A multicellullar model of a feedback network regulating spatial gene expression domains in the shoot apical meristem

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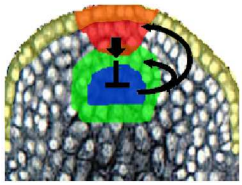
The **shoot apical meristem** (**SAM**) of plants is the biological target for a mathematical model for developmental biology. We present simulations of a stem cell regulating inter-cellular network with emphasis on the regulation of the "organizing center" and activation of the **WUS**-expressing domain. *In vivo* GFP measurments of important proteins are quantified and used as templates for the models.

The shoot apical meristem (SAM) of *Arabidopsis*

- Source of the aboveground part of a plant
- Small (about 10^3 cells)
- Genes important for the development identified

Some genes and known interactions

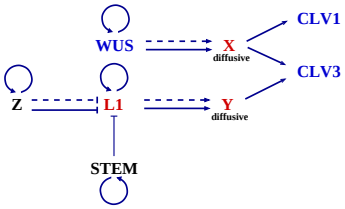
- CLAVATA3 (**CLV3**): stem cell marker
- CLAVATA1 (**CLV1**): receptor kinase
- WUSCHEL (**WUS**): homeodomain, transcription factor
- ATML1 (**ATML1**): homeodomain, transcription factor



The interactions between **CLV3**, **CLV1** and **WUS** partly regulate the development of the SAM, and thereby the complete plant. **WUS** induces both **CLV1** and **CLV3**. On the other hand **CLV3**(ligand) and **CLV1**(receptor) act in a network repressing **WUS** creating a feedback loop for the regulation. Genes exist which are expressed only in the L1 layer (such as **ATML1**). The signal inducing **WUS** expression is unknown and a hypothesis for that is introduced in this work.

A network for regulating CLV3

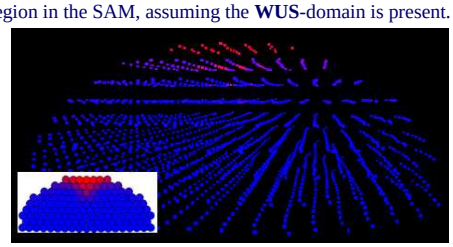
How can **WUS** regulate **CLV3** when the expression domains do not overlap?



A partly hypothesized network, where the inducing signal from WUS is combined with an L1-originating signal for CLV3 activation. **X** is suggested by experiments, but unknown. **L1** and **Y** have genes with analogous expression patterns (ATML1 and ACR4).

Simulation

In previous work we have demonstrated that the model network is able to produce expressions mimicking the stem cell region in the SAM, assuming the **WUS**-domain is present.



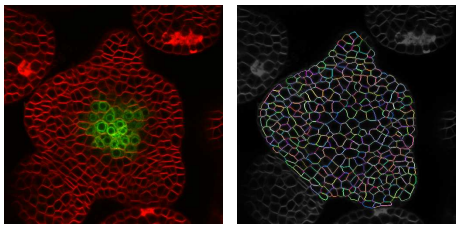
Simulation of a 3D nongrowing SAM of 1765 cells. The final (stable) expression level of the stem cell marking **CLV3** is shown.

Acknowledgements

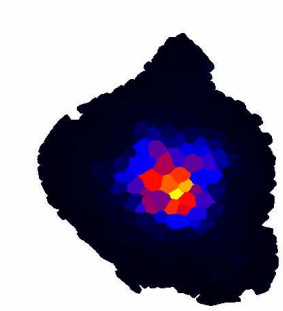
HJ was in part supported by Knut and Alice Wallenberg Foundation through Swegene. Experimental meristem work was supported by a U.S. NSF grant to EMM. Additional support provided by NSF:FIBR award number EF-0330786.

Quantifying *in vivo* GFP-data

We are developing software for automatic extraction of cell data from a stack of GFP images of living plants. This can be used to create templates of cell positions, cell sizes, and protein/mRNA concentrations at a (sub)cellular level.



