A Computational Model of Cellular Morphogenesis in Plants

Tim Rudge[1] and Jim Haseloff

Department of Plant Sciences, University of Cambridge, Cambridge CB2 3EA, U.K
[1] Present address: ARC Centre for Complex Systems and Advanced Computational Modelling Centre, University of Queensland, QLD 4072, Australia
timrudge@maths.uq.edu.au
jim.haseloff@plantsci.cam.ac.uk

Abstract. Plant morphogenesis is the development of plant form and structure by coordinated cell division and growth. We present a dynamic computational model of plant morphogenesis at cellular level. The model is based on a self-reproducing cell, which has dynamic state parameters and spatial boundary geometry. Cell-cell signalling is simulated by diffusion of morphogens, and genetic regulation by a program or script. Each cell runs an identical script, equivalent to the genome. The model provides a platform to explore coupled interactions between genetic regulation, spatio-mechanical factors, and signal transduction in multicellular organisation. We demonstrate the capacity of the model to capture the key aspects of plant morphogenesis.

1 Introduction

Plant morphogenesis is the formation of shape and structure by coordination of cell shape, growth, and proliferation by mitosis. The control mechanisms involved in regulating morphogenesis are complex, and reverse engineering them from experimental data is an extremely difficult task. Thus, computational and mathematical models are becoming increasingly important tools for developmental biologists.

1.1 Plant Morphogenesis

Plant cells are enclosed in a semi-rigid cell wall composed of cellulose microfibrils, other polysaccharides and proteins[1]. They secrete an extracellular matrix which binds the walls of neighbouring cells into fixed relationships - each cell is firmly bound to its neighbours[1]. The partition between two cells is *double-walled*, with each cell's wall having independent composition, architecture and properties [2]. Division of plant cells occurs, after the chromosomes have been duplicated and a phragmoplast formed, by the synthesis of a new wall segment splitting the cell into two halves[1].

Cells maintain hydrostatic pressure, or turgor, which produces strain in the walls[1]. Growth is an interplay between this pressure driven stretching of walls and

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biosynthetic processes that modify the walls' protein structure [3]. Enzymes and other agents maintain the mechanical strength and extensibility of cell walls until they cease growing [4]. The two processes occur on different time scales; biosynthesis occurring over hours or days, and elastic stretching over seconds or minutes [3]. The interface between two neighbouring plant cells is typically linear in cross-section (figure 2), suggesting minimal pressure differences between neighbours.

Plant cells are often polar, that is they organise their behaviour along directional axes. Growth and division are coordinated with respect to these axes.

Factors that regulate cell behaviour (e.g. via gene expression), such as hormones, transcription factors and other molecules are transported from cell to cell. These signalling pathways have been shown to play a role in directing many developmental processes including cell shape, growth, movement, and proliferation [5], as well as cell polarity orientation[6, 7].

Signalling and other mechanisms provide key positional information, which coordinates differential cell behaviour. Although lineage plays some role in cell fate specification, it seems positional information is of primary importance in plant development [8, 9].

1.2 Modelling Approaches

Several approaches to modelling cell shape mechanics and multicellular organisation have been developed. Although not particular to plant cells, these models provide a basis on which to elaborate specific techniques for examining plant morphogenesis.
Fleischer and Barr [10] used a spherical geometric representation of cells, concentrating their efforts on cell proliferation and subsequent multi-cellular organisation. They took a rule-based approach to cell behaviour and used diffusion for cell-cell signalling. Although useful, the spherical (or any other primitive-based) model cannot properly capture the range of cell-cell relations that forms the basis for the intercellular signalling that is thought to direct plant growth. In particular, the Fleischer representation limits cells to sphere packing arrangements, which are not typical of plant cells.

Honda et al [11, 12] and Kawasaki and Okuzuno [13] developed 2- and 3-dimensional vertex methods for modelling cell shape. These models describe the cell-boundary as a collection of linear (2D) or polygonal (3D) faces. They considered the effects of cell division in [12], but did not consider cell behaviour in any detail. The linear representation of cell-cell interfaces fits well with observed plant cell shapes, and allows realistic multi-cell arrangements.

