technology (KAUST) Award KUK-013-04, J.R.K. was funded by the Royal Society and Wolfson Foundation, and A.B. was funded by a Royal Society University Research Fellowship. J.G and C.G. were funded by the Institut de Biologie Computationnelle de Montpellier and the Morphogenetics Inria Project-Lab.
The integration of hormonal signaling networks and mobile microRNAs is required for vascular patterning in *Arabidopsis* roots.

Supporting Information

November 28, 2013

Supplementary Figures

Figure 1: The tissue representation is taken from a cross section through the meristematic region of a primary root. Cell vertices, cell walls and cell types are manually drawn from an image based on *IAA2::GUS* staining. Scale bar = 10 µm.
Figure 2: Split channel images of PIN1::PIN1::GFP, PIN3::PIN3::GFP and PIN7::PIN7::GFP. The red channel shows propidium iodide which stains the plasma membrane and the green channel shows GFP. As reported [1] [2] [3] PIN1 is present throughout the stele and PIN7 is present in the procambium, phloem and associated pericycle cells. Whilst there is almost no variation in the patterns of PIN1 and PIN7, some variability exists in the PIN3 pattern and it is sometimes present in a broader domain. However, it is consistently strong in the protoxylem associated pericycle cells. Scale bars = 20 µm.
Figure 3: Characterization of PINs with immunolocalization. This allowed us to develop detailed z stacks without the samples bleaching. PIN1 is detected with α-PIN1 antibody, whereas PIN3::PIN3:GFP and PIN7::PIN7:GFP are detected with α-GFP antibodies. Using this approach we detected PIN1 in the endodermis (labeled end), however it was not localized to any lateral membranes in these cells. We observed PIN1 on the lateral membranes of the pericycle cells (pc). Here it appears to localize to the radial membranes (i.e. directing auxin between the pericycle cells) and the centripetal membranes (i.e. directing auxin into the stele). We did not observe any PIN1 on the centrifugal membranes in the pericycle cells. In the intervening procambial stem cells (ipcs) we observed signal on all lateral membranes. In contrast within the xylem cells (xy) we did not see any lateral localization of PIN1 and the signal was purely localized to the basal membrane. As before, we observed signal in the protoxylem-associated pericycle cells. This appeared to include partial centripetal localization. We also observed some PIN3 signal in the protoxylem. However, this was not accompanied with any observable lateral localization. We observed radial and centripetal localization of PIN7 in the pericycle cells (suggesting that auxin could be directed between these cells and into the vascular cylinder). We observed lateral localization of PIN7 on what appeared to be the radial, centripetal and centrifugal membranes.
Figure 4: Further analysis of the PIN7 localization by CellSeT [4]. CellSeT segments confocal images and assigns unique identities to each cell and walls between cells. The software then samples the fluorescence offset between the red (propidium iodide stained cell wall) and the green (PIN7:GFP) channels, and produce Gaussian plots of each. It will determine any offset between these Gaussians and report this at sub-pixel accuracy. The offset illustrates the direction in which PINs are localized on the plasma membrane and this information is returned with a confidence value based on the number of offset pixels. Based on this technology, we were able to determine the polarity of PIN7 on the lateral membranes of procambial cells. (A) In the first instance we tested the localization of PIN7 in procambial cells on the walls flanking the xylem axis. As we do not observe any PIN7 within the xylem axis, we expected all of the PIN to be localized on the centrifugal side of this cell wall (i.e. within the procambial cells). We observed this localization 94% of the time (n=46). Furthermore, the 6% of cells where CellSeT reported the PIN to be present in the xylem cells were supported by very low confidence values (0.16 pixels compared with 0.93 pixels). The white lines show which side of the cell wall the GFP signal is predicted and an associated offset value. The gray lines show predictions that are supported with pixel offset values below 0.1. (B) Our confocal images and immunolocalizations suggest that PIN7 was present on all lateral membranes in procambial cells. These were based on observing the confocal images by eye. However, when we see PIN7 associated with a cell wall between two cells expressing PIN7, it isn’t possible to be sure whether PIN7 is localized on both or just one of the plasma membranes. We reasoned that if PIN7 was present on both cell membranes then it would predict the offset in the cell with the strongest expression of PIN7. However, as PIN7 levels appear to be approximately equal in each of the procambial cells we reasoned that if PIN7 was present in both plasma membranes this would be evident from CellSeT because there would be no clear consensus about which way the PINs would be localized and because the offset values would be relatively low. We observed that in 57% of the cases CellSeT called the offset to be in the centrifugal direction (n=76) and in both the cases where CellSeT predicted either a centrifugal and centripetal offset, these were supported by somewhat lower offset values (0.62 and 0.65 pixels respectively). Taken together these results strongly support our previous observations showing the localization of PIN7 on all lateral membranes in procambial cells.
Figure 5: Documentation of PIN3 localization with CellSeT. The top left image shows the raw confocal image. The software segments the image and identifies and assigns unique numbers to each cell. The user manually assigns cells of interest with GFP expression and selects these cells along with the neighboring cells. Although there is considerable variation, the PIN3 accumulating on the boundary between pericycle cells and their neighbors is predominantly localized on the pericycle-side of the cell wall suggesting that these cells export PIN to their neighbors. We observed this in 70% of pericycle-neighbor boundaries (n=64). An example of a marked up image showing the localization of PIN3 is shown in the left panel. The lower images zoom in on one of the xylem poles. The cell numbered “99” is a protoxylem cell, and the cells numbered “98” and “106” are protoxylem-associated pericycle cells.

Figure 6: Schematic showing the localization of PINs 1, 3 and 7 used in this study. The cell types are annotated in the same way as FigS1 (phloem = yellow, procambium = green, xylem = blue, pericycle = red and endodermis = orange). The red arrows demonstrate the polarity of the PINs and the direction in which they will transport auxin.
In this section we develop our multicellular mathematical model of vascular patterning in a realistic geometry, as used to generate Supplementary Movies 1-9. Initially, the tissue representation is derived by manually drawing the cell walls and the distribution of auxin efflux transporters PIN1, PIN3 and PIN7 using the open source software Inkscape. Cell wall geometry is reproduced from a digital photograph of a root cross section. The subcellular location of the PINs is based on confocal images (see Supplementary Figures 2 and 3), and in the case of PIN3 and PIN7 has been determined using the CellSeT software [4]. The framework used for the model itself is OpenAlea, an open source software project for plant architecture modeling[5]. Libraries and tools in OpenAlea are primarily based on the use of the high level, object-oriented script language, Python. The .xml format file encoding the cell wall structure plus PIN locations produced by Inkscape is converted into a format compatible for use in OpenAlea using a separate Python script. Once this is done, the sub-cellular interaction network, as summarised in Figure 1 and Supplementary Figure 9, is then coded into ordinary differential equations (ODEs) and embedded in every cell in the realistic tissue structure using the OpenAlea software. The ODE model is derived below, and a table summarizing the species included in the model is reported in Supplementary Table 1.
Figure 9: Schematic representation of the network regulating vascular patterning. Green solid arrows indicate gene activation, red solid arrows indicate gene inhibition, the dashed green arrow indicates the transport on auxin mediated by PIN7 and the purple segment represents mutual degradation between miRNA165/166 and PHB mRNA.

### Table 1: Table summarising the species included in the mathematical model defined by Eqs. (1)-(6).

<table>
<thead>
<tr>
<th>Species symbol</th>
<th>Species name</th>
<th>Species type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aux</td>
<td>Auxin</td>
<td>hormone</td>
</tr>
<tr>
<td>CK</td>
<td>Cytokinin</td>
<td>hormone</td>
</tr>
<tr>
<td>ARR5m</td>
<td>Arabidopsis response regulator 5</td>
<td>mRNA</td>
</tr>
<tr>
<td>ARR5p</td>
<td>Arabidopsis response regulator 5</td>
<td>protein</td>
</tr>
<tr>
<td>AHP6m</td>
<td>Arabidopsis histidine phosphotransfer 6</td>
<td>mRNA</td>
</tr>
<tr>
<td>AHP6p</td>
<td>Arabidopsis histidine phosphotransfer 6</td>
<td>protein</td>
</tr>
<tr>
<td>IAA2m</td>
<td>indole-3-acetic acid inducible 2</td>
<td>mRNA</td>
</tr>
<tr>
<td>IAA2p</td>
<td>indole-3-acetic acid inducible 2</td>
<td>protein</td>
</tr>
<tr>
<td>PIN1p</td>
<td>Arabidopsis thaliana PIN-formed 1</td>
<td>protein</td>
</tr>
<tr>
<td>PIN3p</td>
<td>Arabidopsis thaliana PIN-formed 3</td>
<td>protein</td>
</tr>
<tr>
<td>PIN7m</td>
<td>Arabidopsis thaliana PIN-formed 7</td>
<td>mRNA</td>
</tr>
<tr>
<td>PIN7p</td>
<td>Arabidopsis thaliana PIN-formed 7</td>
<td>protein</td>
</tr>
<tr>
<td>PHBm</td>
<td>Phabulosa</td>
<td>mRNA</td>
</tr>
<tr>
<td>PHBp</td>
<td>Phabulosa</td>
<td>protein</td>
</tr>
<tr>
<td>miRNA165/6</td>
<td>microRNA 165/6</td>
<td>micro RNA</td>
</tr>
<tr>
<td>CKIN</td>
<td>Cytokinin inhibitor</td>
<td>unknown</td>
</tr>
</tbody>
</table>

**Sub-cellular interactions.** Our model is based on the following biological hypotheses. All genetic loci are transcribed and translated in every cell and all the species are degraded in every cell except miR165/6 (which is transcribed only in the endodermis) and CKIN (which is transcribed only in the metaxylem). In all other tissues the transcription rates of CKIN and miRNA₆₆₅/₆₆₆, PCKINₐₘ, are set to zero. PIN1, PIN3 and PIN7 are located as experimentally observed on the cell walls represented in Supplementary Figure 7 and auxin is actively transported between cells in which at least one of the PIN proteins is present.

