Chapter 4

Summary of Findings

The aim of this work was to better understand the mechanism that controls organ formation in the Arabidopsis shoot apical meristem. To accomplish this I investigated three aspects of meristem growth and development. First, I tracked cell divisions to understand the factors controlling new wall placement. Second, I measured the affect that cell size plays in controlling placement of new organs (phyllotaxis). Third, I investigated how the size of the meristem affects the phyllotaxis.

4.1 Cell Divisions

The morphology of the meristem is dependent on cell divisions and the placement of new walls. To understand how the new walls are positioned I tracked divisions in live plants using time-lapse confocal microscopy. I then converted the image data into 3D coordinates representing cell boundaries and used a mathematical model to understand the properties that best predict the placement of new walls.

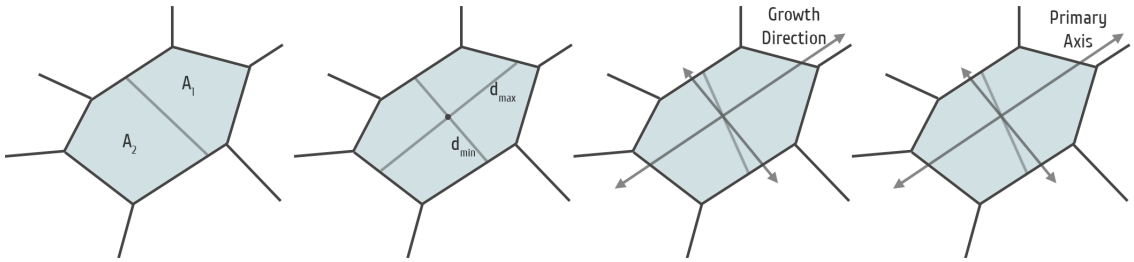
I looked at four geometric properties that could potentially affect wall placement and could also be measured in the collected data.

1. Daughter Cell Area (A). Measurement of how close the areas of the two daughter cells are from being equal.
2. New Wall Length (L). Measurement of how close the new wall is from the shortest possible wall that travels through the center of the mother cell.

3. Growth Direction (g). Measurement of how close the new wall is to being perpendicular to the direction of most growth.

4. Primary Axis Direction (e). Measurement of how close the new wall is to being perpendicular to the primary axis (longest direction) of the mother cell.

Figure 4.1: Heuristics of cell division.



(A) (L) (g) (e)

Each of those measurements was fed into a corresponding function that compares how close the observed location of the wall (Θ_1) is to the 'ideal' location (Θ_2). The ideal location was the location that minimized a particular geometric property, such as the position where both daughter cells would be exactly equal in area (V_A).

$$V_A = \left(\frac{A_1 - A_2}{A}\right)^2 \tag{4.1}$$

$$V_L = \frac{(d - d_{min})^2 + c_L\Delta^2}{(d + d_{min})^2} \tag{4.2}$$

$$V_e = (w \cdot e)^2 + \frac{\varepsilon_g \Delta}{d} \tag{4.3}$$

$$V_g = (w \cdot g)^2 + \frac{\varepsilon_g \Delta}{d} \tag{4.4}$$

Each of the four equations were part of a larger potential function (Equation 4.5). Each of the four components was given its own weight, w_i. An optimization algorithm found the optimal weight for each component that minimized the overall potential, essentially indicating which geometric properties are most important for determining the positions of new cell walls.

$$V(\Theta_1, \Theta_2) = \sum_{A,L,e,g} w_i V_i(\Theta_1, \Theta_2) \tag{4.5}$$

The optimal weight vector was determined to be $w = (V_A, V_L, V_e, V_g) = (0.68,\ 0.73,\ 0,\ 0)$. This indicates that the minimization of differences in daughter cell area (V_A) and minimization of new wall length (V_L) best explain the placement of the new wall. Growth direction (V_g) and the length

of the cell (V_e) are not needed for predicting the location of the new wall.

This result is interesting because our observations show more than 75% of new walls are within 30 degrees of being perpendicular to the primary axis (e), far more than would be expected by random chance, even though the model says the primary axis is irrelevant for predicting wall placement. One explanation is that this is a property that emerges in the process of minimizing differences in daughter cell area and new wall length.

