

MODELING PLANT DEVELOPMENT WITH GENE REGULATION NETWORKS INCLUDING SIGNALING AND CELL DIVISION

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Abstract

The shoot apical meristem of *Arabidopsis thaliana* is an example of a developmental system which can be modeled at genetic and mechanical levels provided that suitable mathematical and computational tools are available to represent intercellular signaling, cell cycling, mechanical stresses, and a changing topology of neighborhood relationships between compartments.

In this paper we present a simplified dynamical 2-dimensional model of a growing plant. Cells in the shoot grow and proliferate, while the number of stem cells at the apex stays constant due to differentiation into tissue cells. Cell types are defined by protein concentrations within the cells, and the dynamics of the differentiation follows from a gene regulation network which includes intercellular signals.

Short Title: Modeling Plant Development

1. Introduction

Developmental systems in biology are complex multicellular systems which require multiple tools to be fully understood. In this paper we show how a mathematical model of biological components can be used to simulate qualitative behavior of a growing plant. The model consists of cells which grow and proliferate, mechanical interactions between cells, and an underlying genetic network describing the dynamics of the cell states. Important for the dynamics is the availability of signaling between neighboring cells.

The model is applied to the shoot apical meristem (SAM), from which the complete aboveground adult plant is derived. Cells in the SAM retain the ability to divide throughout the life of the shoot, while differentiation of these cells into mature cell types balance the size of the SAM, which stays close to constant throughout shoot life. Cell fates are dependent on the cell positions, and signaling between neighboring cells is believed to play a major role in the differentiation process.

Simulations result in a growing plant, where the SAM is pushed upwards while the stem of the plant is expanding. The stability of the model

and its underlying assumptions are discussed along with recent data suggesting a more complicated genetic network with feedback control for regulating the stem cell population within the SAM.

2. Methods and Algorithms

2.1 The Shoot Apical Meristem

Consider the shoot apical meristem (SAM) of *Arabidopsis thaliana* which is a model organism among plants (The Arabidopsis Genome Initiative, 2000). The SAM is the source of the complete aboveground part of the organism. It forms during embryogenesis and retains a nearly constant size and shape from germination, throughout the life of the shoot that it is producing. Among its products are secondary and higher order SAMs that produce branches. The SAM contains a dynamically stable spatial pattern of meristematic regions, despite cell division that causes individual cells or their daughters to move into different regions (Figure 1A; Meyerowitz, 1997). The central zone (cz) is at the very apex, the peripheral zone (pz) is on the sides, and the rib meristem (rib) is in the central part of the meristem.

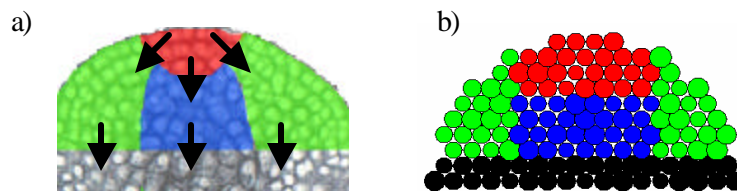


Figure 1. Meristem cell regions. a) Section of a meristem where meristematic cell regions are indicated by colors. Red – central zone, blue – rib meristem, and green – peripheral zone. The arrows show the displacement and differentiation path for cells. b) Initial configuration for a simulation run where cells are colored by marker genes (colors as in A).

The slowly dividing central zone cells are thought to be the ultimate stem cell population and provide cells for the maintenance of the meristem. The peripheral zone is where new leaf or flower primordia are initiated, while the rib meristem provides cells for the formation of the stem. Each of these zones has zone-specific gene expression, where for example the central zone is the domain of expression of the *CLAVATA3* gene (Fletcher, 1999).

It is known that cell positions are more important than cell lineages for the cell fates in the SAM (Scheres, 2001). Experiments where a single cell is marked show that its descendents can end up as parts of different tissues (Jegla and Sussex, 1990). In the root meristem, laser ablation studies have shown that if a cell is separated from the more mature cells, it remains arrested in a not fully differentiated state (van den Berg et al, 1995). These features are used as basic assumptions in the model presented in this chapter.