Based on the above mentioned hypotheses, we model transcription via the following reactions:

\[
\begin{align*}
\emptyset & \overset{p_{AHP6m}}{\longrightarrow} AHP6_m, \\
\emptyset & \overset{p_{IAA2m}}{\longrightarrow} IAA2_m, \\
\emptyset & \overset{p_{ARR5m}}{\longrightarrow} ARR5_m, \\
\emptyset & \overset{p_{PHBm}}{\longrightarrow} PHB_m,
\end{align*}
\]

\[
\begin{align*}
AHP6_m & \overset{d_{AHP6m}}{\longrightarrow} \emptyset, \\
IAA2_m & \overset{d_{IAA2m}}{\longrightarrow} \emptyset, \\
ARR5_m & \overset{d_{ARR5m}}{\longrightarrow} \emptyset, \\
PHB_m & \overset{d_{PHBm}}{\longrightarrow} \emptyset.
\end{align*}
\]

where \(p_{AHP6m}, p_{IAA2m}, p_{ARR5m}, p_{PHBm}, p_{ARR7m}, p_{PHBm}\) are transcription rates, \(d_{AHP6m}, d_{IAA2m}, d_{ARR5m}, d_{PHBm}\), are degradation rates.
are degradation rates and $F_{AHP6_m}$, $F_{IAA2_m}$, $F_{ARR5_m}$ are Hill functional forms given by:

$$F_{AHP6_m}([\text{Aux}]_i, [\text{PHB}]_i) = \frac{([\text{Aux}]_i/\theta_{AUX})^{m_{AUX}}}{1 + ([\text{Aux}]_i/\theta_{AUX})^{m_{AUX}} + ([\text{PHB}]_i/\theta_{PHB})^{m_{PHB}}},$$

$$F_{IAA2_m}([\text{Aux}]_i) = \frac{([\text{Aux}]_i/\theta_{AUX})^{m_{AUX}}}{1 + ([\text{Aux}]_i/\theta_{AUX})^{m_{AUX}}} ;$$

$$F_{ARR5_m}([\text{Aux}]_i, [\text{Ck}]_i) = \frac{([\text{Ck}]_i/\theta_{CK})^{m_{CK}}}{1 + ([\text{Ck}]_i/\theta_{CK})^{m_{CK}} + ([\text{AHP6}]_i/\theta_{AHP6})^{m_{AHP6}}},$$

(1)

where $i$ is the cell index, $\theta_{AUX}$, $\theta_{CK}$, $\theta_{PHB}$, $\theta_{AHP6}$ are the binding thresholds of the relevant proteins, $m_{AUX}$, $m_{CK}$, $m_{AHP6}$ are Hill coefficients. PIN1, PIN3 and PIN7 protein concentrations are divided among the cell walls where these proteins are present (see Supplementary Figure 7). In Supplementary Movies 1-4, their total concentration within each cell containing PINs is fixed to the unitary value, whereas in Supplementary Movies 5-9 only PIN7 is present and its mRNA concentration is governed by the Hill function:

$$F_{PIN7_m}([\text{ARR5}]_i) = \frac{([\text{ARR5}]_i/\theta_{ARR5})^{m_{ARR5}}}{1 + ([\text{ARR5}]_i/\theta_{ARR5})^{m_{ARR5}}} ,$$

(2)

where $i$ is the cell index, $\theta_{ARR5}$ is $\text{ARR5}$ binding threshold and $m_{ARR5}$ its Hill coefficient.

We suppose that CKN mRNA is produced only in the metaxylem via:

$$\emptyset \xrightarrow{pC{\text{CKIN}}_m} \text{CKIN}_m, \quad \text{CKIN}_m \xrightarrow{dC{\text{CKIN}}_m} \emptyset,$$

and miRNA165/166 only in the endodermis via:

$$\emptyset \xrightarrow{p{\text{miRNA}}} \text{miRNA165/166}, \quad \text{miRNA165/166} \xrightarrow{d{\text{miRNA}}_p} \emptyset,$$

where $pC{\text{CKIN}}_m, dC{\text{CKIN}}_m, p_{\text{miRNA}}, d_{\text{miRNA}}$ are production and degradation rates. PHB mRNA and miRNA165/166 accelerate each other’s degradation at rate $d_{\text{miRNA/mRNA}}$ as follows [6]:

$$\text{PHB}_m + \text{miRNA165/166} \xrightarrow{d_{\text{miRNA/mRNA}}} \emptyset.$$

From the above reactions we obtain the following system of ordinary differential equations:

$$\frac{d[\text{AHP6}]_i}{dt} = p_{AHP6_m} F^{(i)}_{AHP6_m} - d_{AHP6} [\text{AHP6}]_i,$$

$$\frac{d[IAA2]}{dt} = p_{IAA2_m} F^{(i)}_{IAA2_m} - d_{IAA2} [I AA2]_i,$$

$$\frac{d[\text{ARR5}]}{dt} = p_{ARR5_m} F^{(i)}_{ARR5_m} - d_{ARR5} [\text{ARR5}]_i,$$

$$\frac{d[\text{PIN7}]}{dt} = p_{PIN7_m} F^{(i)}_{PIN7_m} - d_{PIN7} [\text{PIN7}]_i,$$

$$\frac{d[\text{PHB}]}{dt} = p_{PHB_m} - d_{PHB}[\text{PHB}]_i - d_{miRNA/mRNA}[\text{PHB}]_i [\text{miRNA165/166}]_i,$$

$$\frac{d[\text{CKIN}]}{dt} = \begin{cases} p_{C{\text{CKIN}}_m} - dC{\text{CKIN}}_m [\text{CKIN}]_i, & \text{if } i \text{ in metaxylem}, \\ 0, & \text{otherwise}, \end{cases}$$

(3)

Translation of these mRNAs is modelled assuming the following reactions:

$$\text{AHP6}_m \xrightarrow{p_{AHP6}} \text{AHP6}_m + \text{AHP6}_p,$$

$$\text{IAA2}_m \xrightarrow{p_{IAA2}} \text{IAA2}_m + \text{IAA2}_p,$$

$$\text{ARR5}_m \xrightarrow{p_{ARR5}} \text{ARR5}_m + \text{ARR5}_p,$$

$$\text{PIN7}_m \xrightarrow{p_{PIN7}} \text{PIN7}_m + \text{PIN7}_p,$$

$$\text{PHB}_m \xrightarrow{p_{PHB}} \text{PHB}_m + \text{PHB}_p,$$

$$\text{CKIN}_m \xrightarrow{p_{C{\text{CKIN}}}} \text{CKIN}_m + \text{CKIN}_p,$$

$$\text{AHP6}_p \xrightarrow{d_{AHP6}} \emptyset,$$

$$\text{IAA2}_p \xrightarrow{d_{IAA2}} \emptyset,$$

$$\text{ARR5}_p \xrightarrow{d_{ARR5}} \emptyset,$$

$$\text{PIN7}_p \xrightarrow{d_{PIN7}} \emptyset,$$

$$\text{PHB}_p \xrightarrow{d_{PHB}} \emptyset.$$
where $p_{AHP6_p}, p_{IAA2_p}, p_{ARR5_p}, p_{PIN7_p}, p_{PHB_p}, p_{CKIN_p}$ are translation rates and $d_{AHP6_p}, d_{IAA2_p}, d_{ARR5_p}, d_{PIN7_p}, d_{PHB_p}, d_{CKIN}$ are degradation rates. From these reactions we obtain the following system of ordinary differential equations:

$$
\begin{align*}
\frac{d[AHP6_p]}{dt} &= p_{AHP6_p} [AHP6_m]_i - d_{AHP6_p} [AHP6_p]_i, \\
\frac{d[IAA2_p]}{dt} &= p_{IAA2_p} [IAA2_m]_i - d_{IAA2_p} [IAA2_p]_i, \\
\frac{d[ARR5_p]}{dt} &= p_{ARR5_p} [ARR5_m]_i - d_{ARR5_p} [ARR5_p]_i, \\
\frac{d[PIN7_p]}{dt} &= p_{PIN7_p} [PIN7_m]_i - d_{PIN7_p} [PIN7_p]_i, \\
\frac{d[PHB_p]}{dt} &= p_{PHB_p} [PHB_m]_i - d_{PHB_p} [PHB_p]_i, \\
\frac{d[CKIN_p]}{dt} &= p_{CKIN_p} [CKIN_m]_i - d_{CKIN_p} [CKIN_p]_i.
\end{align*}
$$

(4)

**Transport and diffusion.** The modeling of transport and diffusion is inspired by the auxin transport model developed in [7]. We denote by $V_i$ ($\mu m^2$) the volume of cell $i$ and by $N_i$ the set of neighboring cells of cell $i$. If $i$ and $n$ are two neighboring cells, $S_{i,n}$ ($\mu m^2$) denotes the area of the exchange surface, and $[PIN1_p]_{i,n}$, $[PIN3_p]_{i,n}$ and $[PIN7_p]_{i,n}$ are the respective levels of PIN1, PIN3 and PIN7 on the membrane of cell $i$ facing cell $n$. The sum of each of the membrane bound PINs in a given cell is equal to the total intracellular level of that PIN ($[PIN1_p]_i$, $[PIN3_p]_i$ and $[PIN7_p]_i$), and is distributed between each of the walls with PIN according to the ratio of their surface areas.

We assume auxin, cytokinin and miRNA165/166 to be passively transported between cells, with background permeabilities $P_{Aux}, P_{Ck}, P_{miRNA}$. Auxin is additionally transported actively by PIN carriers with rate $T_{Aux}$. Assuming that cytokinin biosynthesis is inhibited by CKIN protein, we model such inhibition by the saturating form:

$$
F_{Ck}([CKIN_p]_i) = \frac{1}{1 + ([CKIN_p]_i/\theta_{CKIN})^{m_{CKIN}}}.
$$

(5)

where $\theta_{CKIN}$ is the binding threshold of the protein CKIN and $m_{CKIN}$ a Hill coefficient. We assume that auxin and cytokinin biosynthesis and degradation occurs in every cell of the root section inside the endodermis and, to account for their transport through the phloem, we enable our model to have different hormone biosynthesis rates in this particular tissue. More precisely, auxin and cytokinin biosynthesis and degradation are governed by the reactions:

$$
\begin{align*}
\emptyset & \xrightarrow{P_{Aux}} \text{Aux}, & \text{Aux} & \xrightarrow{d_{Aux}} \emptyset, \\
\emptyset & \xrightarrow{P_{Ck}} \text{Ck}, & \text{Ck} & \xrightarrow{d_{Ck}} \emptyset,
\end{align*}
$$

where $P_{Aux}, P_{Ck}$ are biosynthesis rates and $d_{Aux}, d_{Ck}$ are degradation rates. From the above considerations we obtain the following system of ordinary differential equations:

$$
\begin{align*}
\frac{d[Aux]}{dt} &= P_{Aux} - d_{Aux} [Aux]_i \\
&\quad - \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} P_{Aux} ([Aux]_i - [Aux]_n) \\
&\quad - \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} T_{Aux} ([PIN1_p]_{i,n} + [PIN3_p]_{i,n} + [PIN7_p]_{i,n}) [Aux]_i \\
&\quad - ([PIN1_p]_{i,n} + [PIN3_p]_{i,n} + [PIN7_p]_{i,n}) [Aux]_n, \\
\frac{d[Ck]}{dt} &= P_{Ck} F_{Ck} - d_{Ck} [Ck]_i - \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} P_{Ck} ([Ck]_i - [Ck]_n), \\
\frac{d[miRNA165/166]}{dt} &= P_{miRNA} - d_{miRNA} [miRNA165/166]_i \\
&\quad - d_{miRNA/mRNA} [PHB_m]_i [miRNA165/166]_i \\
&\quad - \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} P_{miRNA} ([miRNA165/166]_i - [miRNA165/166]_n).
\end{align*}
$$

(6)
where \( i \) is the cell index and \( p_{miRNA} = 0 \) in all cells outside the endodermis.

The experimental evidence suggest that auxin and cytokinin marker genes are absent from the endodermis. To reflect this in the model additional degradation terms are added to the equations for auxin and cytokinin in (6):

\[
- \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} P_{\text{Aux}}^{\text{end}} [\text{Aux}]_i,
\]

\[
- \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} P_{\text{Ck}}^{\text{end}} [\text{Ck}]_i,
\]

for \( i \) in the endodermis. These terms also account for loss of auxin and cytokinin to the outer root tissues not included in the tissue geometry and, in the case of auxin, from top-down transport in the primary root. To keep auxin and cytokinin at a low level in the endodermis we set \( P_{\text{Aux}}^{\text{end}} = 20.0 \cdot \mu m \cdot \mu M^{-1} \cdot s^{-1} \) and \( P_{\text{Ck}}^{\text{end}} = 100.0 \cdot \mu m \cdot \mu M^{-1} \cdot s^{-1} \).

