SUPPLEMENTARY INFORMATION

1 Supplementary Notes

1.1 Mapping of the position of periclinal cell divisions

As previously reported, the cell identity in phloem/procambium is not fully correlated with the cell lineage. For example, the cell at the protophloem sieve element (PSE) position undergoes two types of periclinal cell divisions, in which PSE initially generates procambium cell files inward, and the later produces metaphloem sieve element (MSE) (Extended Data Fig. 1a). In addition, companion cells (CC) are produced by the divisions in procambial cells laterally adjacent to PSE (Extended Data Fig. 1a). In order to categorize each periclinal cell division based on the position of cells, we therefore classified the phloem/procambium cells into five groups based on their position relative to PSE and pericycle (Fig. 1a). The cells surrounding the PSE were classified into two group, PSE-lateral neighbour (PSE-LN), a cell touching both PSE and pericycle, and PSE- internal neighbour (PSE-IN), a cell touching PSE but not pericycle (Fig. 1a). The intervening procambial cells non-adjacent to PSE were classified into two categories, outer procambial cell (OPC), a cell adjacent to pericycle, and internal procambial cell (IPC), a cell touching neither PSE nor pericycle. By comparing the cell pattern in segmented images (Extended Data Fig. 1b), 273 periclinal cell divisions from 13 independent wild-type roots were mapped, resulting in 60 events in PSE, 142 events in PSE-LN, 39 events in OPC, 6 in PSE-IN and 26 events in PX position, respectively (Extended Data Fig. 1c). The number of cells in each position is different, for example, two in PSE and four in PSE-LN. Also the number of OPC or IPC increases after the periclinal division in PSE or PSE-LN during development, and therefore we counted the number of cells in each cell category in each cross-section and calculated the mean number of cells in a given cell type (Extended Data Fig. 1d). The number of events per cell in each group was calculated by diving the number of events by the mean cell number of each group during development (Fig. 1b and Extended Data Fig.1).

1.2 Redundancy of *PEAR* genes

In *Arabidopsis*, the family of DOF-domain transcription factors comprises 36 members (Extended Data Fig. 3b). Analysis of *pear1*, its closest homolog *pear2* or the double *pear1pear2* mutants did not reveal phenotypes similar to the *pPEAR1[XVE]::icals3m* line

affected in symplastic movement between phloem and procambium cells (Extended Data Fig 2a-e), suggesting broader functional redundancy within the family of DOF TFs. In order to understand the range of this effect we generated multiple combinatorial mutants covering multiple phloem specific/abundant members of the DOF family.

As a first approach we generated combinatorial mutants with the close homologs of *PEAR1* (Extended Data Fig. 3b). Knocking-out up to five genes from the *PEAR1* clade (*pear1 pear2 obp2 obp3 dof2.2* (At2g28810)), did not result in a phenotype resembling the icals3m line (Extended Data Fig. 5b). At the same time, screening of the transcriptional reporters of DOF family members identified *DOF5.6/HCA2*, *DOF3.2/DOF6* and *DOF5.3/TMO6* as genes expressed specifically/abundantly in the early phloem position (Extended Data Fig. 3d). We measured expression levels of these genes in the *pear1 pear2* mutant background and found that transcript levels of *HCA2*, *DOF6* and *TMO6* were elevated (Extended Data Fig. 3e), suggesting a compensation mechanism among more distantly related *PEAR* genes. In this scenario, the phenotype of the double mutant might be obscured by the increased expression level of other *PEAR* genes and only absence of all of them would result in strong phenotypes.

