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Modelling meristem development in plants

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Meristems continually supply new cells for post-embryonic plant development and coordinate the initiation of new organs, such as leaves and flowers. Meristem function is regulated by a large and interconnected dynamic system that includes transcription networks, intercellular protein signalling, polarized transport of hormones and a constantly changing cellular topology. Mathematical modelling, in which the dynamics of a system are simulated using explicitly defined interactions, can serve as a powerful tool for examining the expected behaviour of such a system given our present knowledge and assumptions. Modelling can also help to investigate new hypotheses *in silico* both to validate ideas and to obtain inspiration for new experiments. Several recent studies have used new molecular data together with modelling and computational techniques to investigate meristem function.

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Introduction

Because of the often regular and symmetrical patterns generated by plants, plant architecture has fascinated not only biologists but also mathematicians and artists alike for centuries [1]. Over the past couple of years, however, mathematical and modelling approaches to understanding plant development have gained fresh momentum [2,3], partially as a result of inexpensive computing power but also because of the rapid increase in detailed molecular data related to plant development.

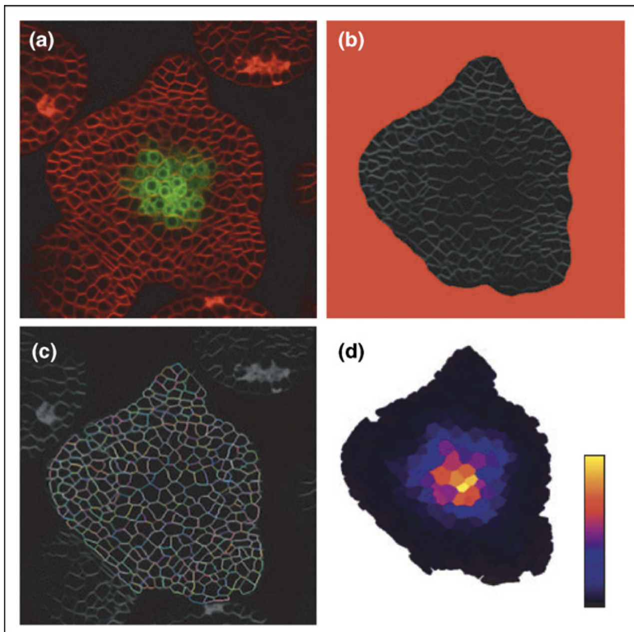
The origin of many plant developmental patterns can be traced back to meristems that are located at the growing tips of roots and shoots, from which most post-embryonic structures are derived. In this review, we focus on how modelling and computational techniques have recently been combined with detailed molecular data to help understand meristem development and function.

Meristem maintenance

One of the astounding features of meristems is their ability to maintain a specific cellular structure and growth pattern throughout the lifetime of the plant, which can last thousands of years in some cases. Experimental studies have shown that the size of the shoot apical meristem (SAM), in particular the region of undifferentiated cells at the very apex, is regulated through a negative-feedback loop between the transcription factor WUSCHEL (WUS) and a small secreted peptide CLAVATA3 (CLV3). *WUS* is expressed in the organising centre, a small subapical region in the interior of the shoot. It promotes stem cell identity and positively regulates *CLV3* transcription. CLV3, in turn, acts together with the receptor kinase CLV1 to repress *WUS* expression, thus creating a negative feedback loop [4–8]. Although this feedback loop provides an intuitively simple system for regulating the size of the *WUS* expression domain, and hence the proliferation of stem cells in the SAM, it leaves several questions open, including the question of how the pattern of the *WUS* expression domain is specified.

The *WUS* expression pattern is intriguing because it is capable of reorganising itself after disruption by laser ablation [9], mirroring the ability of meristems in general to self-organise after wounding. Self-organisation is also a property exhibited by reaction–diffusion systems, as proposed by Turing [10] and by Gierer and Meinhardt [11]. As a test to see how well such a scheme might work to control *WUS* expression, Jönsson *et al.* [12••] examined a mathematical model in which *WUS* expression was placed under the control of a reaction–diffusion mechanism. The model was simulated on a two-dimensional (2D) cellular template extracted from a transverse confocal microscope section of the SAM centre (Figure 1a–c). The model created one maximum of *WUS* expression within the meristem region, which was centred within the tissue using a hypothetical repressive signal emanating from the epidermal (or L1) cell layer. Model parameters were tuned by comparing the output of the model directly with real *WUS* expression, also extracted from the template data (Figure 1d). When the central cells that express *WUS* were removed from the model to simulate the effect of laser ablation, *WUS* expression reappeared on either side of the ablated region in a similar fashion to the observed experimental response. An alternative model, using constitutive expression modulated by the repressive signal from the L1, failed to re-organise *WUS* expression in this