In Supplementary Movie 9 we include the influx carrier AUX1 and we model auxin transport as follows:

\[
\frac{d[\text{Aux}]_i}{dt} = p_{\text{Aux}} - d_{\text{Aux}}[\text{Aux}]_i - \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} P_{\text{Aux}} ([\text{Aux}]_i - [\text{Aux}]_n)
\]

\[- \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} T_{\text{Aux}} ([\text{PIN7}]_{p,i,n} [\text{Aux}]_i - [\text{PIN7}]_{p,n} [\text{Aux}]_n),
\]

\[- \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} T_{\text{AUX1}} ([\text{AUX1}]_{p,i,n} [\text{Aux}]_n - [\text{AUX1}]_{p,i,n} [\text{Aux}]_i),
\]

where \( T_{\text{AUX1}} \) is the influx rate due to AUX1 expression. AUX1 is localised on all the membranes of the phloem cells and is constantly expressed in the phloem, its unitary concentration being proportionally divided on the cell walls.

**Parameters.** As in other mathematical analyses of signaling networks, only partial information is known about the parameter values of the model [8], [9]. As in [8], the parameter values have been specified as follows. The parameters relating to auxin transport efficiency (\( T_{\text{Aux}} \)) and auxin background permeability (\( P_{\text{Aux}} \)) have been analyzed in [10], [11] and we use the same values for those parameters (see Supplementary Table 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T_{\text{Aux}} )</td>
<td>Transport efficiency of PIN proteins</td>
<td>20.0 \cdot \mu m \cdot \mu M^{-1} \cdot s^{-1}</td>
</tr>
<tr>
<td>( P_{\text{Aux}} )</td>
<td>Auxin background permeability</td>
<td>1.0 \cdot \mu m \cdot s^{-1}</td>
</tr>
<tr>
<td>( P_{\text{Ck}} )</td>
<td>Cytokinin background permeability</td>
<td>10.0 \cdot \mu m \cdot s^{-1}</td>
</tr>
<tr>
<td>( P_{miRNA} )</td>
<td>miRNA background permeability</td>
<td>1.5 \cdot \mu m \cdot s^{-1}</td>
</tr>
<tr>
<td>( p_{\text{Aux}} )</td>
<td>Auxin biosynthesis rate</td>
<td>0.06 \cdot \mu M^{-1} \cdot s^{-1}</td>
</tr>
<tr>
<td>( p_{\text{Ck}} )</td>
<td>Cytokinin biosynthesis rate</td>
<td>2.0 \cdot \mu M^{-1} \cdot s^{-1}</td>
</tr>
<tr>
<td>( p_{\text{CKIN}p} )</td>
<td>CKIN protein translation rate</td>
<td>1.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( p_{\text{AHP6p}} )</td>
<td>AHP6 protein translation rate</td>
<td>1.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( p_{\text{IAA2p}} )</td>
<td>IAA2 protein translation rate</td>
<td>10.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( p_{\text{ARR5p}} )</td>
<td>ARR5 protein translation rate</td>
<td>10.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( p_{\text{PIN7p}} )</td>
<td>PIN7 protein translation rate</td>
<td>5.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( p_{\text{PHBp}} )</td>
<td>PHB protein translation rate</td>
<td>1.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( d_{\text{Aux}} )</td>
<td>Auxin degradation rate</td>
<td>1.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( d_{\text{Ck}} )</td>
<td>Cytokinin degradation rate</td>
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</tr>
<tr>
<td>( d_{\text{CKINp}} )</td>
<td>CKIN protein degradation rate</td>
<td>1.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( d_{\text{AHP6p}} )</td>
<td>AHP6 protein degradation rate</td>
<td>1.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( d_{\text{IAA2p}} )</td>
<td>IAA2 protein degradation rate</td>
<td>10.0 \cdot s^{-1}</td>
</tr>
<tr>
<td>( d_{\text{ARR5p}} )</td>
<td>ARR5 protein degradation rate</td>
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<tr>
<td>( d_{\text{PIN7p}} )</td>
<td>PIN7 protein degradation rate</td>
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</tr>
<tr>
<td>( d_{\text{PHBp}} )</td>
<td>PHB protein degradation rate</td>
<td>1.0 \cdot s^{-1}</td>
</tr>
</tbody>
</table>

Table 2: Default values of the parameters regulating translation and cell-to-cell communication.
Table 3: Default values of the parameters regulating transcription. The Hill coefficients have been set greater than one to reflect a non-linear response of genes to activators and repressors. Where known, these have been chosen to reflect the presence of multiple binding sites within a promoter or the number of genes required for a given response. For example, both AHP6 and IAA2 contain multiple Auxin Response Elements (AuxREs).

Following [10], we assume that the ratio between rates of active and passive transport to be 1/20. The other parameters are unknown: initially, as proposed in [8], all unknown parameters were set to be unity of their particular unit; subsequently, some have been modified in the light of experimental observations. We explored a range of values for the production and degradation of PHB mRNA and miR, and selected realistic values from this range that reproduced the patterns observed in [12]. We subsequently examined the effect that altering these values had (see section 2) and found that quite significant changes were required in order to disrupt the gradient of PHB. Due to the partial arbitrariness of the parameter choice, and to test the robustness of the model results, in the following sections we develop local and global sensitivity analyses of our signaling network model in single cells, taken from different tissues, and we analyze the effects of parameter perturbations in the two-dimensional geometry. The default parameter values and units used in the multicellular model are reported in Supplementary Tables 2, 3. Variations around these values have been applied to produce Supplementary Movies 1-7 and are reported in the legends of the Supplementary Movies. With this choice of parameters the model evolves to a stable pattern in approximately 15 seconds simulated time (see Supplementary Movies 1-7). A typical simulation result is presented in Supplementary Movie 6.
2 Exploration of parameter space in PHB - miRNA165/6 sub model

The spatial distribution of PHB protein is modified in the model solely via the interaction and mutual degradation of miRNA and PHB mRNA. Because of this the equations for both in (3) and (6) may be considered as a separate sub-model defining the spatial expression of PHB mRNA. Figure 10(a) shows the steady state distribution of PHB mRNA using the default parameter values given in Table 3. The robustness of model PHB expression pattern to parameter changes was tested by considering the mean mRNA values in five concentric cell layers as shown in Figure 10(b). These were then plotted relative to the peak PHB expression in the central layer (L1) for a variety of parameter values. The distribution for the default parameter set is shown in figure 10(c). PHB mRNA distributions for variations in each of the default parameter values for $d_{miRNA/mRNA}$, $p_{PHBm}$, $d_{PHBm}$, $P_{miRNA}$, $d_{miRNA}$ and $p_{miRNA}$ are shown in Figure 11.

Figure 10: (A.) Steady state PHB protein in the wild type root cross section using the default parameter values in Table 3 with the original model (Equations (3) and (6)). (B.) Schematic root cross section showing cell layer groupings used to produce the mean value plots in C and in Figure 11. (C.) Mean PHB mRNA and miRNA in each of the cell layer groupings L1-L5 for both the original model (red), and the model given by Equations (7a) and (7b) in which miRNA is not degraded by the interaction with PHB mRNA (blue). For PHB mRNA each bar is plotted relative to the value in L1 using the original model, while for miRNA each bar is plotted relative to the value in L5 with the original model.

To demonstrate the importance of the mutual degradation of both the miRNA and PHB mRNA in establishing a sharp boundary to the domain of PHB expression an alternative model was considered in which while the miRNA still accelerates the degradation of PHB mRNA, it is itself no longer consumed in this reaction, as follows:

$$\frac{d[miRNA]_i}{dt} = p_{miRNA} - d_{miRNA}[miRNA]_i - \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} P_{miRNA} ([miRNA]_i - [miRNA]_n), \quad (7a)$$

$$\frac{d[PHBm]_i}{dt} = p_{PHBm} - d_{PHBm}[PHBm]_i - d_{miRNA/mRNA}[PHBm]_i[miRNA]_i, \quad (7b)$$

where the model variables and parameter values are as previously defined. For this alternative model the mean PHB mRNA in each cell layer L1-L5 is plotted, relative to the L1 value using the full model, for each of the parameter sets as shown in Figures 10(c) and 11. In each case, removing the mutual degradation of miRNA and PHB mRNA and replacing with just the miRNA promoted degradation of PHB mRNA removes the sharp boundary between strong and weak PHB expression in adjacent cell layers.
EXPLORATION OF PARAMETER SPACE IN PHB - MIRNA165/6 SUB MODEL

Figure 11: Alterations in parameter space in the PHB mRNA/miRNA submodel. Red bars: Mean steady state PHB mRNA using the original model (Equations (3) and (6)) in each of the cell layers L1-L5 (defined in Figure 10(b)), relative to the value in L1, for half and double each of the default parameter values for $d_{miRNA/mRNA}$, $p_{PHB}$, $d_{PHB}$, $P_{miRNA}$, $d_{miRNA}$ and $p_{miRNA}$ given in Table 3. In each case the remaining parameter values are kept to the default values. Blue bars: As for plot shown with red bars but using the model in which miRNA is not degraded by the interaction with PHB mRNA (Equations (7a) and (7b)). The bars are normalised to the value in L1 found using the original model.

2.1 Continuous approximation of the PHB - miRNA165/6 sub model

2.2 Model derivation

To examine further the relevance of mutual degradation in creating a sharp boundary of PHB mRNA we assumed that the root cross section has approximately radial symmetry with respect to geometry, production and degradation of PHB and miRNA and analyzed the evolution of $[\text{miRNA}] (r, \theta, t)$ and $[\text{PHB}_m] (r, \theta, t)$ on a circle of radius $\bar{R}$ with $r \in [0, \bar{R}]$, $\theta \in [0, 2\pi]$ and $t \in \mathbb{R}^+$. We considered a two-dimensional discrete model comprising the interaction between miRNA and PHB mRNA and neglecting the other molecular interactions. The model assumes $N_r$ cells along any radius for a fixed angle, $N_\theta$ cells along any circumference for a fixed radius and is given by the equations

$$\frac{\partial [\text{miRNA}](r, \theta, t)}{\partial t} = p_{\text{miRNA}}(r) - d_{\text{miRNA}}[\text{miRNA}](r, \theta, t) - d_{\text{miRNA/mRNA}}[\text{miRNA}](r, \theta, t)[\text{PHB}_m](r, \theta, t) + D\Delta_d[\text{miRNA}](r, \theta, t),$$

$$\frac{\partial [\text{PHB}_m](r, \theta, t)}{\partial t} = p_{\text{PHB}_m} - d_{\text{PHB}_m}[\text{PHB}_m](r, \theta, t) - d_{\text{miRNA/mRNA}}[\text{miRNA}](r, \theta, t)[\text{PHB}_m](r, \theta, t),$$

where $\Delta_d$ is a discrete Laplacian operator accounting for the circular geometry,

$$\Delta_d[\text{miRNA}](r, \theta, t) := \frac{[\text{miRNA}](r - \delta_r, \theta, t) - 2[\text{miRNA}](r, \theta, t) + [\text{miRNA}](r + \delta_r, \theta, t)}{\delta_r^2} + \frac{1}{r} \frac{[\text{miRNA}](r + \delta_r, \theta, t) - [\text{miRNA}](r - \delta_r, \theta, t)}{2\delta_r} + \frac{1}{r^2} \frac{[\text{miRNA}](r, \theta - \delta_\theta, t) - 2[\text{miRNA}](r, \theta, t) + [\text{miRNA}](r, \theta + \delta_\theta, t)}{\delta_\theta^2},$$

