On the Mechanism of Wing Size Determination in Fly Development

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1 Statement of the Problem

Have you ever wondered why our cells stop growing or how they "know" we have reached the correct size? Many researchers have searched for the mechanism that controls the growth of cells. Hufnagel, Teleman, and their team attempted to determine and understand how a "growing tissue knows when it has achieved its correct final size" [1] with regards to the wing of Drosophila (a type of fruit fly). In other words, what causes the tissue to cease growing and the cells to stop diving? They also explored how the tissue stops growing evenly, even though the morphogen gradient is not uniform throughout the cell. In our paper, we will explore others' work on the subject along with what Hufnagel, Teleman, and their group have researched in order to understand this process. If these mechanisms were understood, we may better understand and possibly control cells that grow and divide too much, or cease too early.

2 Background

This mechanism has been studied by many other scientists as well. For example, Stephen Day and Peter Lawrence [2] searched for this mechanism in the Drosophila. Their conclusion was that the steepness of the morphogen gradient determines whether or not a cell will grow and divide. Morphogen is any type of signaling molecule that acts on cells to cause a specific response, working in a concentration-dependent manner. This means that whether the cell responds to the morphogen is based on the amount of morphogen being supplied to the cell. The specific morphogen in the Drosophila wing causing cell growth is known as decapentaplegic, or Dpp. There is a strip of cells located in the center of the wing from which the Dpp diffuses out of and moves toward the edge of the tissue, creating a gradient, (see Figure 1 A,B). Day and Lawrence believed that as the wing grows this "strip" of Dpp is stretched, reducing the gradient and causing the Dpp to fall below the threshold needed for continued division and growth. This conclusion was found to be false by Hufnagel and his team. His group found that found that the length of the Dpp strip is independent of the disk size [Figure 1 D].

Other models have been proposed that do not depend on the morphogen gradient, but are instead based on the idea of cell growth dependent on positional values. A cell's positional value is it's spatial coordinates, or where it is located in relation to its neighbors. As cells divide, the neighbors change and cells are moved around, lowering the positional value. When the positional value of a cell is too low, below a specific threshold, cell growth stops. However, experiments have been conducted, such as the one in Germany [4], which showed that cells moved from the location governed by it's positional value would still grow based on the concentration of Dpp.

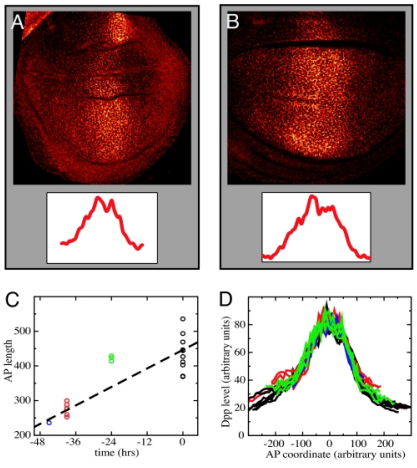

Figure 1. This figure, from the paper by Hufnagel and Teleman [1], shows the "strip" of cells containing Dpp, running vertically down the imaginal disc (A and B). The dpp is represented by the fluorescence in the wing. The brighter the color, the higher the concentration of Dpp. The concentration lessens as the Dpp moves toward the edges of

the wing, creating the gradient. C shows the growth of the anterior-posterior length in the imaginal disc. The different colored circle represent different stages of the fly during development. D is a graph of the level of Dpp plotted against the length of the imaginal disc. The colors correspond to various ages of the Drosophila. From the overlaid graphs, Hufnagel's team concluded that the length size of the imaginal disk is independent of the level of Dpp produced.

3 Proposal

The work performed by Hufnagel, Teleman, and their team further confirmed that cell growth is governed by the morphogen gradient. As long as the gradient stayed above a certain level, cell growth would continue. Hufnagel and his team hypothesized that tissues stopped growing uniformly, not due to morphogen or positional value thresholds, but instead due to negative feedback from mechanical stress on the cells. Negative feedback is when the outcome of a process influences the process to change. In this instance, the morphogen that causes cells to grow and divide also causes the cells to become more 'squished', placing stress on the cells and eventually causing the cells to stop growing. We will continue by looking at the english and mathematical representations of the models.