To test this hypothesis we generated multiple combinatorial *pear* mutants using available KO lines for *PEAR1*, *PEAR2*, *DOF6*, *OBP2* and *HCA2*. The intermediate triple and all quadruple mutant combinations did not show a narrow root phenotype (Extended Data Fig. 5b), however, when combining five mutations together we found that around 30 per cent of the quintuple *pear1 pear2 obp2 dof6 hca2* mutants displayed reduced root growth. These roots showed a reduction in the number of the procambial cell files, and about 30 per cent of them resembled the phenotype of roots impaired in the symplastic communication around PSE (*pPEAR1[XVE]::icals3m*, Extended Data Fig.2c). In those roots some of the procambial cell files did not undergo any periclinal divisions. Specifically comparison of all the intermediate quadruple mutants with a quintuple mutant indicates the importance of each individual gene in the regulation of phloem and procambium proliferation. Expression of *PEAR1*, *PEAR2*, *OBP2* and *HCA2* genes under their native promoters significantly increased the number of vascular cell files suppressing the strong phenotype of the quintuple mutant. The analysed lines expressing *DOF6* showed a relatively weak phenotype suppression effect in the quintuple mutant background.

Since the penetrance of the strong phenotype in the quintuple *pear1 pear2 obp2 dof6 hca2* mutants was not very high, we introduced a mutation in *TMO6* gene, a closest homolog of *DOF6* expressed abundantly in phloem and upregulated in the *pear1 pear2* double mutant background (Extended Data Fig. 3e). After introducing CRISPR-*Cas9*

generated loss-of-function allele of *TMO6* in *pear* quintuple (resulting in the *pear1 pear2 dof6 tmo6 obp2 hca2* hextuple mutant), even less cell divisions occurred in the root vasculature, reducing the variability between roots. The number of procambial cell files in the differentiated zone of the *pear* hextuple mutant (~10) closely corresponds to the number of procambial initials (~7) suggesting that almost all periclinal cell divisions are abolished in this mutant background. Importantly, we did not observe any cell division phenotype in the single *tmo6* mutant (Extended Data Fig. 5b), suggesting that *TMO6* functions redundantly with the other *PEAR* genes. To further support the importance of *PEAR* genes in the process of periclinal division of the procambial cells, we expressed them individually under their native promoters in the hextuple mutant background. *PEAR1*, *DOF6* and *TMO6* suppressed the strong phenotype of *pear* hextuple mutant confirming the role of *PEAR* genes in regulation of this process (Extended Data Fig.5d). Expression analysis of the *pPEAR2::PEAR2-VENUS* in the *pear* hextuple mutant revealed lack of *PEAR2* promoter activity in the early phloem. Since most of the periclinal divisions are concentrated around the early phloem cells, *pPEAR2::PEAR2-VENUS* did not suppress this phenotype in the *pear* hextuple mutant. By contrast, suppression was very clear in *pear* quintuple where *PEAR2* promoter is active in the early phloem cells (Extended Data Fig. 5d).

Because of the strong effect of *tmo6* mutation in the hextuple mutant background, we investigated its influence on the *pear1*, *pear2* and *dof6* mutants. Double mutants *pear1 tmo6*, *pear2 tmo6* and *dof6 tmo6* did not show the strong phenotype observed in the quintuple or hextuple mutants. The triple mutant *pear1 pear2 tmo6* showed a strong phenotype but not to the extent of the hextuple mutant (Fig 2f). This phenotype was also strongly variable suggesting that although *TMO6* plays an important role, the contribution from other *pear* mutants is required for a strong hextuple mutant phenotype. Furthermore, we have established the phenotypes for *pear1 pear2 dof6 tmo6* quadruple and *pear1 pear2 dof6 tmo6 hca2* quintuple mutant (Fig. 2f and Extended Data Fig. 5b). These mutants largely resemble the *pear1 pear2 dof6 tmo6 hca2 obp2* hextuple mutant, indicating that the mobile PEAR1, PEAR2, DOF6 and TMO6 proteins play a major role in regulating radial growth, while HCA2 and OBP2 play a more minor role.