(9)
$D$ is the diffusion coefficient of $[\text{miRNA}]$, $r = i \cdot \delta_r = i \cdot \frac{R}{N_r}$ and $\theta = j \cdot \delta_\theta = j \cdot \frac{\pi}{N_\theta}$, where $i = 1, \ldots, N_r$ and $j = 1, \ldots, N_\theta$ are the cell indexes, $\delta_r, \delta_\theta$ are the radial and angular spacings, and where reactions and parameters are defined as in Section 1. Approximate estimates measured from cross sections in root regions similar to the one represented in Supplementary Figure 1 suggest that on average $\bar{R} = 45 \, \mu m$. We assume that miRNA is transcribed in a region close to the boundaries, representing the endodermis, at rate:

$$p_{\text{miRNA}}(r) := \begin{cases} 32.5 \, \mu M \cdot s^{-1} & \text{if } 0 \, \mu m \leq r \leq 5 \, \mu m \text{ or } 40 \, \mu m \leq r \leq 45 \, \mu m, \\ 0 \, \mu M \cdot s^{-1} & \text{if } 5 \, \mu m \leq r \leq 40 \, \mu m. \end{cases}$$

Accounting for radial symmetry and assuming that $\delta_r, \delta_\theta \ll 1$, we then derive a continuum limit and analyze how the steepness of $[PHB_m]$ profile varies when we vary the degradation rate $d_{\text{miRNA}/mRNA}$ in two model variants. In the first variant, we assume that miRNA and $PHB$ mRNA mutually degrade one another following the equations:

$$\frac{\partial [\text{miRNA}](r, t)}{\partial t} = p_{\text{miRNA}}(r) - d_{\text{miRNA}}/[\text{miRNA}](r, t) - d_{\text{miRNA}/mRNA}[\text{miRNA}](r, t)[PHB_m](r, t) + D\Delta[\text{miRNA}](r, t), \quad (10a)$$

$$\frac{\partial [PHB_m](r, t)}{\partial t} = p_{PHB_m} - d_{PHB_m}[PHB_m](r, t) - d_{\text{miRNA}/mRNA}[\text{miRNA}](r, t)[PHB_m](r, t), \quad (10b)$$

where $\Delta$ is the usual Laplacian operator, namely in the radially symmetric case

$$\Delta[\text{miRNA}](r, t) = \frac{\partial^2[\text{miRNA}]}{\partial r^2} + \frac{1}{r} \frac{\partial[\text{miRNA}]}{\partial r}, \quad (11)$$

with $0 < r < \bar{R}$, with the following conditions at $r = 0, \bar{R}$

$$\frac{\partial [\text{miRNA}](\bar{R}, t)}{\partial r} = 0, \quad (12a)$$

$$[\text{miRNA}](0, t) < \infty, \quad (12b)$$

and with initial conditions given by

$$[\text{miRNA}](r, 0) = 0, \quad [PHB_m](r, 0) = 0. \quad (13)$$

In the second variant we assume that only $PHB$ mRNA is degraded by miRNA as follows:

$$\frac{\partial [\text{miRNA}](r, t)}{\partial t} = p_{\text{miRNA}}(r) - d_{\text{miRNA}}/[\text{miRNA}](r, t) + D\Delta[\text{miRNA}](r, t), \quad (14a)$$

$$\frac{\partial [PHB_m](r, t)}{\partial t} = p_{PHB_m} - d_{PHB_m}[PHB_m](r, t) - d_{\text{miRNA}/mRNA}[\text{miRNA}](r, t)[PHB_m](r, t), \quad (14b)$$

with the boundary and initial conditions given by Eqs. (12), (13).

### 2.3 Analytical solutions

The time dependent solution of equation (14a) and the steady states of equations (14) can be derived analytically. The steady state of equation (14a) follows by substituting into the equation

$$0 = p_{\text{miRNA}}(r) - d_{\text{miRNA}}/[\text{miRNA}](r) + D\Delta[\text{miRNA}](r) \quad (15)$$

the Fourier-Bessel series expansions

$$p_{\text{miRNA}}(r) := \sum_{n=0}^{\infty} p_n J_0(\lambda_n r), \quad [\text{miRNA}](r) := \sum_{n=0}^{\infty} m_n J_0(\lambda_n r), \quad (16)$$

where $J_0(\lambda_n r)$ is the zero-th Bessel function of the first kind, $0 < \lambda_0 < \ldots < \lambda_n < \ldots$ are the infinite solutions of the equation $J_0(\lambda_n \bar{R}) = 0$ and

$$p_n := \frac{\langle p_{\text{miRNA}}(r), J_0(\lambda_n r) \rangle}{\|J_0(\lambda_n r)\|^2}, \quad m_n := \frac{\langle [\text{miRNA}](r), J_0(\lambda_n r) \rangle}{\|J_0(\lambda_n r)\|^2}$$
are the coefficients of the Fourier-Bessel series with
\[ (f(r), g(r)) := \int_0^R f(r)g(r)rdr, \quad \|f\|^2 := \langle f, f \rangle, \quad f, g : (0, R] \to \mathbb{R}. \]

From the identity \( \Delta J_0(\lambda_n r) = -\lambda_n^2 J_0(\lambda_n r) \) it follows that \( m_n = \frac{p_n}{D\lambda_n^2 + d_{miRNA}} \) and, from the latter, we deduce that the steady state solution is given by
\[ [miRNA](r) = \sum_{n=0}^{\infty} \frac{p_n}{D\lambda_n^2 + d_{miRNA}} J_0(\lambda_n r). \]

The corresponding time dependent solution is
\[ [miRNA](r) = \sum_{n=0}^{\infty} p_n \frac{1 - e^{-(D\lambda_n^2 + d_{miRNA})t}}{D\lambda_n^2 + d_{miRNA}} J_0(\lambda_n r). \]

At steady state, equation (14b) supplies an algebraic equation for \([PHB_m]\) in terms of \([miRNA]\) which can be written as
\[ [PHB_m](r) = \frac{p_{PHB_m}}{d_{PHB_m} + d_{miRNA}/mRNA}[miRNA](r). \]

The case with mutual degradation of \( PHB \) mRNA and miRNA given by equations (10) cannot be solved analytically (cf. Levine et al. [6]) but it can be clarified further by an asymptotic analysis of the limit in which \( d_{miRNA}/mRNA \) becomes large, so that miRNA and \( PHB \) mRNA cannot coexist at significant levels at the same location. In this case, a sharp-interface problem then results in the limit, with sharp drops off in miRNA and \( PHB \) mRNA levels either side of the interface. In particular, if
\[ d_{miRNA}/mRNA[miRNA] \gg d_{PHB_m}, \]
then in the miRNA rich region and at steady state, on neglecting the independent degradation term in Eq. (10b) one has
\[ p_{miRNA}(r) - p_{PHB_m} - d_{miRNA}[miRNA](r) + D\Delta [miRNA](r) = 0. \]

Although formally the solution of Eq. (20) on the full domain reduces to Eq. (17) with
\[ p_n = \frac{\langle p_{miRNA}(r) - p_{PHB_m}, J_0(\lambda_n r) \rangle}{\|J_0(\lambda_n r)\|^2} \]
this solution approximates the steady state solution of Eq. (10a) only in the region satisfying the condition (19). Conversely, assuming that in the \( PHB \) mRNA rich region the effect of miRNA diffusion is negligible and that at any cellular position the decay of miRNA and \( PHB \) mRNA is dominated by coupled degradation within a ‘strong interaction limit’ defined by the inequality
\[ \max \left\{ \frac{d_{miRNA}/mRNA \cdot p_{miRNA}(r)/d_{miRNA}}{d_{PHB_m}}, \frac{d_{miRNA}/mRNA \cdot p_{PHB_m}/d_{PHB_m}}{d_{miRNA}} \right\} \gg 1, \quad \forall r \]
the estimate of \( PHB \) mRNA concentration at steady state derived in one dimension (cf. Levine et al. [6]) for an mRNA concentration of an unspecified gene under these conditions still holds in two dimensions:
\[ [PHB_m](r) \approx \frac{[p_{PHB_m} - p_{miRNA}(r)]_+}{d_{PHB_m}} \]
with \([x]_+ = \max \{0, x\}\).

### 2.4 Numerical solutions

In order to express the steepness of \([PHB_m]\) quantitatively we calculated numerically its spatial derivative \( \frac{\partial [PHB_m]}{\partial x} \) on a diameter \( D = [-R, R] \) at a point \( x \in I = [-\bar{R}, 0] \) such that
\[ [PHB_m](\bar{x}) = \frac{\max_{x \in I}([PHB_m]) + \min_{x \in I}([PHB_m])}{2} \]
and we considered its absolute value $|\frac{\partial [PHB_m]}{\partial x}(\bar{x})|$ as an estimate of $[PHB_m]$ sharpness. In both variants we maintained transcription and degradation rates as defined in Supplementary Tables 2, 3 and we fixed $D = 1.5 \mu m^2 \cdot s^{-1}$. In Figure 12a we show the steady state solutions of equations (10) and (14) together with a segment which is tangent to $[PHB_m]$ at $\bar{x}$. The slope of this segment is clearly higher when mutual degradation occurs. The importance of mutual degradation in generating a steep gradient of $[PHB_m]$ is further highlighted in Figure 12b which shows how PHB sharpness ($|\frac{\partial [PHB_m]}{\partial x}(\bar{x})|$) varies in the steady state solutions of equations (10) and (14) when changing $d_{miRNA/mRNA}$. Whereas PHB sharpness reaches a plateau at higher values of $d_{miRNA/mRNA}$ when miRNA is not degraded by PHB mRNA, mutual degradation causes a pronounced increase in $|\frac{\partial [PHB_m]}{\partial x}(\bar{x})|$ when increasing $d_{miRNA/mRNA}$.

![Figure 12](image.png)

Figure 12: Continuous approximation of the PHB - miRNA165/6 sub model. (a) Steady state solutions of Eqs. (10) (solid lines) and (14) (dashed lines) along a diameter. Steady state solutions of miRNA are presented in blue, whereas steady state solutions of PHB mRNA are shown in green. The red segments are tangent to $[PHB_m]$ at $\bar{x}$ and their slope highlights that when mutual degradation is present the profile of PHB mRNA is steeper. (b) PHB mRNA sharpness ($|\frac{\partial [PHB_m]}{\partial x}(\bar{x})|$) of the steady state solutions of Eqs. (10) (red line) and (14) (blue line) at increasing values of $d_{miRNA/mRNA}$. Mutual degradation causes PHB mRNA sharpness to increase more dramatically than when miRNA is not degraded by PHB mRNA.

3 Sensitivity analysis

Mathematical models of signaling networks often comprise of a large number of nonlinear differential equations describing the interaction network which, in turn, rely upon a large number of parameters associated with the reaction rates. Since the input/output relationships in such models may be not intuitive, sensitivity analysis algorithms may help identifying which parameters play a key role by ordering the relevance of parameter variations (input) in modifying the variation in the model variables (output). In order to investigate how the variables of the mathematical model defined by Eqs. (1)-(6) vary when choosing parameter values that differ from the default value reported in Supplementary Tables 2 and 3 we apply three types of sensitivity analysis.

Firstly, using a local sensitivity analysis, we analyze in one-dimension how each individual component of the sub-cellular interaction network is affected by variation of each parameter from its default value, in two representative tissue types (inside and outside the metaxylem), allowing us to identify which variables are most affected by parameter perturbations. The parameters are then ranked by the average sensitivity of the model variables to parameter perturbations allowing us to identify which parameters have the strongest influence on the components of the interaction network.

We then apply a global sensitivity analysis, which ranks the sensitivity of parameters over a much wider region of the parameter space. This finds that, in general, the parameters whose rankings in the global analysis differ most from those computed by the local analysis are ranked lower by the local sensitivity algorithm than the global sensitivity algorithm. This suggests that their optimised values do not reside within a sensitive region of the
parameter space and that the signalling pathway is relatively robust to parameter variation [14].

We finally select the parameters that affect the variables of the sub-cellular network most strongly, and analyze the sensitivity of the model variables in every tissue within the full multicellular model to variation in these parameters.