Figure 1



Segmentation of 2D cell geometry and *WUS* expression from confocal data. **(a)** Original transverse confocal optical section through the *Arabidopsis* inflorescence meristem. The cell membranes (red) are stained with FM4-64. GFP localised in the endoplasmic reticulum under the control of the *WUS* promoter is also shown (green). **(b,c)** Cells extracted from the membrane data by removing the background (b), and using a watershed-like algorithm for segmentation (c). **(d)** Quantitative estimation of WUS expression from GFP intensities in segmented cells. Expression levels are indicated from black (low intensities) to yellow (high intensities). Reproduced from [12••] with permission from Oxford University Press, Copyright 2005.

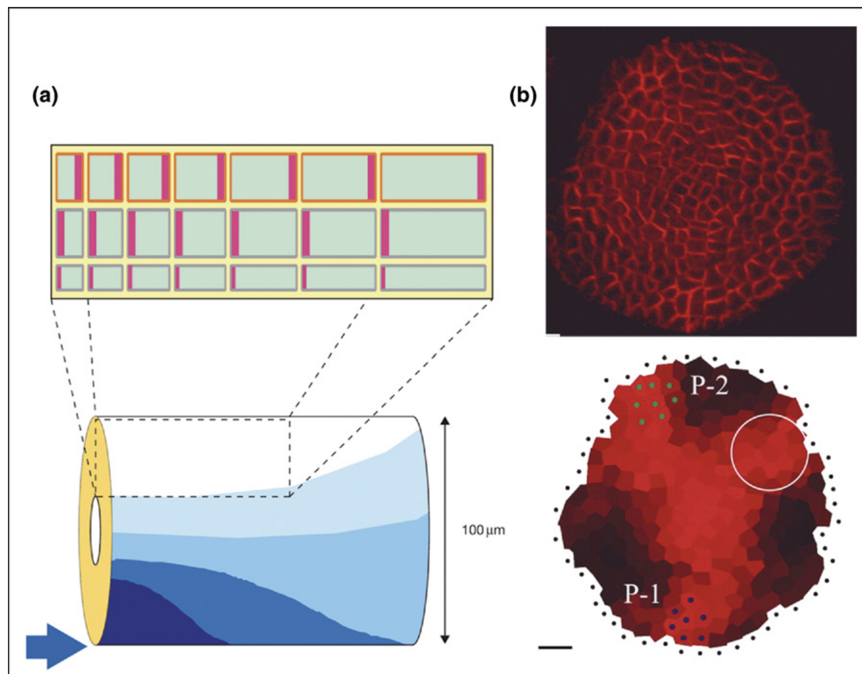
way. Although this model is abstracted from the biology to a considerable degree, it suggests that the basic mechanism of long-range inhibition together with local reinforcement could account for the self-organisational properties of not only *WUS* expression but also meristems in general. Although CLV3 is a prime candidate for a WUS-induced long-range inhibitor, recent experiments also show that members of the homeodomain zipper (HD-ZIP) class of transcription factors play a repressive role in regulating *WUS* expression [13,14]. It will be interesting to test whether the expression of these genes, like that of *CLV3*, is also dependent on WUS activity and whether models can be used to discriminate between different scenarios of long-range inhibition.

How is auxin distributed in meristems?

Auxin flow within the root meristem is coordinated by multiple members of the PIN-FORMED (PIN) family of auxin efflux mediators, and by the auxin influx mediator AUX1 [15•,16]. Swarup *et al.* [17••] combined experiments and modelling to investigate the role of auxin in mediating gravitropism. A three-dimensional (3D) model, corresponding to the elongation zone proximal to the meristem, was constructed using the stereotypical arrangement of root cells and detailed distribution patterns of PIN1, PIN2 and AUX1 (Figure 2a). The model used these data, together with the chemiosmotic transport theory [18,19], to show that the expression of *AUX1* in the epidermal cells should be sufficient to mediate the apical transport of auxin from the root tip with only moderate diffusion. Given an asymmetric pulse of auxin from the root apex in response to an altered gravity vector, epidermal cells are predicted to maintain this asymmetry throughout the elongation zone to promote differential growth. In fact, Swarup *et al.* [17••] go on to predict that PIN2 should not be required in the epidermis for this transport function because low levels of PIN1 in these cells should suffice. Instead, PIN2 is suggested to be necessary only for auxin efflux into the epidermis from the lateral root cap. This study represents an important step towards modelling the flux of auxin throughout the root meristem. It would be interesting to see whether such a complete model might account for the specific auxin-induced expression and protein degradation patterns of the various PIN proteins.