1.3 Uncoupling the cell division and cell differentiation effects of the *pear* mutants

In addition to the reduction of vascular cell number, we made observations of cells that had not cleared the cytoplasm (characteristic to sieve element differentiation) in some of the *pear* combinatorial mutants. To assess the status of phloem, we were assaying phloem transport and unloading using the CFDA dye (Oparka et al., 1994). We found strong

transport defects in the *pear* hextuple mutant, indicating problems in the functionality of phloem. In contrast, we observed functional CFDA transport and unloading in the narrow roots of *pear1 pear2 dof6 obp2 hca2* quintuple mutant (Extended Data Fig. 5e). Thus, the problems in the PEAR mediated periclinal cell divisions can be dissected from the apparent defects in phloem differentiation. Furthermore, to exclude possibility that defects observed in phloem function and differentiation in the *pear* hextuple mutant influence the rate of periclinal divisions, we counted the number of vascular cell files at an early stage of plant development, before phloem transport becomes active. Previous work has showed that the activation of phloem transport occurs only around two days after germination, when phloem becomes fully functional as a consequence of PSE enucleation (Bauby et al. 2007) and the procambium/phloem tissue proliferation stage precedes developmentally the final differentiation of PSE (Furuta et al., 2014). We observed reduced number of vascular cell files in the postembryonic root of 1.5 days old hextuple mutant seedlings (Extended Data Fig. 5f), thus dissecting the cell proliferation and differentiation aspects of the *pear* hextuple mutant phenotype.

1.4 Bisymmetric auxin-cytokinin response pattern in root

Previous work indicated that auxin-induced cytokinin production in the xylem axis triggers the periclinal cell divisions in a non-cell autonomous manner in the flanking phloem/procambial domain (De Rybel et al., 2014). As described in Fig. 1, the periclinal cell divisions are concentrated around the PSE, whereas no periclinal cell division was observed in those internal procambial cells (IPC, Fig. 1a and b). Here we further dissected the dynamics of the hormonal response domain during procambial development. Auxin signalling maximum was formed in xylem cells already at initial stage (Extended Data Fig. 7a') and maintained during procambial development (Extended Data Fig. 7a'' and a'''). By contrast, we found that the domain of high cytokinin response is more dynamic. At initial stage, high cytokinin response was activated at PSE and its neighbouring cells (Extended Data Fig. 7a'), and this high cyokinin response domain was maintained during proliferative phase (Extended Data Fig. 7a'-a''). Only at a later stage of development, we observed that cytokinin response domain becomes concentrated in procambial cells neighbouring the xylem axis (Extended Data Fig. 7a''').

1.5 Interaction of PEAR1 and cytokinin signalling during embryogenesis

As described in main text, we revealed the interaction of PEAR1 and cytokinin sigalling in post-embryonic root vascular tissue (Fig. 3a-e). We further studied this interaction and its dynamics during embryogenesis where the root vascular cells are initiated. During

embryogenesis, high cytokinin response is initiated in vascular cells of upper lower tier (ult) at the early heart stage (Extended Data Fig. 7e and f), and only at the late heart stage is the characteristic bisymmetric pattern of cytokinin output established (Extended Data Fig. 7g and h). In *wol* embryos, activation of cytokinin response in vascular tissue does not occur and a radial auxin response pattern is maintained (Extended Data Fig. 7j-l). *PEAR1* transcription pattern was highly correlated with cytokinin signalling during embryogenesis, except for its broad expression in the early globular stage (Extended Data Fig. 7m-o). By contrast, in *wol* embryos, where no cytokinin response was detected within vascular cells, *PEAR1* transcription was initially observed in the globular *wol* embryo (Extended Data Fig. 7p) but was gradually attenuated after heart stage (Extended Data Fig.7q-r).