4 The Model

The model for this project makes several key assumptions about the biology and cell structure of cells in the imaginal disc. Firstly, it is assumed that Dpp is the only morphogen required to promote cell growth. The other morphogen, Wingless (Wg), is not assumed to impact cell development. The cells in this model begin in mechanical equilibrium, where the total energy of the system is minimal. This value depends on the location of the cell, the perimeter of the cell, and the stress from its neighboring cells. The cell geometry is structured as polygons, as shown in Figure 2. This is a two dimensional model. Each cell has its own position, shape and height dependent on its mechanical equilibrium.

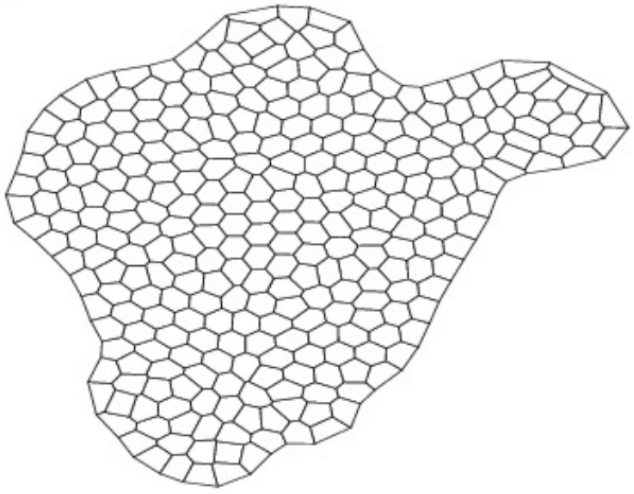

Figure 2, (Hufnagel and Teleman [1]) This figure shows the structure of the cells in this model. Each polygon represents a cell.

When a cell divides, it is assumed that the division is a random Poisson process with a fixed rate. This rate will depend only on the morphogen concentration and the mechanical stress in the cell. It is also assumed that upon cell division, the daughter cells grow to their full size instantaneously, and are in mechanical equilibrium. This process continues until the induced mechanical stress forces the cells to stop growing, completing the wing.

5 Mathematical Model

As discussed in the previous section, the first assumption in the tissue growth arrest model concerns the morphogen distribution and gradient. Only the Dpp morphogen was considered in this model with the assumption that is it secreted by a single cell, at a constant rate, in the middle of the tissue. The time necessary to establish the spatial morphogen profile is short compared to the rates needed for cell growth and cell division. The spatial dynamics are governed by diffusion and a constant degradation rate. Thus, the result is a fixed morphogen distribution profile that decays exponentially with distance to the source. The static axial morphogen gradient takes the form:

$$M(r) = me^{-r/l},$$

Where r is the position, m is the morphogen amplitude, and l is the characteristic length scale. (Recall from section *2 Background*, the characteristic length scale is determined by the length of the strip of cells from which the Dpp diffuses from and moves towards the edge of the tissue, creating the gradient.)

With respect to mechanical equilibrium, it is assumed that the cell layer reaches this state, with the cells adhering to each other, on a time scale faster than that of cell growth and division. The surface tension term (depending on the perimeter of the cell), a bulk contribution term (defining the preferred volume of the cell), a term coupling neighboring cells, and a term that regulates the stiffness of the cell's thickness all comprise the mechanical properties of a cell in the tissue layer. The equilibrium positions of a tissue with N_C cells and N_V vertices are found by the following Hamiltonian:

$$H(r_1,\ldots, r_{NV}, \xi_1,\ldots, \xi_{NC}) = \sum_{\alpha=\text{cell}} [\, \rho_\alpha + a(V_\alpha - V_0)^2 + b \sum_{\beta=\nu(\alpha)} (\xi_\alpha - \xi_\beta)^2 + c(\xi_\alpha - 1)^2]$$

where ρ_α is the cell perimeter, V_α is the cell volume, and ξ_α is the cell height. With $V_\alpha = A_\alpha \xi_\alpha$, the cell area A_α is determined by the positions of its vertices, r_i. $\nu(\alpha)$ denotes the collection of all cells adjacent to cell α. Deviations of V_α from V_0 are accounted for by a, b imposes a penalty on the variation of ξ_α between adjacent cells, and c controls deviations of ξ_α from an unstressed value (without loss of generality, Hufnagel and his team take $\xi_\alpha = 1$).