In the SAM, PIN1 expression and localisation in the epidermal layer appear to be important for determining the auxin distribution in relation to where new primordia are formed ([20,21••]; see below). However, PIN1 localisation patterns in these cells are harder to interpret than PIN1 localisation patterns in the root. Some cells are not clearly polarised and PIN localisation patterns change constantly as primordia development proceeds around the meristem periphery [21••]. To try to deduce auxin distribution patterns in these cells, de Reuille *et al.* [22••] used confocal imaging to visualise and document PIN1 immunolocalization patterns in the L1 (Figure 2b). After hand-marking these patterns such that each side of each cell was designated to either contribute or not contribute transport to the adjoining cell, they introduced a simplified model for PIN1-dependant auxin flow (not based on the chemiosmotic transport mechanism) that included diffusion. Encouragingly, their simulations predicted auxin peaks at positions in which new primordia were about to form, as has been shown to occur experimentally. Unexpectedly, their model also predicted high auxin levels at the shoot apex (Figure 2b). The authors were able to support this prediction experimentally: they successfully detected indole acetic acid (IAA) specifically in the meristem apex using an IAA-specific monoclonal antibody and gas chromatography–mass spectrometry (GCMS). By showing that the synthetic auxin reporter DR5 was not sensitive to exogenous auxin in this region, the authors concluded that perception of auxin is suppressed at the level of primary auxin-response genes at the meristem centre; however, contradictory results are presented in [23••]. Finally, the simulations of their flow model led de Reuille *et al.* [22••] to hypothesise that auxin levels increase at new primordial positions because of

Figure 2



Modelled auxin distribution pattern in the root and shoot. **(a)** Illustration of the cylindrical template, representing the root elongation zone used in Swarup *et al.* [17••]. The 3D model incorporates the epidermis, cortex, and endodermis cells (top, middle and bottom row of cells, respectively) and auxin transport mediator locations illustrated in the inset (PIN1/PIN2, pink color [located basipetally and apically in the cells]; AUX1, orange color [located at all cell membranes in the epidermal cells]). The blue shades illustrate auxin concentrations in the epidermis when auxin is deposited at the lower side from the lateral root cap (arrow). (Adapted with permission from Macmillan Publishers Ltd: Nature Cell Biology [17••], Copyright 2005.) **(b)** Transverse section of the SAM showing anti-PIN1 immunolabelling (top) and simulation output showing auxin concentrations (bottom) from de Reuille *et al.* [22••].The cells at the location of a new primordia (circle) as well as the apex have high auxin concentrations. Two older primordia (P-1, P-2) are defined by the authors and indicated with dots within the cells. These cells are modeled with an extra depletion, and the dots outside the template indicates where auxin is supplied in the simulation. Scale bar represents 20 µm. (Modified from [22••] with permission from National Academy of Sciences, Copyright 2006.)

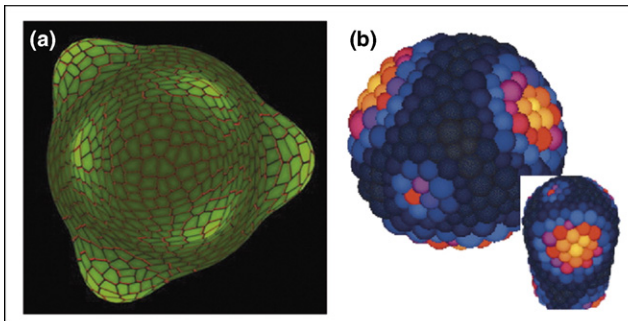
overaccumulation of auxin in the meristem centre and depletion of auxin by neighbouring primordia. The question of how PIN1 polarity is coordinated to produce this flow pattern is left unanswered.