We take a hybrid approach, using a similar geometric representation to [12] and [13], with the diffusion signalling used in [10]. We add anisotropic cell behaviour and a system for specifying arbitrary models of genetic regulation.

2 Computational Model

Each cell is defined by a set of dynamic state parameters (including morphogen levels, growth rate, etc.) and a closed boundary. The state of the cell determines its behaviour at any point in time. Cell behaviour is expressed as the transformation of

cell state parameters to proceed to a new state. These transformations are defined in the genetic script, which is the same for each cell.

The boundary of the cell describes its shape, and is decomposed into a set of *walls*. Each wall is the interface between two cells. Morphogens move from one cell to the other via the wall, and both of the adjacent cells have equal influence on its properties. Cell growth is the process of increasing the lengths of the walls to varying degrees.

The state parameters provide feedback between the cell shape, cell-cell signalling, and the regulation of cell behaviour. The nature of the coupling of these feedback processes is arbitrarily defined in the genetic script.
The model is iterated as follows:

1. Execute genetic script for each cell
2. Iterate the morphogen diffusion system over N time-steps
3. Adjust the mechanical properties of the walls
4. Find the equilibrium wall configuration
5. Repeat from step 1

2.1 Cell Shape

We model cell geometry in 2-dimensions – approximating a layer of cells. The cell walls are modelled as *two* linearly elastic elements (springs) bound together at the end points. Each of the adjacent cells influences the properties of only one of these springs. Each spring has stiffness K and natural length L_n determined from the state parameters of the appropriate cell. Simulated forces are computed at each vertex as shown in figure 1A. The magnitude of the turgor force is PL, where P is the cell pressure and L is the wall length.

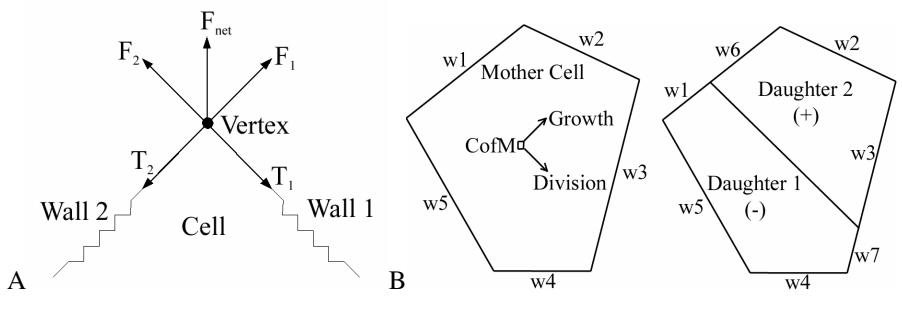

Fig. 1. (A) Forces on a vertex due to turgor (F) from one cell, and elastic tension (T). (B) Cell division consists of inserting a new wall across the centre of mass of the cell. Existing walls *w1* and *w3* are split in two by the division.

Cell growth is achieved by increasing the natural lengths of the springs associated with the growing cell, simulating biosynthesis of wall materials. At each time step the cells' growth rates are used to compute the natural lengths of each of the springs, using:

$$L_n^{t+1} = L_n^t + \lambda(L^t - L_n^t) \tag{1}$$

where λ is a function of growth rate defined on [0,1] (see next section), and superscripts denote time step.

Synthesis of wall constituents continually maintains wall strength [4]. In order to model this we impose the limit on L_n:

$$L_n^{t+1} = Max\left\{L_n^t, L_n^t + \lambda(L^t - L_n^t), (1-\varepsilon_o)L^t\right\} \quad (2)$$

where ε_o is the maximum strain the wall tolerates without reinforcing its structure.

The model assumes that cell growth occurs at a much slower rate than that at which forces propagate through the cell wall matrix. This means that a kind of temporary equilibrium can be assumed at each time step, such that the walls have rearranged themselves so as to minimize the forces on them. We use a Runge-Kutta algorithm [14] to find the equilibrium vertex positions at each time-step.

2.2 Cell Polarity

We maintain a pair of orthogonal unit polarity vectors, $\hat{\mathbf{v}}_1$ and $\hat{\mathbf{v}}_2$, for each cell. These are initialised arbitrarily, and then updated each time step to reflect the rotation of the cell as it grows. Cell growth, division, and the separation of morphogens after division, are each organised about one of the polarity vectors (which one to use is determined by the genetic script).