3.1 Sub-cellular network (local sensitivity analysis).

Following the approach proposed in [15], [16], we analyse the steady state sensitivity of the sub-cellular interactions defined by Eqs. (1)-(6) to perturbations from the default set reported in Supplementary Tables 2 and 3, with the exception of parameters associated with transport and permeability ($T_{Aux}$, $P_{Aux}$, $P_{Ck}$, $P_{miRNA}$) and parameters associated with PIN7 concentration ($pp_{PIN7}$, $d_{PIN7}$). We have not included the endodermis in this analysis because it does not contain PIN7 and there is no significant variation in the expression of network components within this tissue.

Under these conditions, we can investigate two possibilities; namely, when a cell is located in the metaxylem (where CKIN is expressed) and when it is located outside this tissue (where CKIN is not expressed). The steady-state sensitivity for the $k$-th element (state or reaction rate) $X_k$ with respect to the parameter $p_j$ is defined by:

$$S_{k,j} := \frac{X^{ss}_k(p_j + \delta p_j) - X^{ss}_k(p_j)}{\delta p_j},$$

where $X^{ss}_k(p_j)$ is the function returning the steady-state for this element (state or reaction rate) for the given parameter $p_j$ and $\delta p_j$ is a perturbation of this parameter [16]. The normalised sensitivity index, defined by:

$$S^{(n)}_{k,j} := \frac{p_j}{X^{ss}_k(p_j)} |S_{k,j}|,$$

estimates the sensitivity of the steady-state value $X^{ss}_k(p_j)$ to perturbation of the parameter $p_j$, relative to the absolute values of both the steady-state and the parameter under evaluation. This index is calculated for all the model species and parameters under a relative parameter perturbation of 1% as defined by default in the Systems Biology Toolbox for Matlab, SBToolbox [15]. Since nominal parameter values of zero lead to normalized sensitivities of zero for these parameters, and nominal steady-state values of zero lead to infinite normalised sensitivities, in these cases the normalized sensitivities for these parameters and states are not determined.

All parameters associated with cell geometry are set to zero, these being the transport and permeability parameters ($T_{Aux}$, $P_{Aux}$, $P_{Ck}$, $P_{miRNA}$) and parameters associated with PIN7 concentration ($pp_{PIN7}$, $d_{PIN7}$). In addition, when considering a cell outside the metaxylem, parameters associated with CKIN ($p_{CKIN_m}$, $P_{CKIN_p}$, $d_{CKIN_m}$, $d_{CKIN_p}$, $\theta_{CKIN}$, $m_{CKIN}$) are removed as this component is not present outside this tissue. The other parameter values are as reported in Supplementary Tables 2 and 3. The steady states represent changes in protein and mRNA levels when parameters are perturbed. The SBToolbox default relative parameter perturbation has been applied, this being set to be 1% of the parameter default value, [15]. The results of this evaluation for each parameter and model species, in typical cells outside and within the metaxylem, are given at [http://www.cpib.ac.uk/vascularmodel, Figures 1-4](http://www.cpib.ac.uk/vascularmodel).

In Supplementary Figure 13 we show a MinMax view of the magnitude of normalised steady-state sensitivities in a typical cell external to the metaxylem (Supplementary Figure 13a) and within the metaxylem (Supplementary Figure 13b). Bars extend from the minimal to the maximal values of all the sensitivities of the variables (species) of the model given by Eqs. (1)-(6) and reported in Supplementary Table 1. The names associated with the parameter ranks are reported in Supplementary Tables 4, 5. Blue lines show the mean value of the sensitivities of the model variables, whereas red lines their median value. Parameters are ranked by the mean values of the variable sensitivities in decreasing order.

The parameters that affect the average variable sensitivity the most outside the metaxylem are those associated with auxin expression and binding (see Supplementary Figure 13a and Supplementary Table 4). Within the metaxylem however, parameters associated with CKIN, auxin and cytokinin expression and binding have a stronger influence on the model output.

In Supplementary Table 6 we present the parameters that affect the average variable sensitivity the most in both cell types (inside and outside the metaxylem) by evaluating the sum of their ranks in the two cell types. We identify $m_{Aux}$, $d_{Aux}$, $p_{Aux}$, $\theta_{Aux}$, $P_{Ck}$, $d_{Ck}$, $\theta_{Ck}$, $m_{Ck}$ as such parameters. Perturbation of these parameters will be analyzed include this parameter in the comparison among different tissues reported in Supplementary Table
Table 4: Table summarising the parameter names associated to the parameter ranks reported in Supplementary Figure 13a.

6; nevertheless, because of its high rank in the metaxylem we will also investigate the effect of its perturbation in two dimensions in section 3.3.

3.2 Sub-cellular network (global sensitivity analysis).

A local sensitivity analysis can provide information about the influence of varying parameters on the model output in a small range around chosen default parameters. In order to explore a much wider range of parameter choices, we now analyze the sensitivity of the same model used in the previous section by applying a variance-based global sensitivity algorithm.

In contrast to regression-based methods, variance-based methods do not assume linearity or monotonicity in the input-output relationships and are well suited to analyze models of non-linear differential equations [14]. Among these methods a well established algorithm is the eFAST algorithm [15], [14], [17], [18], [19].

The eFAST method is a variance decomposition method which partitions the variance of the model output, determining what fraction of the variance can be explained by variation in each input parameter [19]. The parameter space is sampled along curves defined by the transformation function:

\[
\hat{x}_i = \frac{1}{2} + \frac{1}{\pi} \arcsin (\sin (\omega_i y + \phi_i)),
\]

where each parameter varies at frequency \(\omega_i\) and random phase shift \(\phi_i\) with a scalar variable \(y \in (-\pi, \pi)\). With this transformation the model output function can be expressed as a Fourier series with respect to \(y\) [14].

The overall variance of the output function is decomposed into summands of the square of Fourier series coefficients which are calculated by Monte Carlo integrations on the sampled parameter space. As a result of this decomposition, two sensitivity indexes are then defined for each parameter: a first-order sensitivity index and a total effect sensitivity index. The first-order sensitivity index \(S_i\) of a parameter \(i\) is calculated as the variance at the frequency uniquely associated with a parameter divided by the total variance. More precisely, the variance \(s_i^2\) is firstly calculated from the Fourier coefficients at the frequency of interest, the first-order sensitivity index \(S_i\) is then defined as

\[
S_i = \frac{s_i^2}{s_{total}^2}
\]

The first-order sensitivity index represents the fraction of the model output variance which is explained by the input variation of a given parameter. The total effect sensitivity index \(S_{T_i}\) is then estimated as follows. eFAST first computes the summed sensitivity index of the entire complementary set of parameters, namely the set of all

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Table 4: Table summarising the parameter names associated to the parameter ranks reported in Supplementary Figure 13a.
Figure 13: MinMax view of the magnitude of normalized steady-state sensitivities in a typical cell external to the metaxylem (a) and within the metaxylem (b). Bars extend from the minimal to the maximal values of all the sensitivities of the variables (species) of the model given by Eqs. (1)-(6) and reported in Supplementary Table 1. The names associated with the parameter ranks are reported in Supplementary Tables 4, 5. Blue lines show the mean value of the model variables, whereas red lines their median value.
### Table 5: Table summarising the parameter names associated to the parameter ranks reported in Supplementary Figure 13b.

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</tr>
<tr>
<td>3</td>
<td>$p_{CK}$</td>
<td>24</td>
<td>$p_{AHP6_m}$</td>
</tr>
<tr>
<td>4</td>
<td>$d_{CK}$</td>
<td>25</td>
<td>$d_{AHP6_m}$</td>
</tr>
<tr>
<td>5</td>
<td>$p_{Aux}$</td>
<td>26</td>
<td>$d_{PHB_p}$</td>
</tr>
<tr>
<td>6</td>
<td>$d_{Aux}$</td>
<td>27</td>
<td>$p_{PHB_p}$</td>
</tr>
<tr>
<td>7</td>
<td>$\theta_{CK}$</td>
<td>28</td>
<td>$p_{IAA2_m}$</td>
</tr>
<tr>
<td>8</td>
<td>$\theta_{Aux}$</td>
<td>29</td>
<td>$d_{IAA2_m}$</td>
</tr>
<tr>
<td>9</td>
<td>$m_{Ck}$</td>
<td>30</td>
<td>$d_{miRNA/mRNA}$</td>
</tr>
<tr>
<td>10</td>
<td>$d_{CKIN_m}$</td>
<td>31</td>
<td>$p_{AHP6_p}$</td>
</tr>
<tr>
<td>11</td>
<td>$p_{CKIN_m}$</td>
<td>32</td>
<td>$d_{AHP6_p}$</td>
</tr>
<tr>
<td>12</td>
<td>$d_{CKIN_p}$</td>
<td>33</td>
<td>$d_{PHB_m}$</td>
</tr>
<tr>
<td>13</td>
<td>$p_{CKIN_p}$</td>
<td>34</td>
<td>$\theta_{PHB}$</td>
</tr>
<tr>
<td>14</td>
<td>$p_{ARR5_m}$</td>
<td>35</td>
<td>$p_{IAPN7_m}$</td>
</tr>
<tr>
<td>15</td>
<td>$d_{ARR5_m}$</td>
<td>36</td>
<td>$p_{IAA2_p}$</td>
</tr>
<tr>
<td>16</td>
<td>$\theta_{CKIN}$</td>
<td>37</td>
<td>$d_{IAPN7_m}$</td>
</tr>
<tr>
<td>17</td>
<td>$p_{ARR5_p}$</td>
<td>38</td>
<td>$d_{IAA2_p}$</td>
</tr>
<tr>
<td>18</td>
<td>$d_{ARR5_p}$</td>
<td>39</td>
<td>$m_{AHP6}$</td>
</tr>
<tr>
<td>19</td>
<td>$m_{ARR5}$</td>
<td>40</td>
<td>$m_{PHB}$</td>
</tr>
<tr>
<td>20</td>
<td>$p_{PHB_m}$</td>
<td>41</td>
<td>$\theta_{AHP6}$</td>
</tr>
<tr>
<td>21</td>
<td>$p_{miRNA}$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 6: Table summarising the most sensitive parameters in a cell within and outside the metaxylem as evaluated by the sum of the parameter ranks in both tissues. Parameters whose sum of ranks is minor than the arbitrary threshold of 30 are reported, these being associated with auxin and cytokinin expression and binding.

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Parameter rank not in metaxylem</th>
<th>Parameter rank in metaxylem</th>
<th>Sum of ranks (&lt; 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$m_{Aux}$</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>$d_{Aux}$</td>
<td>2</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>$p_{Aux}$</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>$\theta_{Aux}$</td>
<td>3</td>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>$p_{CK}$</td>
<td>11</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>$d_{CK}$</td>
<td>12</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>$\theta_{CK}$</td>
<td>13</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td>$m_{Ck}$</td>
<td>14</td>
<td>9</td>
<td>23</td>
</tr>
</tbody>
</table>
parameters except for parameter $i$. $S_{Ti}$ is then calculated as the remaining variance after the contribution of the complementary set $S_{ci}$ is removed:

$$S_{Ti} = 1 - S_{ci}$$

In such a way, $S_{Ti}$ evaluates also higher-order, nonlinear interactions between the parameter of interest and the complementary set of parameters.

In what follows we evaluate the total effect sensitivity indexes of the model variables considering the two subcellular cases and nominal parameter values that we analyzed in the previous section. More precisely, we applied the eFAST algorithm as encoded in the Systems Biology Toolbox for Matlab, SBToolbox [15] by maintaining the default algorithm parameter values (1000 Monte Carlo simulations, one order of magnitude of parameters range from the nominal parameter value).