1.6 Analysis of PEAR1/2 downstream targets

Studying the expression patterns of PEAR1/2 downstream targets by *in silico* analysis and reporter constructs revealed that most of PEAR1/2 targets are expressed in PSE and its surrounding cells, indicating that *PEAR* genes control their targets in a non-cell autonomous manner. This result highlights that the mobility of the PEAR1/2 proteins is important for their function (Fig. 2g-h and Extended Data Fig.6a-i). In order to dissect the function of PEAR1/2 targets, we performed a statistical overrepresentation test for Gene Ontology (GO) terms of the PEAR1/2 direct targets using the PANTHER (protein annotation through evolutionary relationship) classification system (http://www.pantherdb.org/). However, no statistically significant results were found. In addition, we could not find genes previously shown to regulate cell proliferation (Supplementary Table 2), suggesting that PEAR1/PEAR2 control radial growth through still uncharacterized genes. By overexpressing some of the targets we found *SMXL3* to be able to induce periclinal cell division (Extended Data Fig.6j).

Supplemental references

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Supplementary Modelling Information

1 Model aims and philosophy

To date there have been several mechanisms shown to be capable of generating either gradients or sharp domains of differential gene expression. Although some aspects of the interaction between PEAR proteins and HD-ZIP III's are similar to other patterning mechanisms based on transcriptional regulation, the observation that HD-ZIP III has a dual effect on both *PEAR* transcription and PEAR mobility appeared to be a new type of interaction that had not been explored before. Therefore, through this modelling approach, we explore the effect that these processes have on regulating the spatial domains of both PEAR and HD-ZIP III within the procambium during phloem specification.

In order to model a single boundary between domains of PEAR and HD-ZIP III, we consider only a single generic *PEAR* gene and a single generic *HD-ZIP III* gene rather than modelling individual family members. As this patterning process occurs in a single dimension (i.e. from the centre of the root to the margin) we chose to model this as a multi-compartment model in one dimension, with compartments representing either cell wall or sub-cellular space. In addition to PEAR and HD-ZIP III, the model also considers auxin, cytokinin and miRNA165/6 as these are all components that have been shown to regulate either PEAR or HD-ZIP III (Donner et al. 2009; Carlsbecker et al. 2010; Miyashima et al. 2011). In this model auxin and miRNA165/6 can be considered as inputs to the model, with cytokinin distribution leading from the auxin distribution, while predictions for PEAR and HD-ZIP III spatial distribution can be considered as the key outputs originating from this study. As there have been a suite of models predicting the localisation of auxin, cytokinin and miRNA165/6 during root vascular patterning (Muraro et al. 2014; De Rybel et al. 2014; el Showk et al. 2015; Mellor et al. 2016), we do not seek to reproduce these findings and instead impose the spatial patterning of these three components as has been described in previous models. As formulated, the model only requires spatial positioning of an auxin source at one end of the template (in the metaxylem) and a miRNA source at the other end of the template (at the outer edge of the stele) in order to produce the spatial distribution of cytokinin, HD-ZIP III and PEAR.

1.1 Inputs: Auxin and miRNA

In this model we impose an auxin maximum in the xylem axis, in a similar way to that shown in mathematical models of root vascular patterning and supported by experimental data of response markers. Rather than incorporating auxin transporters into our model, we limit auxin production to the metaxylem and set a low diffusion parameter relative to auxin degradation so that the vast majority of auxin remains in the metaxylem, but that a low level exists in adjacent cells. The final auxin distribution is similar to previous models (Muraro et al. 2014; De Rybel et al. 2014; el Showk et al. 2015).

The effect of miRNA on the spatial distribution of the HD-ZIP III transcription factor PHB is modulated by the interaction and mutual degradation with a miRNA and has been explored mathematically in Muraro et al. (2014). In this model, the miRNA was produced in response to *SHR* in the endodermis and could then diffuse into the stele.

Here, we simplify this by assuming a constant source of miRNA at the outer edge of the stele. The miRNA can then diffuse within the stele and degrade (and be degraded by) HD-ZIP III.