This result is also interesting in a historical context as a number of scientists have commented on how different geometric features dictate new wall locations. In particular, Julius von Sachs concluded that new walls split the area of the daughter cells equally while Léo Errera determined that the walls find a minimal length. Both of those assertions were confirmed in this study. Wilhelm Hofmeister determined that new walls form perpendicular to the direction of the primary axis which was determined here to be not true. Although, as just mentioned this phenomenon does seems to emerge frequently as a result of "Sach's Rule" and "Errera's Rule."

4.2 Cell Size

The shoot apical meristem is a complex and dynamic system. To better understand which of the many components plays a major role in determining the phyllotaxis pattern I built a large computer model based on other meristem models that tried to incorporate all of the components known to be involved in the patterning process. By running this model many times and changing the values of the parameters I determined that theoretically the two most important factors controlling phyllotaxis were cell size and meristem diameter.

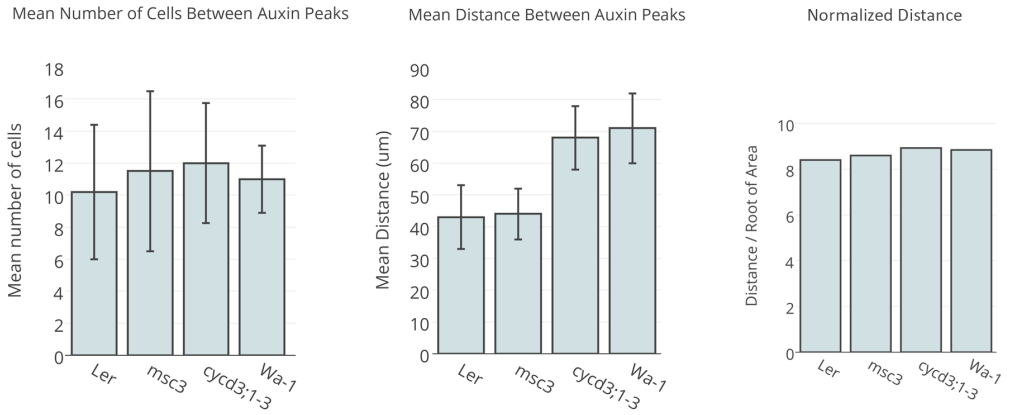
In our current understanding of phyllotaxis (the placement of organs around the meristem), the hormone auxin is pumped out of the cell towards the neighboring cell with the highest concentration of auxin. This creates a positive feedback loop where auxin accumulates in some areas and is depleted from the surrounding areas. New organs form in areas of high auxin. In the model diffusion of auxin across the cell is instantaneous and as a result the limiting factor is the transport rate across the membrane. So the localization of auxin is mostly dependent on the number of walls around the meristem. But this model property does not necessarily represent reality. It is possible that auxin takes considerable time to diffuse through the cytoplasm. If intracellular diffusion is the limiting factor then the number of membranes around the meristem should be irrelevant. In this case the

absolute distance between organs should be consistent regardless of how large or small the cells in a particular mutant are.

To test this I measured cell sizes and the distances between organs in mutants that had cells of varying sizes. The mutants *Wa-1* and *cycD3;1-3* had larger cells than wild type while *msc3* had smaller cells. I used confocal microscopy followed by 3D image processing algorithms to extract the dimensions of the cells and morphology of the meristems. These data were then used to make the measurements of cell size and organ positions.

I measured the diameters of cells and the distances and number of cells between subsequent organs. The mean number of cells between organs within a given genotype was between 10 and 12 but the individual measurements had a much higher variability. The absolute distance between organs in large-cell mutants was about twice that of wild-type-sized plants. When I divided the distances between organs by the root of the cell areas (which should be approximately the width of the cell) the distances between organs was very consistent between genotypes. These results indicate that the assumption of the model was correct. The limiting factor in auxin movement is transport across the membrane rather than intracellular diffusion. Further, cell size does impact the placement of organs. Although, the effect is not very noticeable on the macroscopic scale as the meristems in large cell mutants are also enlarged which compensate by having approximately the same *number* of cells as wild type.

Figure 4.2: Effect of cell size on auxin patterning in the shoot apical meristem.