Since data showing a high degree of cooperativity have Hill coefficients of up to 10 [20], [21] and a perturbation of one order of magnitude would lead to Hill coefficients up to 50, we do not include in the global sensitivity analysis these parameters. Perturbations of the Hill coefficients from their nominal values were however investigated locally in the previous section.

As with the local sensitivity analysis, in Supplementary Figure 14 we show a MinMax view of the magnitude of the total effect sensitivities in a typical cell external to the metaxylem (Supplementary Figure 14a) and within the metaxylem (Supplementary Figure 14b). Bars extend from the minimal to the maximal values of all the sensitivities of the variables (species) of the model given by Eqs. (1)-(6) and reported in Supplementary Table 1. The names associated with the parameter ranks are reported in Supplementary Tables 7 and 8. Blue lines show the mean value of the model variables, whereas red lines their median value.

Because of the variation in the search space between the local and global sensitivity analyses, the relative influence of parameter perturbations on the model variables, which we evaluate as parameter ranks, may differ between the two. Supplementary Table 9 lists the parameters outside the metaxylem with the greatest difference in rank between the two analyses (> 10 ranks), and Supplementary Table 10 lists those parameters that differ most in rank within the metaxylem. Comparing the ranking of the parameter sensitivities of the local analysis with the global analysis in this way, we found that in general, the parameters whose rankings differ most between the two analyses are ranked lower by the local sensitivity algorithm than the global sensitivity algorithm.

The result of this comparative analysis means that the parameters whose associated sensitivity is most influenced by increasing the search space of their perturbed values are relatively less sensitive to local perturbations around their default value than to global perturbations comprising a wider parameter range, suggesting that their default values do not reside within a sensitive region of the parameter space. Consequently, the signalling pathway appears to be operating in a region of parameter space more robust to stochastic variation in gene regulation affecting these parameters [14] than if their default values were chosen in other regions of the search space.

In cases where the difference in ranked influence between the global and local sensitivity is small, this suggests that the parameters sampled in the smaller search space in the local analysis do not have a greater relative influence on the model output than when parameters are sampled in the wider search space using the global sensitivity algorithm. In other words, in relative terms, the choice of the default parameter values is not greatly affecting the sensitivity of the model to small perturbations in these parameters.

### 3.3 2D model of vascular patterning.

Using the results of the sensitivity analysis of the one-dimensional model developed in sections 3.1, 3.2, we now evaluate in our multicellular model given by Eqs. (1)-(6), and the tissue representation reported in Supplementary Figures 1 and 2, the sensitivities of the parameters that most effect the model variables in one dimension, both in the metaxylem and outside the metaxylem. The selected parameters are $p_{Aux}$, $p_{Ck}$, $d_{Aux}$, $d_{Ck}$, $\theta_{Aux}$, $\theta_{Ck}$, $m_{Aux}$, $m_{Ck}$ and $m_{Ck/N}$, the latter of which is most important in the metaxylem. We also analyze perturbations in transport, permeability, PIN7 protein production and protein degradation rates, as these were not included in the 1D sensitivity analysis, and the parameters affecting mutual degradation of PHB mRNA and miRNA165/6 as a further support of the analysis performed in Section 2. Parameters were perturbed 10% above their default values reported in Supplementary Tables 2 and 3.

In Supplementary Figures 16 - 18 we show the mean of the normalized sensitivity index given by Eq. (24) for all cells of each cell type, calculated for each model component, for each parameter listed above. As expected intuitively, $p_{Aux}$ and $d_{Aux}$ play the strongest role in the sensitivity of auxin and of the proteins associated with auxin response (IAA2, AHP6), and $p_{Ck}$ and $d_{Ck}$ influence mainly the sensitivity of cytokinin, ARR5 and PIN7. Parameters associated with protein-DNA binding and Hill coefficients present an analogous effect on the network...
Figure 14: MinMax view of the magnitude of the total effect sensitivities in a typical cell outside the metaxylem (a) and within the metaxylem (b). Bars extend from the minimal to the maximal values of all the sensitivities of the variables (species) of the model given by Eqs. (1)-(6) and reported in Supplementary Table 1. The names associated with the parameter ranks are reported in Supplementary Tables 7, 8. Blue lines show the mean value of the model variables, whereas red lines their median value.
### Table 7: Table summarising the parameter names associated to the parameter ranks reported in Supplementary Figure 14a.

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Parameter rank</th>
<th>Parameter name</th>
<th>Parameter rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_{Aux}$</td>
<td>1</td>
<td>$d_{IAA2m}$</td>
<td>16</td>
</tr>
<tr>
<td>$p_{Aux}$</td>
<td>2</td>
<td>$p_{ARR5m}$</td>
<td>17</td>
</tr>
<tr>
<td>$\theta_{Aux}$</td>
<td>3</td>
<td>$d_{miRNA/mRNA}$</td>
<td>18</td>
</tr>
<tr>
<td>$p_{PHB}$</td>
<td>4</td>
<td>$d_{ARR5p}$</td>
<td>19</td>
</tr>
<tr>
<td>$p_{AHP6m}$</td>
<td>5</td>
<td>$d_{AHP6p}$</td>
<td>20</td>
</tr>
<tr>
<td>$d_{AHP6m}$</td>
<td>6</td>
<td>$p_{IAA2m}$</td>
<td>21</td>
</tr>
<tr>
<td>$d_{CK}$</td>
<td>7</td>
<td>$p_{ARR5p}$</td>
<td>22</td>
</tr>
<tr>
<td>$p_{AHP6p}$</td>
<td>8</td>
<td>$d_{IAA2p}$</td>
<td>23</td>
</tr>
<tr>
<td>$d_{miRNA}$</td>
<td>9</td>
<td>$d_{AHP6p}$</td>
<td>24</td>
</tr>
<tr>
<td>$d_{AHP6p}$</td>
<td>10</td>
<td>$d_{PHBp}$</td>
<td>25</td>
</tr>
<tr>
<td>$p_{PHBp}$</td>
<td>11</td>
<td>$\theta_{ARR5}$</td>
<td>26</td>
</tr>
<tr>
<td>$p_{CK}$</td>
<td>12</td>
<td>$p_{IAA2p}$</td>
<td>27</td>
</tr>
<tr>
<td>$p_{miRNA}$</td>
<td>13</td>
<td>$p_{PIN7m}$</td>
<td>28</td>
</tr>
<tr>
<td>$d_{ARR5m}$</td>
<td>14</td>
<td>$d_{PHBm}$</td>
<td>29</td>
</tr>
<tr>
<td>$d_{miRNA/mRNA}$</td>
<td>15</td>
<td>$\theta_{PHB}$</td>
<td>30</td>
</tr>
</tbody>
</table>

### Table 8: Table summarising the parameter names associated to the parameter ranks reported in Supplementary Figure 14b.

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Parameter rank</th>
<th>Parameter name</th>
<th>Parameter rank</th>
</tr>
</thead>
<tbody>
<tr>
<td>$d_{Aux}$</td>
<td>1</td>
<td>$\theta_{CKIN}$</td>
<td>19</td>
</tr>
<tr>
<td>$\theta_{Aux}$</td>
<td>2</td>
<td>$p_{ARR5m}$</td>
<td>20</td>
</tr>
<tr>
<td>$p_{CKINm}$</td>
<td>3</td>
<td>$d_{ARR5p}$</td>
<td>21</td>
</tr>
<tr>
<td>$p_{Aux}$</td>
<td>4</td>
<td>$d_{IAA2p}$</td>
<td>22</td>
</tr>
<tr>
<td>$p_{PHBm}$</td>
<td>5</td>
<td>$d_{AHP6p}$</td>
<td>23</td>
</tr>
<tr>
<td>$p_{CKINp}$</td>
<td>6</td>
<td>$d_{PHBp}$</td>
<td>24</td>
</tr>
<tr>
<td>$d_{miRNA}$</td>
<td>7</td>
<td>$d_{CKINp}$</td>
<td>25</td>
</tr>
<tr>
<td>$p_{miRNA}$</td>
<td>8</td>
<td>$d_{PHBp}$</td>
<td>26</td>
</tr>
<tr>
<td>$d_{CK}$</td>
<td>9</td>
<td>$\theta_{ARR5}$</td>
<td>27</td>
</tr>
<tr>
<td>$d_{IAA2m}$</td>
<td>10</td>
<td>$p_{IAA2p}$</td>
<td>28</td>
</tr>
<tr>
<td>$d_{AHP6m}$</td>
<td>11</td>
<td>$p_{PIN7m}$</td>
<td>29</td>
</tr>
<tr>
<td>$d_{ARR5m}$</td>
<td>12</td>
<td>$p_{ARR5p}$</td>
<td>30</td>
</tr>
<tr>
<td>$d_{miRNA/mRNA}$</td>
<td>13</td>
<td>$p_{CK}$</td>
<td>31</td>
</tr>
<tr>
<td>$\theta_{CK}$</td>
<td>14</td>
<td>$\theta_{ARR5}$</td>
<td>32</td>
</tr>
<tr>
<td>$p_{PHBp}$</td>
<td>15</td>
<td>$d_{PHBm}$</td>
<td>33</td>
</tr>
<tr>
<td>$p_{IAA2m}$</td>
<td>16</td>
<td>$\theta_{AHP6}$</td>
<td>34</td>
</tr>
<tr>
<td>$d_{CKINm}$</td>
<td>17</td>
<td>$\theta_{PHB}$</td>
<td>35</td>
</tr>
<tr>
<td>$p_{AHP6m}$</td>
<td>18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 9: Table summarising the parameters whose local sensitivity rankings differ most from the global rankings (> 10 ranks) in decreasing order of difference in a cell outside the metaxylem.

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Parameter rank in local analysis</th>
<th>Parameter rank in global analysis</th>
<th>Distance by ranks (&gt; 10 ranks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p_{PHBm}$</td>
<td>23</td>
<td>4</td>
<td>19</td>
</tr>
<tr>
<td>$p_{PHBp}$</td>
<td>27</td>
<td>11</td>
<td>16</td>
</tr>
<tr>
<td>$d_{miRNA}$</td>
<td>21</td>
<td>9</td>
<td>12</td>
</tr>
<tr>
<td>$\theta_{AHP6}$</td>
<td>9</td>
<td>20</td>
<td>11</td>
</tr>
</tbody>
</table>
### Table 10: Table summarising the parameters whose local sensitivity rankings differ most from the global rankings (> 10 ranks) in decreasing order of difference in a cell within the metaxylem.

<table>
<thead>
<tr>
<th>Parameter name</th>
<th>Parameter rank in local analysis</th>
<th>Parameter rank in global analysis</th>
<th>Distance by ranks (&gt; 10 ranks)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$p_{Ck}$</td>
<td>3</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td>$d_{IAA2m}$</td>
<td>29</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>$d_{miRNA/mRNA}$</td>
<td>30</td>
<td>13</td>
<td>17</td>
</tr>
<tr>
<td>$d_{miRNA}$</td>
<td>23</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>$d_{IAA2p}$</td>
<td>38</td>
<td>22</td>
<td>16</td>
</tr>
<tr>
<td>$p_{PHB}$</td>
<td>20</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>$d_{AHP6m}$</td>
<td>25</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>$d_{PIN7m}$</td>
<td>37</td>
<td>23</td>
<td>14</td>
</tr>
<tr>
<td>$d_{CK1Np}$</td>
<td>12</td>
<td>26</td>
<td>14</td>
</tr>
<tr>
<td>$p_{miRNA}$</td>
<td>21</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>$p_{ARR5p}$</td>
<td>17</td>
<td>30</td>
<td>13</td>
</tr>
<tr>
<td>$p_{PHBp}$</td>
<td>27</td>
<td>15</td>
<td>12</td>
</tr>
<tr>
<td>$p_{IAA2m}$</td>
<td>28</td>
<td>16</td>
<td>12</td>
</tr>
</tbody>
</table>

components, with $\theta_{Aux}$ and $m_{Aux}$ influencing mostly IAA2 and AHP6 sensitivity, and with $\theta_{Ck}$ and $m_{Ck}$ affecting mainly ARR5 and PIN7 (see Supplementary Figure 16).