1.2 Outputs: Cytokinin, PEAR and HD-ZIP

Although we do not explicitly set out to predict cytokinin distribution, it emerges from our model. Cytokinin biosynthesis is promoted by auxin response in the xylem axis via MP-dependent activation of *LOG* genes (De Rybel et al. 2014) whilst its activity is repressed in these cells via AHP6 (Mahonen et al. 2006). For this reason we include separate model components representing both the hormone itself (assumed to be produced directly in response to auxin) and a generic cytokinin response gene (assumed to be repressed by auxin directly). It is this cytokinin response that then promotes PEAR production. In this way, while PEAR production may occur wherever the cytokinin response gene is present, it is effectively excluded from the metaxylem due to the auxin source there. As has been done for previous models, we make the assumption that while the cytokinin hormone is free to move via diffusion, the cytokinin response is not.

As we show here that *PEAR* transcription is promoted by cytokinin, we use the cytokinin response (described above) to promote PEAR production in the model. Conversely, we show that HD-ZIP III represses *PEAR* transcription, so the PEAR production rate in the model is negatively affected by the level of HD-ZIP III. Based on the observed movement of translational PEAR reporters, we assume that PEAR can move via diffusion, but that this rate of movement is negatively affected by the presence of HD-ZIP III as supported by experimental determination of the diffusion coefficients.

Though we use a single generic HD-ZIP III protein we assume three independent modules contribute to its production. Firstly we assume a basal constant production rate in all cells, production is then increased in response to auxin and decreased in response to PEAR. Although there are differences in the regulation of individual HD-ZIP III's, e.g. ATHB8 shows a clear auxin induction, we feel that, collectively, these rules reflect the activity of the group. In addition to a miRNA independent rate of degradation (as is included for all model components), we also incorporate a mechanism through which HD-ZIP III and miRNA mutually degrade one another as described above.

Based on these regulatory interactions, we can predict the steady state patterns of cytokinin, HD-ZIP III and PEAR based on two inputs namely auxin and miRNA165/6. This allows us to dissect the network and examine the effects that each regulatory component has on the final pattern.

2 Model Description

2.1 Spatial Domain

The model is solved on a one-dimensional spatial array of discrete compartments representing a cross-section of root tissue from the centre of the stele at the xylem axis to the edge of the stele where phloem is formed. The spatial subdivisions may represent either cell or cell wall compartments, with multiple compartments per cell so that intracellular resolution is present within the model. Either 3, 4 or 5 cells are simulated, subdivided by 2, 3 or 4 walls respectively. All compartments have equal unit widths, with 23 compartments per cell and 2 compartments per cell wall. Each compartment is numbered sequentially from 1 (xylem) to N (outer stele) where N is the total number of compartments. The subset of cellular compartments is denoted K and the subset of wall components denoted W.

2.2 Model Components

The model simulates the evolution over time in a given spatial compartment i of a miRNA (denoted M_i), a generic HD-ZIP III (denoted H_i), PEAR (denoted P_i), auxin (denoted A_i), cytokinin (denoted C_i) and the cytokinin response (denoted R_i). Differentiating cytokinin from the cytokinin response in this way is necessary to capture the dual role of auxin in promoting cytokinin biosynthesis while also repressing the cytokinin response (De Rybel et al. 2014).

The spatial movement of model components via diffusion is modelled using standard discretisations of diffusion operators to simulate flux between adjacent compartments, with the exception of the movement of PEAR. In the case of PEAR to simulate the blocking of PEAR movement by HD-ZIP

III the diffusion coefficient between two adjacent compartments is modified by a decreasing function of the average value of HD-ZIP III in the two compartments:

$$J = D_p \left(\frac{P_{i-1} - P_i}{1 + \left(\frac{H_{i-1}+H_i}{2\phi} \right)^q} \right), \tag{1}$$

where J is the flux of PEAR between compartments i and $i-1$, D_p is the diffusion coefficient in the absence of HD-ZIP III and ϕ and m are additional parameters. For values and definition of these and all other parameters please refer to Table 1.