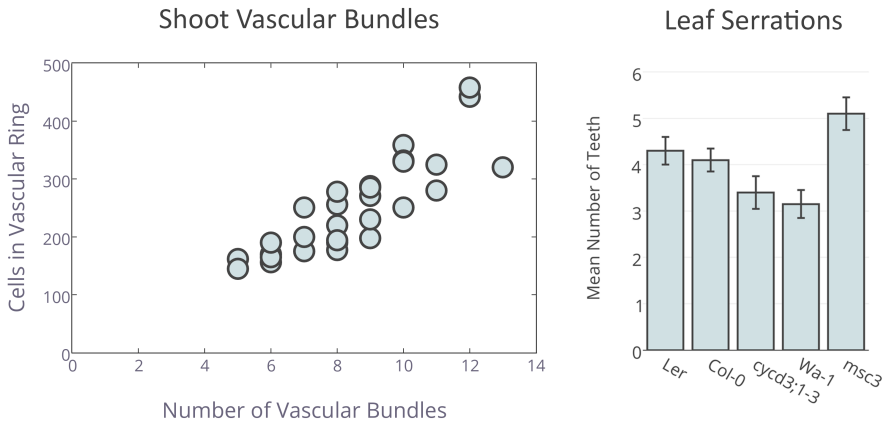
In addition to the meristem, I also explored the effect of cell size on patterning in the roots, leaf margins and shoot vasculature. In the roots, cell size mutants displayed disorganized auxin patterns but overall normal morphology. In areas where auxin would normally accumulate prior to lateral

root formation, my fluorescent auxin reporter was not visible but the lateral roots still formed. This is perplexing and indicates that my model of auxin transport in the meristem is insufficient for explaining root development.

In leaves, auxin is transported around the periphery during early development using many of the same components as in the shoot meristem. In areas of high auxin, serrations form through accelerated growth. If cell size plays a role in auxin distribution here then I would expect cell size mutants to have a corresponding change in number of serrations. *msc3* mutants have leaf cells of approximately half the width of wild type cells. Therefore that mutant should have twice as many serrations as wild type. Likewise, the large cell mutants have cells about twice as wide as wild type and should have about half as many serrations.

The data show that *msc3* mutants have about 25% more serrations and large cell mutants have about 33% fewer. So while the direction of change is as predicted, the *magnitude* of change is not what I would expect. My conclusion on leaf serrations is that the model of cell size controlling auxin distribution is not applicable in this tissue.

Figure 4.3: Effect of cell size on auxin patterning in the shoot vasculature (left) and leaf boundary (right).



In the shoot vasulature, auxin is thought to be transported around the vascular ring causing vascular bundles to form in areas of high auxin. I counted vascular bundles and number of cells around the ring in the same cell size mutants as before. The data show a linear relationship between number of cells in the ring and number of bundles. So it is possible that the transport-limited auxin model is applicable in this tissue.

Overall, the model is able to closely predict the patterning in the shoot meristem and shoot vascu-

lature but fails in the leaves and roots.

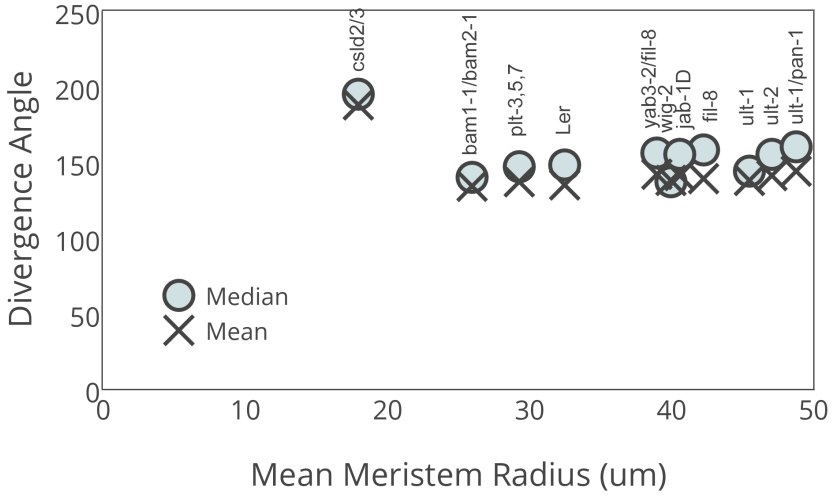
4.3 Meristem Size

The other major hypothesis generated by the computer model is that meristem diameter should affect the phyllotaxis pattern by altering the divergence angles between successive organs. In the model this phenomenon was closely tied to the cell-size phenomenon as increasing the size of the meristem increased the number of cells present. Shrinking the cells had many of the same effects as enlarging the meristem.

