

Proposal for Roeder Lab Rotation

Studying Sepal Development by Confocal Imaging and Computational Modeling

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Conceptual Framework

Sepals are leaf-like structures located at the outermost layer of a flower to protect the developing reproductive organs inside. In each flower of an *Arabidopsis* plant, there are four equal-sized sepals for best protection, making it an ideal model to study organ size control.

In their work, Hong et al. (2016) built computational models to show that spatiotemporal averaging of local variability in growth is necessary to generate robust sepal size. These models start each leaf as a half-round, plastic-like, anisotropic material, and exert turgor pressure on it to drive its deformation. As it reaches a pre-defined height, a countdown timer is triggered, which terminates the program after a pre-defined period of time. This timer was intended to imitate the action of reactive oxygen species (ROS), which is a maturation factor that initiates at the tip and spreads downward to the base. However, this maturation effect is not implemented in their models, which might have caused a deviation in the shape that the models produce from that of real sepals.

Despite this drawback, their models were great in generating predictions that match observations, and it is interesting to see if they could be generalized to model other leaf-like structures, such as a leaf. A quick realization is that the *Arabidopsis* leaf has a petiole, which is absent from the model. Previously, Kate and Antione added a growth factor into the model, which is produced at constant rate at the leaf base and diffuses into the leaf while being degraded on its way. The leaf surface responds to the growth factor according to a hyperbolic-tangent response curve. The model generates mushroom-shaped leaves which do not have petioles either, and it is interesting to see what the shape of the leaf will be if a different response curve is used.

The *Arabidopsis* *BLADE-ON-PETIOLE (BOP) 1* and *2* genes are leaf boundary genes expressed at the leaf base (Khan, Xu, & Hepworth, 2014). They restrict blade outgrowth from the petiole, presumably by activating the *KNOX* repressor *ASYMMETRIC LEAF2 (AS2)* (Jun, Ha, & Fletcher, 2010). They also increase the expression of the signaling peptides CLE5 and CLE6 (DiGennaro, Grienberger, Dao, Jun, & Fletcher, 2018), although the function of these peptides are largely uncharacterized. An interesting hypothesis is that CLE5, CLE6, or some other mobile signal is activated by BOP1, BOP2, or AS2 at the leaf base, which diffuses further into the leaf, inhibiting blade outgrowth and shaping the petiole. It is doable to first test the plausibility of this hypothesis using computational modeling before investigating the identity of this mobile signal.

There is evidence that microRNAs participate in the development of leaf and leaf-like organs (Nag, King, & Jack, 2009; Palatnik et al., 2003). MiR319a is expressed at the base of floral primordium, and at later stages, the proximal region of petal primordium. Surprisingly, loss-of-function mutation of miR319a results in loss of petals and underdeveloped stamens and sepals, indicating a broader region of miR319a action. To test how far a microRNA can travel from the place of its transcription, our lab previously designed a system, where a histone-GFP fusion protein is constitutively expressed, showing nuclear fluorescence, and where a microRNA targeting the GFP sequence is expressed driven by the miR319a promoter. The function of this microRNA is indicated by diminished nuclear fluorescence compared with that in a control plant in which only the histone-GFP fusion is present. By comparing the region of miR319a promoter activity (Nag et al., 2009) with the region where this microRNA functions, we know how far it has traveled.

Research Aims

1. To modify the leaf model in several ways, including implementing the arrest front, changing the response curve to the growth factor, and adding an inhibitory factor, and see whether the shape of the resulting leaf is significantly altered in any way.
2. To image nuclear fluorescence in the floral buds of the control plants (those with histone-GFP only) as a comparison to the plants in which the GFP is silenced with a microRNA.

Research Design

Aim 1: Modifications to the Leaf Model

We will implement the arrest front by increasing the Young's modulus of the tissue beyond the arrest front (probably by a factor of 10), which imitates the effect of ROS in stiffening the cell wall. It can be imagined that this may blunt the final shape of the leaf.

We will change the growth factor response curve from the hyperbolic tangent function to a Hill or step function, as they have a flatter plateau at lower growth factor concentration. This may also produce a more blunt-ended leaf. As a control, we will use a linear response curve, which may cause the leaf to be more sharp-ended.

We will add a growth inhibitor which is produced at the leaf base at a rate faster than that of the growth factor, and which is degraded faster than the growth factor is, so that near the base, the combined effect is growth-inhibiting, and that in the distal region, the combined effect is growth-promoting. We will try different modes in which the growth factor and the growth inhibitor interact, including additive and antagonistic. Hopefully these efforts will reproduce the petiole in the leaf model.

Aim 2: Imaging the Control Plants

We will image the floral buds of the control plants using the Roeder Lab confocal microscope. Because these plants do not have the microRNA that targets GFP, we expect these plants to show strong nuclear fluorescence under the microscope.

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