For simplicity, when modelling the three genes *HD-ZIP III*, *PEAR* and the generic cytokinin response we use a single variable to represent both the mRNA and protein. Gene expression is modelled using combinations of Hill functions, depending on the required regulatory logic. We assume *HD-ZIP III* is activated by three independent modules, one constitutive, one auxin dependent and one PEAR dependent, and so model the transcription rate of *HD-ZIP III* F_h as the sum of a constant, and increasing functions of auxin and PEAR:

$$F_h(A, P) = \lambda \left(1 + \frac{A^{n_h}}{\theta_h^{n_h} + A^{n_h}} + \frac{P^{m_h}}{\psi_h^{m_h} + P^{m_h}} \right), \tag{2}$$

where θ_h and ψ_h are threshold parameters, n_h and m_h Hill coefficents, and λ a proportionality constant.

PEAR is activated by the cytokinin response and repressed by HD-ZIP III so we model its transcription F_p as the product of positive and negative Hill functions:

$$F_p(H, R) = \frac{\theta_p^{n_p}}{\theta_p^{n_p} + H^{n_p}} \times \frac{R^{m_p}}{\psi_p^{m_p} + R^{m_p}}, \tag{3}$$

where θ_p and ψ_p are threshold parameters and n_p and m_p Hill coefficents.

Cytokinin production F_c is modelled as a simple increasing Hill function of auxin:

$$F_c(A) = \frac{A^{n_c}}{\theta_c^{n_c} + A^{n_c}}, \tag{4}$$

where θ_c is the threshold parameter and n_c the Hill coefficent.

Finally the cytokinin response is regulated positively by cytokinin, but negatively by auxin so its transcription rate F_r is given by:

$$F_r(A, C) = \frac{\theta_r^{n_r}}{\theta_r^{n_r} + A^{n_r}} \times \frac{C^{m_r}}{\psi_r^{m_r} + C^{m_r}} \tag{5}$$

where θ_r and ψ_r are threshold parameters and n_r and m_r Hill coefficents.

For all of these production rate functions we make the approximation that the rate of production is equal throughout a given cell. This is done by using the mean value of any given transcription factor within the set of compartments making up that cell in the above functions. We denote these mean cellular values for a given compartment using the $\widehat{\ }$ notation so that for example the rate of cytokinin production in a compartment i is a function of $\widehat{A}_i$, the mean value of auxin in all compartments in the cell containing i.

The modelling of the mutual degradation of miRNA and HD-ZIP III is simulated via mass-action and is similar to that of Muraro et al. (2014). Degradation terms for each model component are included in all cellular compartments.

2.3 Cellular compartments

Combining the above for $1 < i < N$ and $i \in K$ (cellular compartments away from the boundary) we have the following set of ordinary differential equations:

$$\frac{dM_i}{dt} = D_m(M_{i-1} + M_{i+1} - 2M_i) - \mu_m M_i - \eta_m M_i H_i, \tag{6a}$$

$$\frac{dH_i}{dt} = \mu_h \left(F_h(\widehat{A}_i, \widehat{P}_i) - H_i - \eta_h M_i H_i \right), \tag{6b}$$

$$\frac{dP_i}{dt} = \mu_p \left(F_p(\widehat{H}_i, \widehat{R}_i) - P_i \right) + D_p \left(\frac{P_{i-1} - P_i}{1 + \left(\frac{H_{i-1}+H_i}{2\phi} \right)^q} + \frac{P_{i+1} - P_i}{1 + \left(\frac{H_i+H_{i+1}}{2\phi} \right)^q} \right), \tag{6c}$$

$$\frac{dA_i}{dt} = \alpha_i - A_i + D_a(A_{i-1} + A_{i+1} - 2A_i), \tag{6d}$$

$$\frac{dC_i}{dt} = \mu_c \left(F_c(\widehat{A}_i) - C_i \right) + D_c(C_{i-1} + C_{i+1} - 2C_i), \tag{6e}$$

