# Inferring Gene Regulatory Network Models for Plant Stem Cell Regulation

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## ABSTRACT

A model of the Shoot Apical Meristem (SAM) in the plant *Arabidopsis thaliana* is investigated. The model is based on cellular resolution of protein concentrations. There is a gene regulatory network within each cell which also depends on signaling between neighboring cells. Cells grow and proliferate and experience mechanical interactions with neighboring cells. In previous work, we have built a model based on existing experimental knowledge of the regulation of the stem cell region in SAM. The experimentally suggested gene network could not by itself be used to create the expression domains of involved key genes. By extending the model with hypothetical genes with expression domains identical to other known genes, we were able to mimic the expression domains using the model. Here we extend the modeling approach to do a more exhaustive search for networks that are able to simulate expression domains of key genes.

In a dicot plant, there is a continuous flow of cells from the apex of a shoot where the main cell proliferation occurs to the stem and other tissues which are created below the shoot [7, 10]. In this way the above ground part of a plant grows and the shoot apical meristem contains a pool of stem cells at the apex throughout life of the post-embryonic plant. The maintainance of a stable stem cell region within the SAM requires an intrinsic balance between cell proliferation and cell differentiation of cells leaving the SAM. While the origin of this balanced system still is unknown, parts of a genetic network regulating the stem cell region have recently been experimentally illuminated ([1, 2, 3], Figure 1). CLV3, which encodes a putative intercellular protein , is expressed in the stem cells at the apex. Together with the receptor encoding CLV1, a repression of WUS gene expression is created. WUS is expressed in a small region below the apex, and activates expression of both CLV1 and CLV3. This leads to a feedback system regulating the stem cell region in the SAM. The spatial expression domains for the discussed genes, which are illustrated in Figure 1A, indicates that these players are only a part of a larger network for the regulation of the stem cell region in plants. The SAM has also been divided into layers, where the L1 layer is the outermost layer of cells, followed by the one cell layer thick L2, and finally the inner corpus. In the L1 and L2 layers cells are dividing prependicular to the surface, maintaining the layered structure. There are genes known to have expression regions following this layering, e.g. ATML1 which is expressed only in the L1 layer [5].

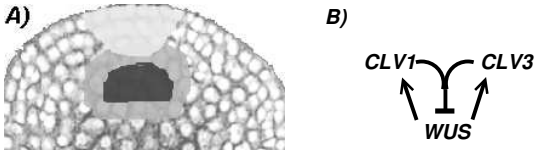


**Figure 1: Shoot Apical Meristem. The regulation of the stem cell region in the SAM is partly regulated by CLV1, CLV3 and WUS. A) Spatial expression domains. CLV3 - light gray, CLV1 - gray, and WUS - dark gray. B) Network indicated by experimental data. CLV3 (ligand) together with CLV1 (receptor) repress WUS, while WUS induces CLV1/3 expression.**

The complexity of a biological system such as the SAM, and the incomplete experimental data, make mathematical models a tool well-suited for illumination of the system. We have created a multicellular model framework to be used on developmental biological systems [4, 9]. Cells are modeled as spheres where the growth is radial. The model incorporates cell growth and proliferation, where the cell cycle can be modeled by a choice from published models. Mechanical interactions between cells are modeled using a truncated spring force, where adhesion between neighboring cells is allowed for. The variation of cell types is modeled with continuous concentrations of proteins, which can affect cellular parameters as e.g. cell growth rates. The protein dynamics is modeled by a neural-network inspired Genetic and Signaling Regulatory Network (GSRN) model [6, 8]

$$\tau_a \frac{dv_a^{(i)}}{dt} = g(u_a^{(i)} + h_a) - \lambda_a v_a^{(i)}, \tag{1}$$

where

$$u_a^{(i)} = \sum_b T_{ab} v_b^{(i)} + \sum_j \Lambda_{ij} \left( \sum_b \hat{T}_{ab} v_b^{(j)} + \sum_{bc} \tilde{T}_{ac}^{(1)} \tilde{T}_{cb}^{(2)} v_b^{(j)} v_c^{(i)} \right) \tag{2}$$

and $g(x)$ is a sigmoidal function. In the equations $v$ represents protein concentration, $a, b$ are indices for the proteins and $i, j$ are indices for cells. The model describes intracellular regulation of proteins (encoded by the $T$ matrix), as well as intercellular interactions between proteins of neigh-

boring cells. The intercellular part contains a direct interaction ($\hat{T}$), and also a ligand-receptor type of interaction ($\tilde{T}^{(1)}\tilde{T}^{(2)}$). The $\lambda$ term is a degradation term, $\tau$ is a time parameter, and $h$ regulates the basal expression. $\Lambda$ describes the connection between individual cells.