In recent experiments, mutant phenotypes have been used to find the roles of different genes in the development of the SAM, to define the mechanisms of communication between different groups of meristematic cells, and to find the patterns of gene regulatory interactions that define the expression domains of the genes. For example, the *CLAVATA3* expression domain has been shown to be partly regulated by the activities of the *CLAVATA1* and *WUSCHEL* genes, as well as by its own activity (Fletcher et al, 1999; Brand et al, 2000; Bowman and Esched, 2000).

2.2 The Underlying Model

In previous work we have introduced a mathematical framework for gene regulation networks combined with cell signaling (Marnellos and Mjolsness, 1998), and the "Cellerator" package for automatic model generation from reaction relationships (Shapiro et al 2001) and regulatory relationships along with cell division (Shapiro and Mjolsness 2001). These tools may be combined to produce models capable of simultaneously representing transcriptional regulation, intercellular signaling, cell division, and mechanical deformation as appropriate to a developmental model. For this study, the model framework is implemented in a C++ program, which is used for the SAM simulations.

Generalizing from (Marnellos and Mjolsness, 1998) and (Mjolsness et al. 1991), we use the combined gene regulation and cell-cell signaling dynamics:

$$\frac{d}{dt}v_a(t) = \frac{1}{t_a} [g(u_a + h_a) - I_a v_a], \quad (1a)$$

where

$$u_a(t) = \sum_b T_{ab} v_b(t) + \sum_{I \in Nbrs} \Lambda^I \sum_b \hat{T}_{ab} v_b^I(t) + \sum_{I \in Nbrs} \Lambda^I \sum_b \sum_c \tilde{T}_{ac}^{(1)} \tilde{T}_{cb}^{(2)} v_c(t) v_b^I(t). \quad (1b)$$

Here v denotes the protein concentrations within a cell. The matrix T represents an intracellular gene regulation network, \hat{T} is an intercellular network, and $\tilde{T}^{(1)}$ and $\tilde{T}^{(2)}$ represent a more detailed intercellular signaling network which separates the connection of receptors and ligands ($\tilde{T}^{(2)}$) from the connection of receptors and nuclear pathway target genes ($\tilde{T}^{(1)}$). The parameter h is used to tune the basal expression level, while Λ determines the degradation rate and t sets a time scale for the reaction. The function $g(x)$ is a

sigmoid function which is able to vary the final output, from an almost linear, to an on-off behavior of the gene expression.

A dynamical neighborhood relation is used to describe the intercellular signaling (ϕ in equation 1). In this case, a simple connection matrix, $\phi \in \{0,1\}$, is used to describe if cells are neighbors ($\phi = 1$), or not, ($\phi = 0$). A pair of cells is defined as neighbors if the distance between them is less than a threshold value, proportional to the radii sum, such that only nearest neighbor cells are connected. Since the cells are moving and dividing, the neighborhood connection matrix is updated at each time step of the simulation.

Cell shapes are approximated as spheres, and a simple model for cell growth and cell division is added, which can be chosen from a variety of published models (Shapiro and Mjolsness, 2001; Goldbeter, 1991; Gardner et al, 1998).

Mechanical interaction between cells is modeled by a softly truncated spring force between cell centers, with a relaxing distance typically set to the sum of the radii of the interacting cells. The cell movement, rather than the acceleration, is proportional to the force, to simulate a highly viscous media (Shapiro and Mjolsness, 2001). While the repelling force is modeled as a standard spring force, the adhesion force is truncated to a given width and strength, reflecting that there is no adhesion between cells that are far apart.

The connection matrix ϕ is also used for optimizing the calculation of the mechanical interaction, only applying the truncated spring force for neighboring cells.