$$\frac{dR_i}{dt} = \mu_r \left(F_r(\widehat{A}_i, \widehat{C}_i) - R_i \right), \tag{6f}$$

where D_m, D_a and D_c are the respective diffusion coefficients of miRNA, auxin and cytokinin, μ_m, μ_h, μ_p, μ_c and μ_r are turnover rates of miRNA, HD-ZIP III, PEAR and cytokinin and the cytokinin response and η_m and η_h are the mutual degradation rates of miRNA and HD-ZIP III. α_i is the production rate of auxin and is set to be zero except for the in compartments in the first cell representing the xylem axis.

2.4 Wall compartments

For the wall compartments ($i \in W$) we set production and degradation equal to zero and only model movement and the mutual degradation of HD-ZIP III and miRNA so that:

$$\frac{dM_i}{dt} = D_m(M_{i-1} + M_{i+1} - 2M_i) - \eta_m M_i H_i, \tag{7a}$$

$$\frac{dH_i}{dt} = 0, \tag{7b}$$

$$\frac{dP_i}{dt} = D_p \left(\frac{P_{i-1} - P_i}{1 + \left(\frac{H_{i-1}+H_i}{2\phi} \right)^q} + \frac{P_{i+1} - P_i}{1 + \left(\frac{H_i+H_{i+1}}{2\phi} \right)^q} \right), \tag{7c}$$

$$\frac{dA_i}{dt} = D_a(A_{i-1} + A_{i+1} - 2A_i), \tag{7d}$$

$$\frac{dC_i}{dt} = D_c(C_{i-1} + C_{i+1} - 2C_i), \tag{7e}$$

$$\frac{dR_i}{dt} = 0. \tag{7f}$$

2.5 Boundary compartments

At the boundary representing the centre of the stele ($i = 1$) we have zero flux boundary conditions so that:

$$\frac{dM_1}{dt} = D_m(M_2 - M_1) - \mu_m M_1 - \eta_m M_1 H_1, \tag{8a}$$

$$\frac{dH_1}{dt} = \mu_h \left(F_h(\widehat{A}_1, \widehat{P}_1) - H_1 - \eta_h M_1 H_1 \right), \tag{8b}$$

$$\frac{dP_1}{dt} = \mu_p \left(F_p(\widehat{H}_1, \widehat{R}_1) - P_1 \right) + D_p \left(\frac{P_2 - P_1}{1 + \left(\frac{H_1 + H_2}{2\phi} \right)^q} \right), \tag{8c}$$

$$\frac{dA_1}{dt} = \alpha_1 - A_1 + D_a(A_2 - A_1), \tag{8d}$$

$$\frac{dC_1}{dt} = \mu_c \left(F_c(\widehat{A}_1) - C_1 \right) + D_c(C_2 - C_1), \tag{8e}$$

$$\frac{dR_1}{dt} = \mu_r \left(F_r(\widehat{A}_1, \widehat{C}_1) - R_1 \right), \tag{8f}$$

and finally at the outer stele boundary ($i = N$) we also have zero flux boundary conditions, except for miRNA which is held fixed at M_{bnd} so that:

$$\frac{dM_N}{dt} = D_m(M_{N-1} - 2M_N + M_{bnd}) - \mu_m M_N - \eta_m M_N H_N, \tag{9a}$$

$$\frac{dH_N}{dt} = \mu_h \left(F_h(\widehat{A}_N, \widehat{P}_N) - H_N - \eta_h M_N H_N \right), \tag{9b}$$

$$\frac{dP_N}{dt} = \mu_p \left(F_p(\widehat{H}_N, \widehat{R}_N) - P_N \right) + D_p \left(\frac{P_{N-1} - P_N}{1 + \left(\frac{H_{N-1} + H_N}{2\phi} \right)^q} \right), \tag{9c}$$

$$\frac{dA_N}{dt} = -A_N + D_a(A_{N-1} - A_N), \tag{9d}$$

$$\frac{dC_N}{dt} = \mu_c \left(F_c(\widehat{A}_N) - C_N \right) + D_c(C_{N-1} - C_N), \tag{9e}$$

$$\frac{dR_N}{dt} = \mu_r \left(F_r(\widehat{A}_N, \widehat{C}_N) - R_N \right). \tag{9f}$$