To test if this happens in reality I measured the sizes of meristems and the divergence angles in a number of mutants with different sized meristems. This required me to develop some new techniques for image processing and analysis.

For determining the size of the meristem I developed a image processing software pipeline that took 3D confocal stacks of meristems, extracted the topology of the meristems and identified important landmark features. To quantify the radius I measured from the apex of the meristem to the nearest crease in between the meristem and the early organ primordium. To quantify divergence angles I initially developed a tomographic imaging technique but soon discovered that the time required per plant was exceeded the time required to measure the angles manually with a divergometer.

Figure 4.4: Mean and median divergence angles as a function of mean meristem radius.

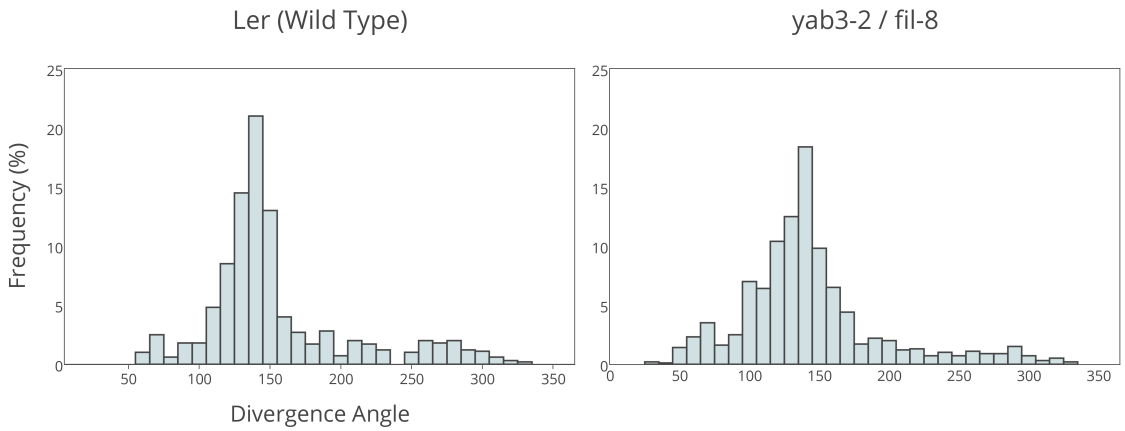


Using these two techniques I measured the meristem sizes and divergence angles of 12 different genotypes. The mean values for these measurements are not particularly interesting. While there

is a wide variety of meristem radii, the mean divergence angle is approximately equal in all but one genotype. In that genotype the meristem has about half the radius of wild type and shows a much larger divergence angle. This is apparent at the macroscopic level as the plants have an obvious alternating pattern where each successive flower is about 180 degrees from the previous flower. Most genotypes appear normal to the naked eye.

The distributions of divergence angles of individual genotypes shows more variability than simply looking at means. In many of the genotypes, like the *yab3-2 / fil-8* for example, the mean remained around the "canonical" 137 degree mark but showed a greater number of angles further away from the mean wild type. These non-canonical divergence angles show that the phyllotaxis is being affected by the changes to the meristem even though the mean remains the same.

Figure 4.5: Comparison of distributions of divergence angles of wild type and a mutant.



So the hypothesis is partially true in that altering the size of the meristem changes the divergence angles. But the pattern is more robust in plants than in the model. Most changes to the meristem result in very minor changes to the phyllotaxis. Although, it is possible to get more noticeable phenotypes as seen in the model by shrinking the meristem considerably. Presumably there is also an upper threshold where the phyllotaxis will significantly change when the meristem gets large enough.

4.4 New Model

The conclusion I have drawn from this research is that our model, while accurately representing reality in some conditions is not sufficiently complete to explain all of the observed phenotypes. As

we gain understanding of more components the model will need to be revised to incorporate these new insights.

Along these lines, one of our colleagues has shown that the size of the central zone is critical for modelling phyllotaxis and that the central zone changes in plants with altered meristem size. The central zone is a region of cells in the center of the meristem where auxin accumulation and differentiation is inactive. Changing the size of this region would change the number of cells auxin needs to be transported through in order to achieve the same divergence angles. So a model that captures this phenomenon would probably be a better representation of reality and help us better understand phyllotaxis in the future.