During mechanical equilibrium, the energy of the system is minimal. The above Hamiltonian (which minimizes the energy of the system) suggests that the volume V_α of the cell should be close to its intrinsic volume $V_{0,a}(t)$, while also minimizing the perimeter and differences in thickness to its neighbors. The intrinsic volume of the cell $V_{0,a}(t)$ is defined by the time dependent cell mass. [1]

The cell geometry is altered by cell division (and resultant growth of daughter cells) and by growth related topical rearrangement of the cells. As described in section *3 The Model*, cells divide at random as a Poisson process, but at a fixed rate, once the cell's volume exceeds a certain threshold value. (Again, without loss of generality, this value is set to 1.)

Newly born cells grow with a rate *k* directly following cell division as:

$$d/dt(V_{0,a}(t)) = k_a \text{ and } V_{0,a}(0) = \underline{V}_{0,a}$$

where $\underline{V}_{0,a}$ is the volume of the cell a directly after division. k_a is the growth rate of cellular volume regulated by both morphogen concentration and mechanical stress. At distances large compared to cell size, the mechanical equilibrium interaction (influenced by cell geometry and proliferation) between cells is reduced to that of continuum elasticity.

The experiment shows that the dependence on position and time arises from its dependence on the morphogen level and on stress. This parameterization can be expressed as:

$$\partial\, \gamma(r,t)=\Gamma(M(r,t),p(r,t))$$

In this equation, M represents the morphogen concentration and p represents the local uniaxial compression stress within the cell layer.

Elastic strain energy is the energy stored in an elastic material upon deformation, which depends on displacement of all direction and transverse strains. The elastic energy caused by displacement can be represented as $E=\frac{K}{2}x^2$, where E is energy, K is the spring constant coefficient, and x is the displacement. By determining the minimal elastic strain energy, we can find the relation between pressure and layer height. The equation of minimal elastic energy is stated as:

$$H=\int d^2r[\tfrac{\mu}{2}(\Delta u_{ab}-\tfrac{\delta_{ab}}{2}\Delta u_{cc})^2+\tfrac{K}{2}(\Delta u_{cc}-\Delta t\gamma)^2+\tfrac{w^2}{2}(\partial_a\Delta\xi)^2+\tfrac{\beta}{2}\Delta\xi(\Delta u_{cc}-\Delta t\gamma)+\tfrac{K_\xi}{2}\Delta\xi^2]$$

And through calculation, this leads to following equation:

$$-w^2\nabla^2\xi(r,t)+\xi(r,y)=ap(r,t)$$

In this equation, ξ represents the layer height and p is the stress. Two major conclusions are drawn from this equation. First, the layer height $\xi(r,t)$ is proportional to the local stress. Therefore the layer height (ξ) is used in the growth model, rather that stress (p) which yields the following model:

$$\gamma=\Gamma(\xi,M)=\Gamma M\,(M)[1-q(\xi-\xi_0)^2\,]$$

Second, the layer height (ξ) cannot vary too rapidly with position and has a characteristic length *w,* below which its variation is suppressed.

6 Author's Results

From the experiment, the authors found that morphogen distribution is independent of disk size but dependent on the parameters involving morphogen production and spreading.

From the mathematical model, it was discovered that when disk boundary reaches the stress threshold, the arrest of cell proliferation throughout the disk is induced by mechanical stress in the tissue.

7 Future Analysis

Future analysis, first and foremost, includes simulating Hufnagel's model using a finite elements method coded in C and implemented in MATLAB. Additionally, extending the model to generalize a more realistic dual morphogen gradient would be advantageous. If these goals proffer too great of ambition, then determining the threshold responsible for arresting cell proliferation may be determined. Also, rudimentary models concerning finite element simulations of epithelial tissues may be worth investigation.

References

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