Cell growth is polarised by defining the function λ in equation 1 as:

$$\lambda = R\left[1 - a\left(\hat{\mathbf{v}} \cdot \hat{\mathbf{w}}\right)^2\right] \quad (3)$$

Where R is the cell growth rate, $\hat{\mathbf{v}}$ is the polarity vector, $\hat{\mathbf{w}}$ is the unit direction of the wall (anti-clockwise with respect to the cell), and a is the degree of anisotropy defined on [0,1]. With $a=0$ we get isotropic growth, and with $a=1$ we get growth mostly in walls aligned closely with the polarity vector $\hat{\mathbf{v}}$.

Cell division is achieved by inserting a new wall positioned to pass through the centre of mass of the cell, and aligned in the direction of the chosen polarity vector. Figure 1B illustrates the process. Two new cells are created on either side of the dividing wall. The morphogen levels of the mother cell are split between the two daughter cells. Each cell receives ½(1+ s_i) (daughter cell 1 in figure 1B) or ½(1- s_i) (daughter cell 2) of the mother cell morphogen, where s_i is the asymmetry factor on [-1,1] defined by the genetic script.

Inheritance of morphogen levels allows the model to capture cell lineage, and asymmetric separation of morphogens makes it possible to consider branching of lineages.

2.3 Genetic Regulation

The genetic script is implemented via an embedded Tcl system. It may perform any sequence of Tcl instructions (e.g. logical conditions, resetting) on the cell state parameters. In addition two procedures are defined. The *divide* procedure instigates

cell division, instructing the spatial model to adjust itself accordingly. The script may also cause the cell to die via a *kill* procedure.

The parameters that define the state of each cell and which are available for transformation by the genetic script are: morphogen production rates, morphogen localisation asymmetry s_i, growth rate R, anisotropy a, growth axis, division axis, and turgor pressure P. Other values are available as *read only* variables: volume V, and morphogen concentrations u_i.

The system is general enough to allow implementation of genetic regulation on many levels, from differential equations to rule-based models.

2.4 Signal Transduction

We consider cell-cell signalling by passive diffusion transport of morphogens produced by the cells. Diffusion allows us to model long-range (high diffusion rate), short-range cell-to-cell (low diffusion rate), and cell-autonomous (zero diffusion rate) signal molecules within the same mathematical framework.

The signalling system is iterated at each cell j for each morphogen i using:

$$dU_i^j(t) = \delta t\left[\gamma_i \sum_{walls}\left(U_i^{neighbour} - U_i^j\right)L + pU_i^j\right] \tag{4}$$

where U_i^j is the morphogen *concentration*, γ_i (units [distance]$^{-1}$[time]$^{-1}$) is the rate of transport per unit length of wall per unit difference in concentration across the wall, L is the length of the wall. The sum is over the walls forming the boundary of cell j and *neighbour* is the cell adjacent to j across each wall. The term pU_i^j is the rate of morphogen production.

3 Results and Discussion

We performed simulated experiments to assess the performance of the model in four key aspects of plant morphogenesis: cell proliferation, coordinated growth, cell lineage, and cell position specification. Parameter settings $P=0.1$, $K=1$ were used throughout.

3.1 Proliferation

Simple cell colonies were generated from initial conditions of a single unit square cell. All cells were grown at the same rate ($R=0.1$) and divided when their volume doubled. Cells inherited polarity from initial vectors: up (axial) and right (lateral). All growth was indeterminate; analysis was limited to the first few hundred time-steps.

Figure 3A shows a colony generated by alternating the cells' division axes and maintaining growth axes perpendicular to it. After each division the growth and division axes were flipped from axial to lateral or vice versa. Cell growth was isotropic. The resulting colony shows regular cell size and shape and its boundary maintains the square shape of the initial cell. The cell walls show the characteristic zigzag wall pattern seen between adjacent cell files in plant roots and shoots (figure 2A).