2.3 The Simulated SAM Network

A model where the cell-cell signaling is the main driver of cell differentiation is defined. Cells that initially correspond to stem cells in the central zone have ability to change state into peripheral or rib meristem cells, when they are neighbors to these cells. Also peripheral zone and rib meristem cells can differentiate when they are neighbors to cells of the stem.

Four genes are introduced as markers of different cell types in the SAM. An intracellular winner-take-all network is introduced (Figure 2a) such that only one of the genes is highly expressed in each cell. This unique expression is achieved by a network in which each gene promotes its own expression, while it represses the expression of the other genes. A cell and its descendants will usually end up in a state where the gene with the highest initial concentration is expressed, while the other genes are not. The cells are initiated with different expressions in the different meristemic regions as shown in Figure 1b.

An intercellular network is also introduced as shown by the dashed lines in Figure 2b. The intercellular network introduces a repression of a selected gene in neighboring cells, together with promotion of its own expression. The result is that a cell can change state if it is neighbor to a

region of more “mature” cells. The intercellular signaling is driving the dynamical differentiation of cells, from central zone cells into peripheral zone and rib meristem cells, and from pz/rib cells into cells of the plant stem.

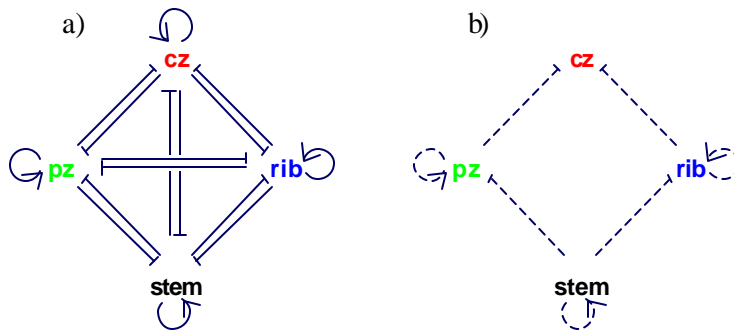


Figure 2. Gene interaction network. Arrows (?) represent upregulation which is implemented as a positive entry in the corresponding T matrix in equation 1, while barred lines (-) represent downregulation (negative T parameter). a) Solid lines represent the intracellular network. b) Dashed lines show the intercellular interactions between neighboring cells.

Cell growth and division is stopped as the cells become part of the stem. This is implemented by using the gene expression within a cell to control the growth parameters. No difference in growth or proliferation rate is implemented for different meristematic regions, although it is again straightforward to control these parameters using the protein concentrations within cells (Jönsson et al, 2002).

3. Results and Discussion

The dynamical behavior of the simplified SAM model is shown in Figure 3. The cells are colored by three of the protein concentrations, where red cells are central zone cells, green are the peripheral zone cells, and blue are rib meristem cells. The cells where the stem marking protein is high in concentration are colored as black cells. Figure 3a shows the simulation close to the initial configuration of Figure 1b. Some of the cells are already in the process of changing state, which is detected by the darker colored cells at the boundaries between different regions.

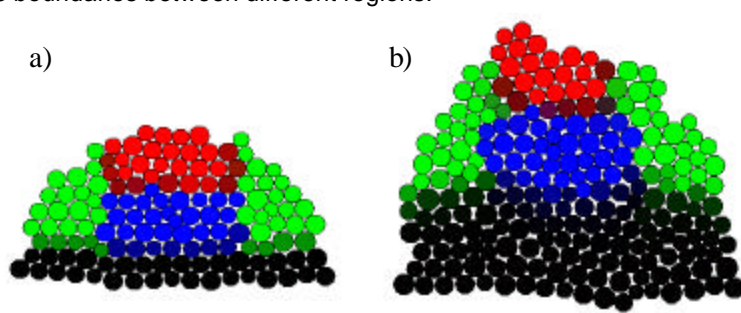


Figure 3. Result of cell division, intercellular signaling, and intracellular gene regulation network dynamics. Colors display the protein concentration related to the marker genes. (a) Start of simulation ($t=300$ in arbitrary units). (b) Result of long-term dynamics ($t=1700$).