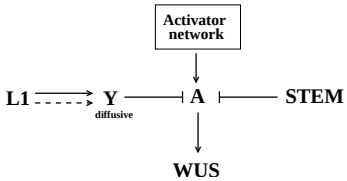
Left shows a horizontal section including a membrane stain (red) and GFP connected to the **WUS** promotor (green). Right shows the extracted walls using a watershed algorithm.



Quantified averaged **GFP** intensity within each extracted cell interpretable as **WUS** concentrations for the cells.

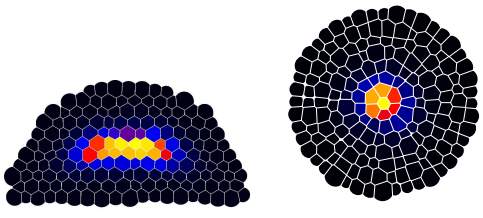
A network for regulating WUS

What is inducing **WUS**-expression?



In this hypothesis, the dynamics of a pattern forming reaction-diffusion model is used (e.g. Meinhardts activator-inhibitor model, or a gene regulatory network (GRN) model with diffusion) to introduce a concentration peak of an activator **A**. The activator induces **WUS** expression. The wavelength of the activator peaks is large enough to allow only one peak within the SAM. The central location of the single peak is assured by a repression of the activator from the L1 layer and in the stem. Data suggests that the L1 layer signal could be **CLV3** diffusing laterally from the apex.

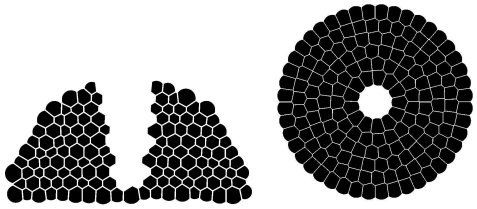
Simulations



2D simulations of vertical (left) and horizontal (right) layers of the SAM including the **WUS** region. **WUS** expression is emerging from a state of no concentrations.

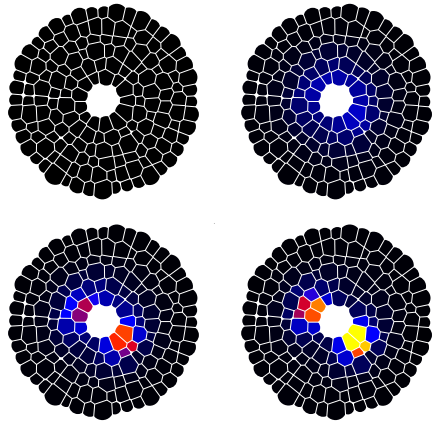
Laser ablation experiment

If the central zone, including the **WUS** expression domain, is removed from the SAM it is reorganizes and either one or two new functional SAMs can be developed (Reinhardt et al 2003). The **WUS** expression first comes on in a ring surrounding the ablated region, and then one region or two regions on opposite sides regain strong **WUS** expression. In the first case one SAM is continued, and in the second the SAM is divided into two new SAMs.



Schematic view of the laser ablation experiment where the cells of the central zone are removed. Left shows a vertical view and right shows a horizontal view.

Simulation



2D simulation of the **WUS** inducing network. The figures show the time evolution of the **WUS** concentration. The dynamics mimic the experimental dynamics of a **WUS** expression starting in a ring around the ablated region and then reorganizes into two confined regions on opposite sides of the ablated cells.

The model

Essential parts of a developmental system are included in a library of mathematical models, and continous differential equations are simulated for cellular variables and molecular concentrations. The model includes:

- Gene regulatory network (GRN)
- Molecular reactions
- Active molecular transport and diffusion
- Cell growth
- Cell cycle/proliferation
- Mechanical cell-cell interactions

http://www.computableplant.org

The aim of the computableplant project is to create a developmental biology simulation-software, and to follow protein dynamics using *in vivo* confocal microscopy. The project also encompasses development of image quantification software, automatized code generation from mathematical models, and model inference algorithms for parameter optimization and computer generated hypotheses.