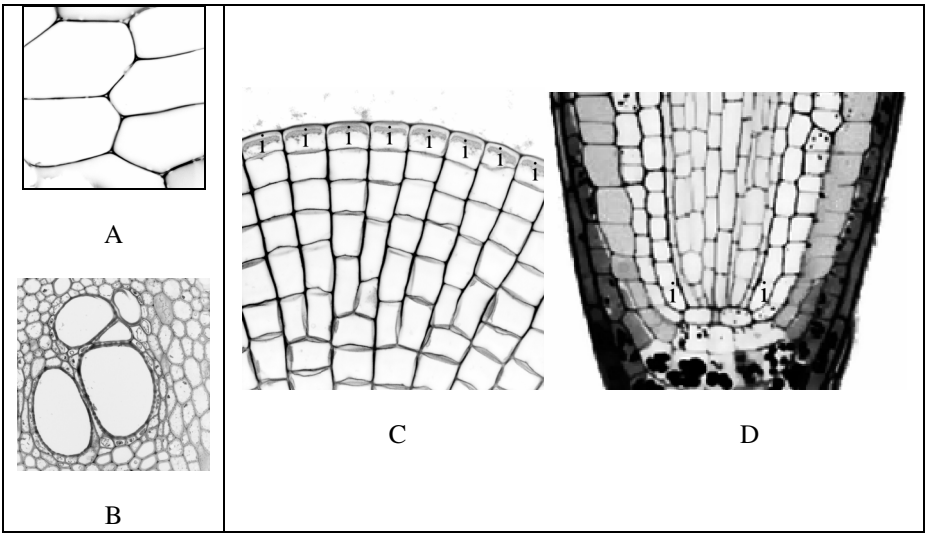

Fig. 2. Examples of cell arrangements in plant tissues. (A) Cell walls show a characteristic zigzag pattern caused by lateral tension forces at T-junctions. (B) Scale range of cell cross-section area, ranging to approximately 1:100. (C, D) Initial or stem cells (i) are maintained at fixed positions in the meristem. (C) Coleochaete scutata (a simple green alga), the stem cells are maintained at the thalus margin. (D) Arabidopsis thaliana root meristem [Reproduced courtesy of Sarah Hodge]), initials remain in fixed positions relative to the root tip after divisions producing other cell types.

Random cell colonies were generated by choosing division axes from a pseudo-random number, with equal probability of axial or lateral division. Again, the cells' growth axes were maintained perpendicular to their division axes. Cell growth was polar, with $a=0.9$.

Figures 3B and 3C show three colonies generated by different pseudo-random seeds. As expected, the cells have a broader range of shapes compared with colonies generated by alternating division. Given equal probability of lateral and axial division, we might expect little average change in colony shape over time. However, overall colony shapes varied widely between random seeds.

The elongated form of figure 3B was caused by constraints on growth imposed by cells on their neighbours. An early sequence of in-line divisions established the long thin shape of the cell colony, which was maintained by the combination of anisotropic growth and coordination of growth by the two-spring model. To illustrate this, consider a line of three cells. If the two end cells are growing along the line and the central cell opposite to the line, growth of the central cell will be retarded. This is because its lateral walls each consist of one growing spring and one non-growing spring. Growth along the line therefore takes place at a much greater rate than lateral growth. In figure 3C similar growth constraints caused an asymmetry in cell-number on the left and right flanks, which was amplified through cell proliferation, causing a dog-leg similar to gravitropism in plant roots.