2.6 Model Parameters

For model parameter values see Table 1. All parameter values are estimates within reasonable bounds based on trial and error in order to demonstate the plausibilty of the model in reproducing experimental observations. Since the model is dimensionless, in the absence of spatial effects, variables are mostly constrained between 0 and 1, and the parameter values are also dimensionless. In particular the Hill thresholds and coefficients represent the relative sensitivity of the different regulatory mechanisms present, with most set to default values of 0.1 and 1 respectively. The relative values of the parameters relating to the mutual degradation of miRNA and HD-ZIP III are similar to those used in Muraro et al. (2014), while values for the turnover rates of model variables have little or no effect on model steady state. Selecting appropriate values for the relative diffusion coefficents is essential in order to observe gradients in the model components over the desired spatial scales, in the experimentally observed positions. While using other parameter values is likely to alter both the position and magnitude of these gradients, we find that small perturbations from the selected parameter set does not significantly affect the overall patterns produced by the model.

Table 1: Nondimensional parameters, with default values.

Diffusion coefficients	
D_m	400
D_p	300
D_a	10
D_c	1000
ϕ	0.005
q	2
Transcription parameters	
λ	0.5
θ_h	1
ψ_h	1
n_h	1
m_h	1
θ_p	0.2
ψ_p	0.1
n_p	4
m_p	1
θ_c	0.1
n_c	1
θ_r	0.1
ψ_r	0.1
n_r	1
m_r	1
Turnover rates	
μ_h	1
μ_p	1
μ_c	1
μ_r	1
miRNA / HD-ZIP III interaction	
η_m	500
η_h	1000
M_{bnd}	1
μ_m	1
Auxin production	
α_i (i in xylem cell)	1
α_i (i not in xylem cell)	0

3 Alternate Cases

3.1 No block on PEAR movement by HD-ZIP III

For the case where the block on PEAR movement in the presence of HD-ZIP III is removed, we simply replace the HD-ZIP III modified diffusion operator given by Equation (1) used in Equations (6c), (7c), (8c) and (9c) with the standard discretised diffusion operator so that in general:

$$\frac{dP_i}{dt} = \mu_p \left(F_p(\widehat{H}_i, \widehat{R}_i) - P_i \right) + D_p \left(P_{i-1} - 2P_i + P_{i+1} \right), \tag{10}$$

with zero flux boundary conditions defined as before.

3.2 No positive feedback on HD-ZIP III expression from PEAR

To omit the positive feedback from PEAR on HD-ZIP III we replace Equation (2) with:

$$F_h(A) = \lambda \left(1 + \frac{A^{n_h}}{\theta_h^{n_h} + A^{n_h}} \right), \tag{11}$$

so that HD-ZIP III production is only dependent on auxin plus a constitutive component.

3.3 No negative feedback on PEAR expression from HD-ZIP III

To omit the negative feedback from HD-ZIP III on PEAR we replace Equation (3) with:

$$F_p(R) = \frac{R^{m_p}}{\psi_p^{m_p} + R^{m_p}}, \tag{12}$$

so that PEAR production is only dependent on the cytokinin response.

4 Model Implementation

The model is implemented using the Python 2.7 programming language with the 'odeint' function from the Scipy package used to solve the differential equations (Jones et al. 2001–) and Matplotlib (Hunter 2007) used to plot the state of the model after $100,000$ timesteps, which we assume to be at steady state.

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