In previous work we have used the described model framework to simulate a gene regulatory network in the SAM where we were able to mimic the stem cell region shown by the resulting CLV3 expression domain [4]. The success of the model requires signaling between cell neighbors and also protein transportation, in this case modeled by diffusion. The model includes gene regulatory network connections not yet verified by experiments, which is essential to achieve the complex expression domains shown in Figure 1A. The main idea to create a stable stem cell region was to use the known promoting signal from WUS and complement it with an activating signal originating from the L1 layer. While the upregulation from WUS is experimentally verified, the regulation from an L1 originating signal is not. The resulting network is one suggestion of how to maintain a constant stem cell region in the SAM. A more thorough search for possible gene regulation networks is possible in this modeling framework, which can precede a more exhaustive experimental search.

We present here a network inference approach for gene regulatory networks within a multicellular growing biological system. Network parameters are optimized by comparing the simulated protein concentrations with time series of template concentrations based on experimental data. The approach is an extension of previous work in Drosophila [6], where now the possibility of general cell proliferation is added. Addition of growth results in a dynamical system with changing cell numbers, positions, and sizes. The comparison with template data is then non-trivial, and one possible solution is to read positional and size data from a template, while dynamically simulating the gene regulatory network within the cells. The simulated proteins are compared to template values to obtain a quality measure for a particular set of network parameters. Finally the quality measure can be optimized with respect to network parameters. The quality measure, should describe similarity with the template, and a typical measure is

$$E = \sum_{t_a}^{N_{time}} \sum_{i}^{N_{cell}} \sum_{k}^{N_{protein}} \sqrt{\left((v_{i,k}(t_a) - v_{i,k}^{(template)}(t_a))^2\right)}, \quad (3)$$

where the protein concentrations $v$ are compared for all proteins in all cells and summed over time points where template data exist. If cell identity, positional and size data are read from the template file, simulated cells correlates exactly with template cells and the measure is well defined. Such an quality measure can be used to optimize parameters of a gene regulatory network using e.g. a special version of simulated annealing as described in [6].

An example of a small network where a template is used to infer gene network parameters is shown in Figure 2. A network of two proteins (X,Y) is simulated, and a template is created (Figure 2A). Figure 2B shows how the quality measure is increased when the system is simulated with parameters around the optimal values used to create the template. Note the "corridor" of good parameter values, where an increased repression on Y from X ($\hat{T}_{YX}$) can be compensated with an increased self activation of Y ($T_{YY}$). Note

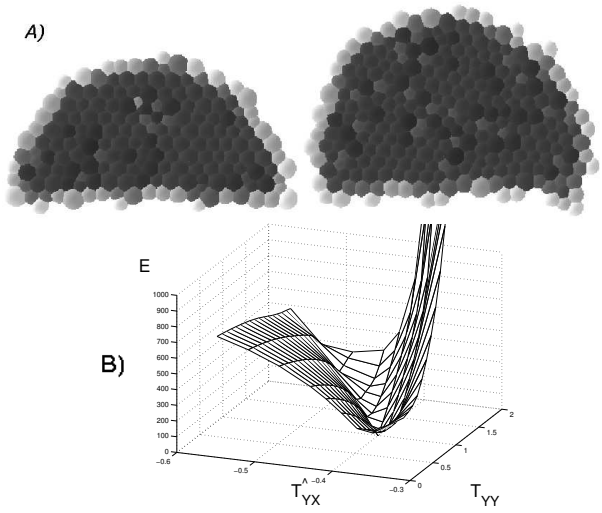


**Figure 2: Two dimensionsional template example with two proteins (X,Y). Protein X represses protein Y intercellular, while X is self inducing. A) Protein Y template concentration at two (out of 20) time points. Concentration is shaded from low (dark) to high (bright), and an L1 expression can be seen. B) Varying the interaction strengths in the gene regulation network model result in an increased value of the quality measure (eq. 3). A simulated annealing approach could be used to find the optima.**

also the lack of repressive signal from the stem, which was included in [4], leading to an expression also in the bottom layer of cells which corresponds to a simulation boundary rather than a surface layer.

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