In Supplementary Figure 15 we show the same chart when perturbing $m_{CKIN}$. As it can be observed by the magnitude of the mean normalized sensitivity (of the order of $10^{-8}$), the sensitivity of the model variables across different tissues of the cross-section to $m_{CKIN}$ in the two-dimensional model is not as noteworthy as in the one-dimensional model in a single cell within the metaxylem.

We then analyzed the sensitivity of the model variables to changes in parameters that are associated with the cellular geometry that could not be analyzed in the one-dimensional model. Supplementary Figure 17 shows the mean normalized sensitivities of the two-dimensional model when perturbing $T_{Aux}$, $P_{Aux}$, $P_{Ck}$, $P_{miRNA}$, $P_{PIN7p}$ and $d_{PIN7p}$. Perturbations in $T_{Aux}$, $P_{Aux}$ influence mainly the sensitivity of auxin, IAA2 and AHP6, with $T_{Aux}$ playing a stronger role in phloem, procambial and xylem cells, while perturbations in $P_{Ck}$ affects mainly cytokinin, ARR5 and PIN7, and perturbations in $P_{miRNA}$ act mostly on miRNA, PHB and AHP6.

In Section 2 the robustness of a sub-model of PHB mRNA and miRNA165/6 mutual degradation was investigated by varying the parameter values associated and observing the spatial pattern generated. We extend this here by analysing the sensitivity of these parameters in the full model given by Eqs. (1)-(6). Supplementary Figure 18 shows the mean normalized sensitivities of the two-dimensional model when perturbing $p_{miRNA}$, $P_{PHBm}$, $d_{miRNA}$, $d_{PHBm}$ and $d_{miRNA/mRNA}$. As expected intuitively, perturbations in these parameters affect mainly the network components that they regulate directly (miRNA, PHBp) or indirectly (AHP6p, ARR5p), in the tissues in which these components are present.

The simulation results at $t = 15$ seconds of our multicellular model given by Eqs. (1)-(6), the tissue representation reported in Supplementary Figures 1 and 2 and the default parameter values reported in Supplementary Tables 2 and 3 show that vascular patterning is maintained under all the parameter perturbations discussed in this section. The model output under each of these perturbations is given at http://www.cpib.ac.uk/vascularmodel, Figures 5-24.

### 4 Steady state analysis

In order to establish and maintain a pattern of gene expression when PIN is free to be expressed throughout the root vasculature, with a homogeneous production rate of both auxin and cytokinin, it must be possible for adjacent cells of a similar size and shape to have significantly different steady state expression of key genes. This can be tested using the model by finding the possible steady states as key model parameters are varied. In particular, the existence of multiple possible steady states within a cell for given production rates of auxin and cytokinin would demonstrate the possibility that a pattern of gene expression within a field of cells could be produced.

It is not practical to compute steady states in the full model with all the spatial information, either analytically or numerically, so to investigate further a number of model simplifications are made. Firstly, rather than the full
Figure 15: Plot representing the mean, calculated for all cells in a particular tissue and for all tissues, of the normalised sensitivity index given by Eq. (24) when perturbing $m_{CKIN}$ 10% above its default value reported in Supplementary Table 3. The sensitivity of the model variables across different tissues to $m_{CKIN}$ is not as noteworthy as in the one-dimensional model in a single cell within the metaxylem being of the order of magnitude of $10^{-8}$.

spatial tissue structure, we consider just two cells of equal volume, with a single interface of unit area between the two. A value for the cell volume of $V = 6$ is used as an approximation of the mean wall surface area to volume ratio found in the full tissue structure. We also assume that the level of PIN at the cell membrane facing the shared interface of a cell $i$ at any time is equal to the level of of PIN protein ($[PIN_{p}]_i$) present in cell $i$ at that time.

While the spatial distributions of both cytokinin and PHB protein are governed by the full model, neither are regulated directly or indirectly in the model by auxin or the genes AHP6, PIN7 or ARR5. Because of this, to investigate model steady states both cytokinin ($Ck$) and PHB protein ($PHB$) are considered as parameter inputs to a simplified sub-model governing AHP6, PIN7 and ARR5. The initial values for Ck and PHB used are the approximate values seen in the xylem pole pericycle cells in the full spatial model, using the default parameter set ($Ck = 0.2$, $PHB = 0.5$). The affect of changing these parameters is investigated in the steady state analysis. Finally, it is not necessary to include $IAA2$ in the model when analyzing the steady states of genes regulating xylem pole specification because it doesn’t regulate the output of any other components. If the cells in the simplified model are denoted by their subscript, we have the following model:

\[
\frac{d[Aux]_1}{dt} = p_{Aux} - d_{Aux}[Aux]_1 - \frac{P_{Aux}}{V} ([Aux]_1 - [Aux]_2) - \frac{T_{Aux}}{V} (\frac{([PIN_{7}]_1[Aux]_1 - [PIN_{7}]_2[Aux]_2)}{2}, \tag{25a}
\]

\[
\frac{d[Aux]_2}{dt} = p_{Aux} - d_{Aux}[Aux]_2 - \frac{P_{Aux}}{V} ([Aux]_2 - [Aux]_1) - \frac{T_{Aux}}{V} (\frac{([PIN_{7}]_2[Aux]_2 - [PIN_{7}]_1[Aux]_1)}{2}, \tag{25b}
\]

\[
\frac{d[AHP6m]_i}{dt} = p_{AHP6m} F_{AHP6m}^{(i)} - d_{AHP6m}[AHP6m]_i, \tag{25c}
\]

\[
\frac{d[ARR5m]_i}{dt} = p_{ARR5m} F_{ARR5m}^{(i)} - d_{ARR5m}[ARR5m]_i, \tag{25d}
\]

\[
\frac{d[PIN7m]_i}{dt} = p_{PIN7m} F_{PIN7m}^{(i)} - d_{PIN7m}[PIN7m]_i, \tag{25e}
\]

\[
\frac{d[AHP6p]_i}{dt} = p_{AHP6p}[AHP6p]_i - d_{AHP6p}[AHP6p]_i, \tag{25f}
\]

\[
\frac{d[ARR5p]_i}{dt} = p_{ARR5p}[ARR5p]_i - d_{ARR5p}[ARR5p]_i, \tag{25g}
\]

\[
\frac{d[PIN7p]_i}{dt} = p_{PIN7p}[PIN7p]_i - d_{PIN7p}[PIN7p]_i, \tag{25h}
\]
Figure 16: Series of plots representing the mean, calculated for all cells in a particular tissue and for all tissues, of the normalized sensitivity index given by Eq. (24). Parameters associated with auxin and cytokinin expression and binding ($p_{Aux}$, $p_{CK}$, $d_{Aux}$, $d_{CK}$, $\theta_{Aux}$, $\theta_{CK}$, $m_{Aux}$, $m_{CK}$) are perturbed 10% above their default value reported in Supplementary Tables 2 and 3. As expected intuitively perturbations in these parameters affect mainly the network components that they regulate directly or indirectly (auxin, IAA2, AHP6 for parameters associated with auxin; cytokinin, ARR5, PIN7 for parameters associated with cytokinin) in the tissues in which these components are present.
Figure 17: Series of plots representing the mean, calculated for all cells in a particular tissue and for all tissues, of the normalized sensitivity index given by Eq. (24). Parameters associated with the cell geometry ($T_{Aux}$, $P_{Aux}$, $P_{CK}$, $P_{miRNA}$, $P_{PIN7p}$, $d_{PIN7p}$) are perturbed 10% above their default value reported in Supplementary Tables 2, 3. $T_{Aux}$, $P_{Aux}$ influence mainly the sensitivity of auxin, IAA2 and AHP6; $P_{CK}$ affects mainly cytokinin, ARR5 and PIN7; perturbations in $P_{miRNA}$ act mostly on miRNA, PHB and AHP6.
Figure 18: Series of plots representing the mean, calculated for all cells in a particular tissue and for all tissues, of the normalised sensitivity index given by Eq. (24). Parameters associated with PHB mRNA and miRNA165/6 ($p_{miRNA}$, $p_{PHB}$, $d_{miRNA}$, $d_{PHB}$, $d_{miRNA/mRNA}$) are perturbed 10% above their default value reported in Supplementary Tables 2, 3. Perturbations in these parameters affect mainly $miRNA$, $PHB_p$, $AHP6_p$, $ARR5_p$ in the tissues in which these components are present.
for $i = 1, 2$, where:

$$F^{(i)}_{AHP6m} = \frac{([Aux]_i/\theta_{Aux})^{m_{Aux}}}{1 + ([Aux]_i/\theta_{Aux})^{m_{Aux}} + (PHB/\theta_{PHB})^{m_{PHB}}},$$

$$F^{(i)}_{ARR5m} = \frac{1 + (Ck/\theta_{Ck})^{m_{Ck}} + ([AHP6p]_i/\theta_{AHP6})^{m_{AHP6}}}{([ARR5p]_i/\theta_{ARR5})^{m_{ARR5}}},$$

$$F^{(i)}_{PIN7m} = \frac{([AHP6p]_i/\theta_{AHP6})^{m_{AHP6}}}{1 + ([ARR5p]_i/\theta_{ARR5})^{m_{ARR5}}}.

Unless stated otherwise, all parameters and variables are as previously defined.

Figure 19 shows the steady states for $auxin$, $PIN7_m$, $AHP6_m$, and $ARR5_m$ in one of the cells for increasing values of $p_{aux}$, the auxin biosynthesis rate (the results are identical in both cells). At low $p_{aux}$ there is a single steady state with low $AHP6$ expression resulting in high $PIN7$ expression in both cells so that the level of auxin is equal in both. At high $p_{aux}$ there is also a single steady state in which the level of auxin is balanced in both cells, but here the high level of AHP6 strongly represses $ARR5$ and so little or no $PIN7$ is expressed in either cell. At intermediate values for $p_{aux}$ there exist two additional stable steady states representing the cases where there is asymmetry in expression between the two cells, with high $ARR5$ and $PIN7$ and low $AHP6$ and auxin in one cell, and vice versa in the other cell. These additional stable branches end with limit points, from which four unstable steady states double back and meet the symmetric steady state branch at two subcritical pitchfork bifurcations, themselves linked by one final unstable steady state. This final unstable steady state represents the range of values for $p_{aux}$ for which gene expression is balanced between the two cells but in which any small perturbation from that steady state is likely to lead to a switch to the case with an asymmetry in gene expression.

Figure 19: Steady states for $auxin$, $PIN7_m$, $AHP6_m$ and $ARR5_m$ (in either cell) for increasing values of the auxin production rate $p_{aux}$ in the model given by equations (25). Red dots indicate limit points, green dots pitchfork bifurcations, solid lines stable steady states, dotted lines unstable steady states.