In Figure 3b, a later time point is shown, where a number of cell divisions have occurred. Also a number of cells have differentiated, resulting in almost constant gene expression regions in the SAM although the individual cells have changed state. In the simulation the plant grows as the number of cells in the stem increases despite that these cells do not divide.

3.1 Stability

As discussed in section 2.1, the gene expression regions of the SAM are quite stable. The gene network is also well designed resulting in a self-organization of the domains. If for example the SAM is bisected, it can form two functioning SAMs with characteristic cell domains (Steeves and Sussex, 1989). The simple model simulated in this paper, where expression domains in the SAM stay constant during growth by an increase of cells due to cell divisions balanced with a decrease due to differentiation, does not reflect this stability. This is illuminated in longer simulations, and Figure 4 shows statistics of the sizes of the gene expression domains for a number of runs at late time points. In each run, initial cell sizes and individual cell cycle periods are varied slightly (cf Figure 1b). It can clearly be seen that the variation of the region sizes increases the longer the simulations run.

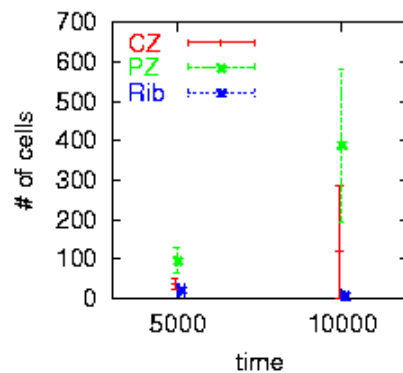


Figure 4. Statistics of the domain sizes for 10 simulations with slightly different initial configurations. The mean and standard deviation (bars) of the distribution is shown at two different time points, showing the increased variation the longer a simulation runs. The time unit is arbitrary (cf Figure 3b where $t=1700$). Red – central zone, blue – rib meristem, and green – peripheral zone.

The instability might be explained by a simple analysis of the model, where the dynamics of the volume, V , of an expression domain is described as in equation 3.

$$\frac{dV}{dt} = aV - bV^{2/3} \quad (3)$$

The first term represents the volume increase due to cell division, proportional to the volume, while the second term is the decrease due to cells changing state at the border towards another domain, proportional to $V^{2/3}$. The equation has two fixed points ($dV/dt=0$) at $V_1^*=0$, and $V_2^*=(b/a)^3$. The second fixed point is the interesting one, but it is unstable, which means that a small deviation from it will result in that the volume either decrease or increase away from the fixed point value. It is possible to tune parameters to stay close to the fixed point for quite a while, but in the long run it is inevitable that the cell domain either disappears, or grows to infinite size. The more troubling behavior of the model is that it will never self-organize into meristematic regions, but can only maintain regions that are initiated at the start of a simulation.

In section 2.1 we described recent experiments which suggest a feedback network between genes expressed in different domains of the SAM. This data suggests a regulation of the stem cell region which might better explain the stability of the stem cell region. In a static simulation, we have also shown that it is possible to create a stable, self-organizing stem cell domain in the SAM using a regulatory network based on this data (Jönsson et al, 2003). Although the new data provides clues for the stability discussion it does not answer all questions, and the stability of the SAM regions remains to a large extent an open problem. The simulations and analysis described in this paper can be seen as an argument for a more complicated set of feedback controls on gene regulation.

3.2 Outlook

We have shown how computer simulations based on a multicellular mathematical model of a developmental system can be used to help qualitative reasoning, in the case of a developmental system. We have addressed the question of cell differentiation of plant cells, and in our simulations, qualitative features of a growing plant are achieved. However, the lack of long-time stability and self-organization of the meristematic regions in the model indicates a more advanced system of interacting genes to create the stable expression regions in the SAM.

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