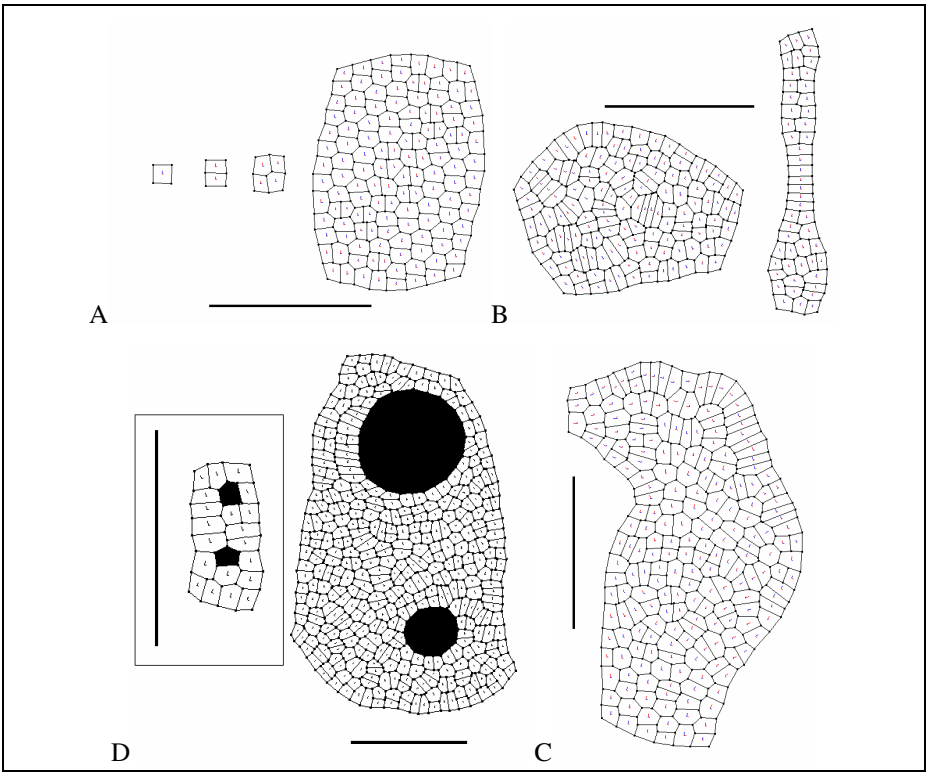

Fig. 3. Indeterminate growth organised by inherited polarity, scale bar shows 10 units. (A) Alternating division axis with growth axis perpendicular: steps 0, 27, 69, and 203 (130 cells). (B,C) Division axis chosen randomly with growth axis perpendicular: clockwise - step 312 (135 cells), step 314 (50 cells), step 390 (210 cells). (D) Growth as in B and C, with two cells (inset) chosen to stop dividing whilst continuing to grow.

Figure 3D shows the effect of manually specifying mitotic inactivity in a few cells. The selected cells (shaded) continued to grow at the same rate but did not divide. The colony showed scale differences in cell size not uncommon in many plants tissues, as illustrated in figure 2B.

3.2 Coordinated Growth

There are several examples of processes in plants (e.g. lateral root development) in which a zone of proliferating cells is established within a mature or slowly growing region. In order to examine this process we triggered proliferation in a single cell and its descendents by injecting a non-diffusing morphogen. The genetic script was configured to trigger growth at $R=0.1$ on presence of this morphogen. Growth was polar ($a=0.9$), and all cells divided on doubling their initial volume.

The effect of the maximum wall strain parameter was examined, keeping all cells turgor pressures equal. Figure 4A shows the results with $\varepsilon_o=0.1$ (approximately the

strain level produced by the cells' turgor). Growth continues indefinitely and surrounding cells are stretched to the point where they divide. With $\varepsilon_o=0.5$ (figure 4B), there is no proliferation and minimal cell growth. These results suggest that zones of varying growth rates could be coordinated via transmission of forces and passive cell wall biosynthesis, without requiring differential turgor.

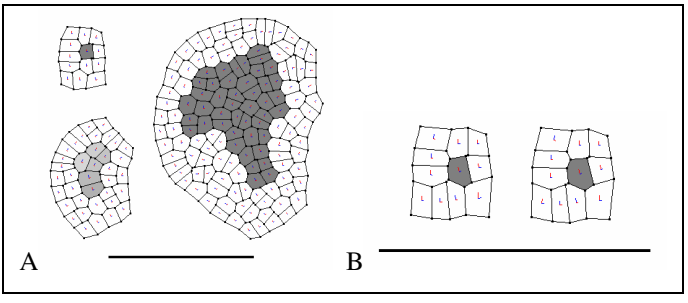

Fig. 4. Cell proliferation zone (shaded) surrounded by non-growing cells (grey tones not significant). Scale bars show 10 units. (A) Cell maximum strain $\varepsilon_o=0.1$; growth is indefinite with surrounding cells stretched: step 0 (13 cells), step 44 (37 cells), step 76 (135 cells). (B) $\varepsilon_o=0.5$, equilibrium reached in right-hand image, cell proliferation constrained.