This result suggests that in the two cell case at least, once an imbalance in $PIN7$ expression has been established between the two cells, there is relatively broad range of auxin production rates within which this imbalance is maintained, providing a hypothetical mechanism for maintaining a pattern. Furthermore, a range of auxin production also exists within which, even when expression is balanced between the two cells, the system is sensitive to small perturbations and will switch to the case where $PIN7$ is strongly expressed in one cell only, providing a hypothetical mechanism for establishing a pattern.
Plotting the steady states for increasing levels of Ck and PHB results in similar, but not identical bifurcation structures as for aux. For PHB, there is no positive lower limit to the bistable region, so that below a threshold value of PHB the system is only stable with high PIN7 in one cell and low PIN7 in the other (Figure 20A). Above another threshold of PHB there exists only one stable steady state in which PIN7 is equal in both cells, and between the two thresholds there is a region in which the system may be stable in either a balanced or imbalanced state. Finally, as with auxin, for low and high Ck there is a single stable steady state where PIN7 is respectively

![Figure 20: Steady states for PIN7m (in either cell) for increasing values of PHB (A.), and Ck (B.) in the model given by equations (25). Red dots indicate limit points, green dots pitchfork bifurcations, solid lines stable steady states, dotted lines unstable steady states.](image)

expressed weakly and strongly in both cells, and for intermediate values multiple steady states are possible (Figure 20B). As with auxin, at the top end of this intermediate Ck range there are two limit points and a subcritical pitchfork bifurcation bounding a range in which PIN7 may either be expressed stably equally in both cells, or strongly in one cell and weakly in the other. Unlike with auxin however, the lower bound of the bistable range occurs at a supercritical pitchfork bifurcation.

5 Mathematical model applied in Supplementary Movies 10-14

In this section, we extend the mathematical model developed in section 1 to include the regulatory effect of SHORT ROOT (SHR) on miRNA165/6. SHR is produced in the vascular cylinder and moves into the endodermis to bind SCARECROW (SCR) [22]. Once in the endodermis, the protein complex SHR-SCR promotes the transcription of miRNA165/6 [12]. Endodermally produced miRNA165/6 then degrades its target mRNA PHB in the endodermis and stele periphery. In our model, we assume that transcription and translation of SHR occurs in all tissues except for the endodermis:

$$\emptyset \overset{p_{SHR}}{\rightarrow} SHR_m,$$

$$SHR_m \overset{p_{SHR}p}{\rightarrow} SHR_m + SHR_p,$$

$$SHR_m \overset{d_{SHR}m}{\rightarrow} \emptyset,$$

$$SHR_p \overset{d_{SHR}p}{\rightarrow} \emptyset.$$

SHR protein is allowed to diffuse and activates the expression of miRNA165/6 in the endodermis. Although this activation would involve binding with SCR, we simplify this redundant process by assuming SHR to directly regulate miRNA165/6 transcription in the endodermis only. Transcription of miRNA165/6 is controlled by the Hill function:

$$F_{miRNA165/6}^{(i)}([SHR]_i) = \frac{([SHR]_i/\theta_{SHR})^{m_{SHR}}}{1 + ([SHR]_i/\theta_{SHR})^{m_{SHR}}},$$

As a result, SHR mRNA and protein levels are governed by the equations:

$$\frac{d[SHR_m]}{dt} = \begin{cases} 0, & \text{if } i \text{ is in the endodermis}, \\ p_{SHR}m - d_{SHR}m[SHR_m], & \text{otherwise}, \end{cases}$$

$$\frac{d[SHR_p]}{dt} = p_{SHR}p - d_{SHR}p[SHR_p] - \frac{1}{V_i} \sum_{n \in N_i} S_{i,n}P_{SHR}(\text{[SHR}_p]_i - \text{[SHR}_p]_n),$$

$$F_{miRNA165/6}^{(i)}([SHR]_i) = \frac{([SHR]_i/\theta_{SHR})^{m_{SHR}}}{1 + ([SHR]_i/\theta_{SHR})^{m_{SHR}}},$$

(26)

(27)
Table 11: Default values of the parameters applied in Supplementary Movies 10-14 together with the default parameters in Supplementary Tables 2 and 3.

whereas the equation for miRNA165/166 takes the form:

$$\frac{d[\text{miRNA165/166}]}{dt} = p_{\text{miRNA}} F_{\text{miRNA165/6}} - d_{\text{miRNA}}[\text{miRNA165/166}]_i - d_{\text{miRNA/mRNA}}[\text{PHB}_m][\text{miRNA165/166}]_i - \frac{1}{V_i} \sum_{n \in N_i} S_{i,n} P_{\text{miRNA}} \left( [\text{miRNA165/166}]_i - [\text{miRNA165/166}]_n \right)$$

(28)

where $i$ is the cell index. In agreement with experimental observations on PIN7 expression (see Figure 4), in Supplementary Movie 12 all vascular cells are allowed the potential to express PIN7. The extended model is then defined by Eqs. (1)-(28) substituting Eq. (28) to the equation for miRNA165/6 transcription in Eqs. (6). The default parameter values of the extended model are defined in Supplementary Table 11 together with the ones reported in Supplementary Tables 2 and 3.

6 Plant Lines

All plant lines were in the Columbia background. the TCSn::GFP line was provided by Bruno Müller [24]. The ARR5::GUS line was published in [25], PIN1::PIN1::GFP, PIN3::PIN3:GFP and PIN7::PIN7:GFP were published in [26, 27], DR5rev::GFP was published in [28], AHP6::GFP in [23], PHB::PHB:GFP in [29], AUX1::AUX1:GFP in [30] and IAA2::GUS in [3].

References

REFERENCES


Movie S1. Simulation of IAA2 expression when PIN1, PIN3, and PIN7 are active. Auxin is actively transported into the xylem axis, where it forms a maximum and activates the expression of IAA2. The parameters associated with cytokinin and all parameters of transcription and translation have been set to zero, except those parameters relating to IAA2 expression. PIN1, PIN3, and PIN7 concentrations have been set to unity. The other parameters have been set to the values reported in SI Appendix, Tables S2 and S3.
Movie S2. Simulation of IAA2 expression when only PIN1 is active. PIN1 is sufficient to reproduce the auxin signaling maximum seen in WT plants. The parameters associated with cytokinin and all parameters of transcription and translation have been set to zero, except for those parameters relating to IAA2 expression. The PIN1 concentration has been set to unity. The other parameters have been set to the values reported in SI Appendix, Tables S2 and S3.

Movie S2
Movie S3. Simulation of IAA2 expression when only PIN7 is active. PIN7 acts redundantly with PIN1 to direct the auxin signaling maximum. The parameters associated with cytokinin and all parameters of transcription and translation have been set to zero, except for those parameters relating to IAA2 expression. The PIN7 concentration has been set to unity. The other parameters have been set to the values reported in SI Appendix, Tables S2 and S3.
Movie S4. Simulation of IAA2 expression when only PIN3 is active. PIN3 activity alone is insufficient to reproduce the auxin signaling maximum seen in WT plants. The parameters associated with cytokinin and all parameters of transcription and translation have been set to zero, except for those parameters relating to IAA2 expression. The PIN3 concentration has been set to unity. The other parameters have been set to the values reported in SI Appendix, Tables S2 and S3.
Simulation of our multicellular model of vascular patterning with all known network components active. In this simulation, there is no mutual degradation present between PHABULOSA (PHB) mRNA and microRNA165/6 (miRNA165/6). As a consequence, the simulation fails to generate a sharp gradient of PHB, which results in altered expression of AHP6. All parameters have been set to their default values, which are reported in SI Appendix, Tables S2 and S3, except for $p_{CKIN_{m}}$ and $d_{miRNA/mRNA}$, which have been set to zero.
Movie S6. Simulation of our multicellular model of vascular patterning with all known network components active and incorporating the mutual degradation between PHB mRNA and miRNA165/6. This simulation is able to recapitulate the experimentally observed gradient of PHB expression, and the simulated patterns of all components are in agreement with the experimental observations except for ARR5, where the simulated expression is also present in the metaxylem. All parameters have been set to their default values, which are reported in SI Appendix, Tables S2 and S3, except for pCKINm, which has been set to zero.

Movie S6
Simulation of our multicellular model of vascular patterning with a cytokinin inhibitor (CKIN) present in the metaxylem. Inclusion of a cytokinin inhibitor into our model with constitutive expression in the metaxylem degrades cytokinin levels, and **ARR5** expression becomes similar to experimental observations. All parameters have been set to the values reported in SI Appendix, Tables S2 and S3.

Movie S7
Movie S8. Simulation of our multicellular model of vascular patterning, in which the phloem is specified as the main source for cytokinin. Specifying the phloem as the main source of cytokinin causes an uneven gradient of ARRS response. We do not observe this uneven response pattern in our experimental analyses. These simulations suggest that a positional bias in the input of hormones is not required. The parameters have been set to the values reported in SI Appendix, Tables S2 and S3, except for the biosynthesis rate for cytokinin $p_{Ck}$ in the phloem, which was set to two times its default value.

Movie S8
Movie S9. Simulation of our multicellular model of vascular patterning containing a phloem-localized auxin influx carrier, AUX1. AUX1 is modeled as a PIN in the inverse direction (influx instead of efflux) with the same transport rate as for the PINs ($T_{\text{AUX1}} = T_{\text{Aux}} = 20.0 \, \mu M^{-1} s^{-1}$). AUX1 is localized on all of the membranes of the phloem cells and constantly expressed in the phloem, its unitary concentration being proportionally divided on the cell walls. The inclusion of such a component has little effect on the overall expression of the reporter genes. All parameters have been set to the values reported in SI Appendix, Tables S2 and S3.

Movie S9
Movie S10. Simulation of our extended model showing the robustness of this genetic network in regulating vascular pattern. In this simulation, the initial conditions relating to the concentration of all network components are set to be equal to the output of simulation SM7 (this distribution of components closely resembles the expression pattern in a WT root). However, all cells are given the potential to express PIN7. The interaction between network components in this parameter space is able to robustly maintain the initial conditions.

Movie S10
Movie S11. Simulation of our extended model testing the effect of increased cytokinin on vascular patterning. The simulation has been set up using the same initial conditions and parameter set as SM12, except that $p_{Ck}$ has been set to 20.0 μM s$^{-1}$. Under similar conditions, when exogenous cytokinin has been applied to growing roots, it has been observed that $AHP6$ expression is lost and that auxin response becomes restricted to the central cells. Increasing the cytokinin levels in this simulation has a similar effect, indicating that our model has the capacity to repattern and that correct maintenance of cytokinin synthesis within a certain threshold is required to maintain a stable vascular pattern.
Movie S12. Simulation of our extended multicellular model of vascular patterning incorporating regulation of miRNA165/6 by SHORT ROOT (SHR). All of the other components and PIN distribution are maintained like in SM7. The pattern is maintained when including SHR-dependent regulation of miRNA165/6. The parameters have been set to the values reported in SI Appendix, Tables S2, S3, and S11.

Movie S12
Movie S13. Simulation of our extended model testing the requirement of SHR for establishing the correct hormonal response pattern. The simulation has been set up using the same initial conditions and parameter set as SM12, except that the transcription rate of SHR ($p_{SHR}$) has been set to zero. Under these conditions, our model predicts a flat field of PHB expression. This pattern of PHB results in a reduction in the level of AHP6, an expansion in the domain of PIN7 so that it becomes approximately radially symmetric, and a restriction in the domain of auxin output to the central cells in the xylem axis.

Movie S13
Movie S14. Simulation of our extended multicellular model of vascular patterning in an shr mutant. In this simulation, we have used the geometry of an shr mutant for the simulations. IAA2 response is restricted to the metaxylem, whereas PIN7 is expressed in an expanded, approximately radially symmetric pattern. The parameters have been set to the values reported in SI Appendix, Tables S2, S3, and S11, except for $p_{SHR_{mut}}$, which has been set to zero.

Movie S14

Other Supporting Information Files

SI Appendix (PDF)