The heart of phyllotaxis — an auxin-mediated spacing mechanism

The positioning of lateral organs (i.e. phyllotaxis) has long been the subject of modelling studies. One of the key findings from these studies is that many, if not most, of the complex patterns of organs observed in nature can be generated by any kind of regular spacing mechanism combined with a gradually expanding generative region, such as a meristem growing over time [24–28]. Recent experimental studies have shown that an essential part of the mechanism involves the transport of auxin, via the asymmetrically localised PIN1 auxin efflux carrier, to positions where primordia are destined to form [29–31]. Thus, a central question is what coordinates PIN1 localisation in such a way? Two recent studies have proposed a mechanism that not only is capable of generating close to observed PIN1 localisation patterns but also is able to generate regularly spaced peaks and troughs of auxin concentration spontaneously [23••,32••]. At the core of these models is a feedback system in which PIN1 protein is localised to the membranes of a cell closest to neighbouring cells that contain the most auxin. Thus, cells with high auxin content polarise their neighbours towards them, further increasing their auxin content until the flux from polar transport is balanced by diffusion.

Although both models are based on the same hypothesis for PIN polarisation, they differ in their approaches. Smith *et al.* [23••] do not always use equations that are easily interpretable in terms of biochemical mechanisms, and to stabilise their patterns, they include additional rules for localising PIN1 that relate abstractly to primordial differentiation. Their model parameters are, however, tuned by comparing the resulting phyllotactic patterns with experimental data from *Arabidopsis*, and a good correspondence is achieved. Last, their model is run on a global growth template defined by the authors to resemble real meristem tissue growth (Figure 3a). By contrast, Jönsson *et al.* [32••] create a dynamic cellular

Figure 3



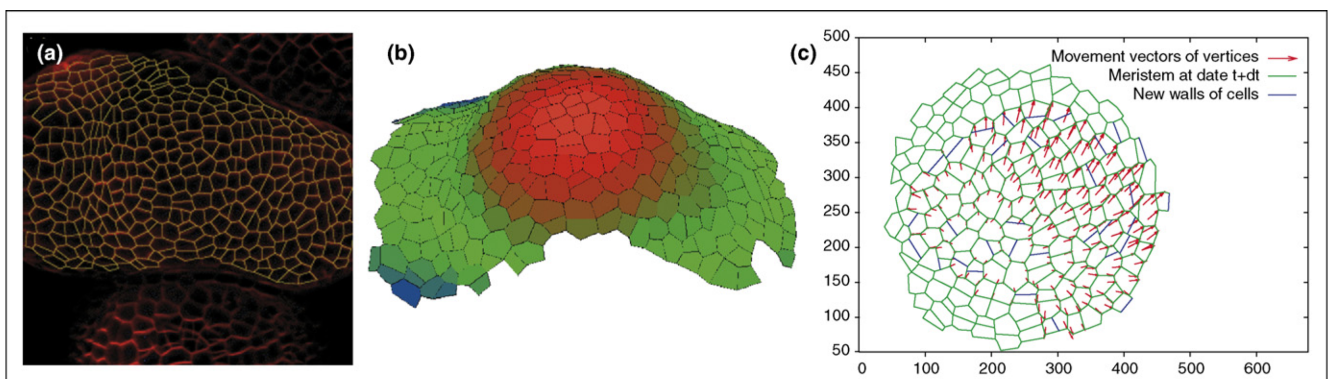
Output from auxin transport models of phyllotaxis. **(a)** Whorled pattern of auxin maxima (light green) and PIN1 distribution (red) generated by the model proposed by Smith *et al.* [23••] on expanding a 2D template mimicking the meristem epidermis. (Modified from [23••] with permission from National Academy of Sciences, Copyright 2006.) **(b)** Spiral pattern of auxin maxima (red and orange) generated by the model proposed by Jönsson *et al.* [32••] on a mechanically growing layer of cells that mimicks the meristem epidermis. Blue indicates low auxin concentrations, red medium and yellow high. Inset shows side view. (Modified from [32••] with permission from National Academy of Sciences, Copyright 2006.)

template using spring mechanics to model growth. Although such a growth model does not appear very 'plant-like', it represents an early attempt to couple real cell wall mechanics to a signalling and gene regulatory model (Figure 3b). The auxin transport model is also defined differently using mechanistic equations, and an experimental template is used together with the chemiosmotic auxin transport theory to estimate parameter values for modelling PIN1 polarisation. This model is also capable of generating phyllotactic patterns, although not as stably as those generated by the model developed by Smith *et al.* [23••]. Overall, the picture that emerges is that PIN1 polarisation by cytoplasmic auxin concentrations in neighbouring cells, even if true, can only be part of the auxin story. Perhaps this is not surprising given the existence of many other players, such as the PINOID (PID) kinase [33], *SHOOTMERISTEMLESS* (*STM*) and *CUP-SHAPED COTYLEDONS*1/2 (*CUC*1/2), that also mediate organ positioning and growth [34]. Nevertheless, these initial models provide us with a novel potential positioning mechanism that is based on up-to-date molecular data and hypotheses that are experimentally testable.