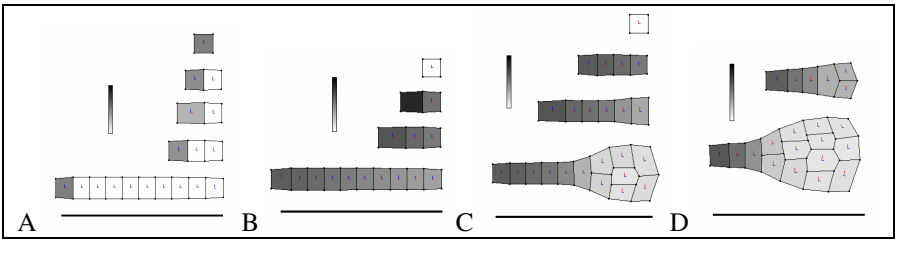

Fig. 5. Morphogens controlling development by inheritance and positional information. In all cases morphogen level shaded from white (zero) to black (one), and scale bar shows 10 units. (A) Inheritance of stem cell character; morphogen inducing 1-D growth and division inherited by only one daughter cell, other cells inactive (steps 0, 156, 242, 311, and 1915). (B) Morphogen gradient; morphogen produced by stem cell, diffuses to other cells where it is degraded (steps 0, 195, 400, 1315). (C,D) Gradient of lateral growth inhibitor; inhibition threshold at 0.5 (C) (steps 0, 461, 832, 1154) and 0.75 (D) (steps 605 and 817).

3.3 Cell Lineage and Positional Information

The relative roles of cell lineage or inheritance, and cell-cell signalling mechanisms and their interactions are important in understanding plant development. We demonstrate both mechanisms in our model independently and in combination.

A stem cell lineage was established using a non-diffusing morphogen with division asymmetry of $s_i=1$ (figure 5A). The morphogen was used to trigger growth and division in 1-dimension. This maintained an active cell at the end of a line of inactive cells, in a similar manner to a plant root- or shoot meristem (figure 2D).

In figure 5B, the stem cell was used to generate a morphogen gradient. The stem cell produced the morphogen shown so as to maintain constant concentration. The morphogen diffused into non-stem cells where it was degraded at a rate proportional to its concentration. The result was an approximately linear gradient along the length of the cell line.

The combination of lineage generated stem-cell and morphogen gradient was used to establish morphogenetic zones along the cell line (figures 5C and 5D). Non-stem cells were scripted to proliferate laterally ($R=0.05$) if the morphogen was below a threshold value. The threshold fixed the distance from the stem-cell up to which lateral growth was inhibited. A higher threshold made the inhibited zone smaller and vice versa. Similar zones of varying growth and division can be identified at characteristic positions across plant meristems (figure 2C,D), and the traffic of plant growth regulators is known to be involved in their delineation.

4 Conclusions

A method has been developed for simulating the particular features of cellular scale plant morphogenesis. The method builds on previous models of cell shape and multicellular organisation with the addition of polar cell behaviour. Although no specific model of genetic regulation has been put forward, a system which can integrate any such model with both a spatio-mechanical and a signalling model has been demonstrated.

The capacity of the system to reproduce key features of plant morphogenesis has been demonstrated using simple rule-based genetic logic. Initial experiments confirm that the model can produce plant-like cell proliferation, coordination of growth zones, and specification of cell behaviour by lineage and position. Thus the system provides a sound basis on which to investigate the complex interactions of all of these elements operating together.

The system has been implemented as interactive software, and as such provides a valuable tool for hypothesis testing in a controlled setting. It also provides a test bed for evaluating models of genetic regulation operating within, as well as controlling cellular development. Work is currently underway on integrating a gene network model into the system, and an evolutionary algorithm for generating networks that produce particular developmental systems.

The results presented here suggest that spatio-mechanical interactions place significant constraints on the shape formation potential of genetic control and patterning. Initial asymmetries in growth were amplified throughout development, and cell growth opposing early growth patterns was constrained. Further work remains to be done in quantifying and analysing these constraints in order to determine the organising potential of mechanical interactions alone, and in combination with genetic regulation in cell colonies.

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