A first step towards the integration of gene regulatory models with morphogenesis and mechanics

So far, we have discussed discrete models that deal with gene expression patterns and the distribution of signalling molecules within 'virtual' cellular templates. Each template has been different, from the static 3D root architecture used by Swarup *et al.* [17••] to the mechanically driven 2D template described by Jönsson *et al.* [32••]. Ideally, the model template should resemble the real plant tissue as closely as possible. Such a template might involve not only creating a cellular architecture but also the use of gene expression patterns and protein distributions and the time-evolution of these data. This is where confocal live-imaging techniques potentially offer significant new advantages for assessing and developing plant developmental simulations. de Reuille *et al.* [35••] used a novel semi-automatic protocol to obtain the cellular architecture of the meristem epidermal layer. This procedure is based on segmenting epidermal cell outlines using a membrane-localised fluorescent dye or green fluorescent protein (GFP) that is then imaged using confocal microscopy (Figure 4a,b). The neighbour relationships can be determined from these data, and by conducting time-lapse imaging, the shapes of these cells can be extracted over time (Figure 4c). Although this type

Figure 4



Four-dimensional segmentation of the shoot meristem epidermis. **(a)** Top view of the meristem epidermis extracted from a stack of transverse confocal images. The background has been removed and the cells manually extracted. **(b)** Three-dimensional view of the epidermis of the meristem. **(c)** Vertex movements as calculated from two consecutive time-points of the surface reconstruction. Note also new cell walls marked in blue. (Modified from [35••] with permission from Blackwell Publishing Ltd, Copyright 2005.)

of data has also been obtained using a simpler non-invasive replica method [36–38], this study potentially represents a first step towards using confocal imaging to extract a multivariable modelling template that includes gene expression patterns and protein localisation data along with cell-shape dynamics.

Conclusions

It is an exciting time for understanding the role of auxin in meristems, and this is reflected in the choice of papers we have focused on in this review. The use of new imaging and perturbation techniques is also providing us with data of unprecedented detail for use in models [21••,39, 40•–42•]. In fact, we feel that these new experimental techniques are of great help in providing the detailed dynamic data required for inspiring mechanistic models and for testing the models adequately.

A challenge not yet addressed in the literature is to link gene regulatory models with realistic models for cell wall mechanics, because only then can models bridge the gap between signalling and morphogenesis. This might not be a trivial task, given the anisotropic and visco-elastic nature of plant cell walls [43], although recent progress is encouraging [44]. Another future challenge, as more and more data become available, is to somehow integrate and combine models and, hopefully, this is where efforts at creating standardised languages such as Systems Biology Markup Language (SBML) can bear fruit [45].

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The authors used an ethanol-inducible gene expression system to overactivate *CLV3* in its normal expression domain in the inflorescence meristem. A sustained *CLV3* induction caused termination of shoot meristem development, whereas a pulsed induction led to a rapid but transient repression of *WUS* expression. Both *WUS* and endogenous *CLV3* expression were measured following the pulsed CLV3 induction using quantitative RT–PCR. This work also helped to characterize the non-linearity and robustness of the CLV–WUS feedback loop by showing that the CLV3 signal strength can vary ten-fold without disrupting shoot meristem development. This paper gives quantitative insight into the dynamics of the CLV3–WUS feedback loop.

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This study documents dynamic changes in auxin transport patterns and cell-type patterning that occur during the regeneration of the *Arabidopsis* root tip after laser ablation. Using time-lapse confocal imaging, the authors find that changes in auxin distribution occur rapidly, followed by changes in cell identity, which in turn are followed by changes in PIN polarity patterns. The *PLETHORA* genes as well as *SHORTROOT* and *SCARECROW* are required for proper re-patterning to occur. By dissecting this process in detail, the authors are able place auxin, cell-fate patterning and polar auxin transport into a linear pathway.

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