SI Text

Computational Model of Growth Mechanics

The initial analysis of the mechanical feedback on tissue growth (1) was based on a continuum description that neglects important aspects of cellular tissues: the discreteness of cell divisions and the finite size of cells. To study the effect of these and to establish their influence on the conclusions obtained via continuum elasticity considerations, we developed and simulated a discrete model of growing tissue. Superficially similar finite element simulations of epithelial tissues have been implemented before (2-6), but these studies did not address the mechanics of growth issues which concern us here.

The discrete model represents the geometry of a patch of a two-dimensional cell layer by a set of polygons sharing edges and vertices. Each polygon represents a cell and is assigned a number representing cell "height" (see SI Fig. 7). Our model involves four main elements.

Mechanical Equilibrium

We assume that on the time scale fast compared with cell growth and proliferation the cell layer reaches mechanical equilibrium with cells adhering to each other. The mechanical properties of a cell in the layer are determined by three terms: (*i*) a surface tension term depending only on the perimeter of the cell (presumed to arise from the cytoskeleton (7, 8), (*ii*) a bulk contribution which defines the preferred volume of the cell, (*iii*) a term that couples neighboring cells and (*iv*) a term that controls the stiffness with respect to the deformation of cell thickness. Specifically, the equilibrium positions of a tissue with N_C cells and N_V vertices are found by minimizing the energy (or the Hamiltonian) given in Eq.**2**:

$$H(r_1,...,r_{N_V},\xi_1,...,\xi_{N_C}) = \sum_{\alpha\in \text{cells}} \left[\rho_\alpha + a(V_\alpha - V_{0,\alpha}(t))^2 + b \sum_{\beta\in \nu(\alpha)} (\xi_\alpha - \xi_\beta)^2 + c(\xi_\alpha - 1)^2 \right], \qquad \textbf{[S1-1]}$$

where ρ_α, V_α, and ξ_α denote the perimeter, volume and the height of cell α. Cell volume is $V_\alpha = A_\alpha \xi_\alpha$ where A_α is the cell area which like the perimeter of a cell are determined by the positions, r_i, of its vertices. $\nu(\alpha)$ in the summation denotes the collection of all cells adjacent to cell α.

According to the above Hamiltonian, the volume V_α of a cell should be close to the intrinsic volume $V_{0,\alpha}(t)$, while minimizing the perimeter and the differences in thickness to its neighbors. The "intrinsic" volume of the cell $V_{0,\alpha}(t)$ is defined by the, in general time-dependent cell mass. The competition between the perimeter, volume and the coupling to the thickness of adjacent cells are controlled by three parameters *a,b,c*. *a*controls deviations of cell volume from $V_{0,\alpha}(t)$ while *b* imposes a penalty on the variation of height ξ_α between neighboring cells. *c* controls deviations of $\xi_\alpha$from the unstressed state which can, without loss of generality be taken to be $\xi_\alpha = 1$. At distances large compared to cell size this interaction between cells reduces to continuum elasticity (8) that provides the correct phenomenological description independent of details on small scales.

Cell Dynamics

Two processes change the geometry of cells in a tissue: (*i*) cell division and the subsequent growth of its daughter cells; and (*ii*) topological rearrangement of cells triggered by growth. We assume that cells divide at random but with the probability that depend on their mechanical state (in the simplest case we assume constant division rate, provided cell size exceeds certain threshold). Division is implemented by introducing a cleavage plane between two opposing cell walls, such that both daughter cells get an equal share of the volume of the mother cell. After the division process, newly born cells grow with a rate κ according to:

$$\frac{d}{dt}V_{0,\alpha}(t) = \kappa_\alpha \text{ and } V_{0,\alpha}(0) = \overline{V}_{0,\alpha}\,, \qquad \textbf{[S1-2]}$$

where $\overline{V}_{0,\alpha}$ is the volume of the cell α right after the division. Here, we consider two alternative models.

Case A: Morphogen concentration and mechanical stress regulate the rate of growth of cellular volume, i.e. κ_α. Division occurs as a Poisson process with fixed rate, once cell volume exceeds certain threshold value,which without loss of generality we can set to one.

Case B: Growth of cell volume occurs relatively fast and can be treated as quasi instantaneous. In this case Eq. **S1-2** is omitted and the intrinsic volume $V_{0,\alpha}(t)$ can be immediately set to one so that daughter cells approach the fixed "adult" cell volume in a single relaxation step after division. All of the dependence on external variables, such as morphogen and mechanical stress, is then lumped into the probability of cell division (per unit time) γ is assumed to depend on morphogen concentration and on mechanical stress.

Addition of cells via cell division and the interaction between neighboring cells necessitate rearrangement of cell contacts. In our model, as in real cell sheets, neighboring cells may move away from each other and cease being neighbors through a T1 topological rearrangement process, illustrated in SI Fig. 7.

Mechanical Feedback

We investigated both implementations of cell growth and division process in out numerical simulations. Cases A and B both behave the in the same way as far as the size determination process is concerned. Case A is more realistic and gives a nontrivial cell size distribution that is interesting to study in its own right. Case B is somewhat simpler to compute with and to present. Hence, here we will concentrate on case B, because of its simplicity. Thus, after a division the cell volume of the daughter cells are set to 1 and the lattice is equilibrated. The division rate of a cell is modeled by a Poisson random process characterized by a rate, which depends on the mechanical stress (or equivalently, on the deformation) and the local morphogen concentration sensed by the cell as shown in Fig. 3. Specifically, the rate of division is parameterized by $\gamma = \Gamma(\xi, M) = \Gamma_M(M)[1 - q(\xi - \xi_0)^2]$ with $\Gamma_M = (M - M_0)\theta(M\text{-}M_0)$. Note that the overall scale of γ is immaterial because it merely sets the units of time in the simulation. (The dependence of the results on the "stiffness" of the feedback, q, is given in Fig. 4*M*.)

Morphogen Distribution

In the wing disc, cell proliferation requires joint presence of Wg and Dpp. However, for our purpose of illustrating possible interplay between the effects of spatially varying morphogen and mechanical feedback, we do need to simulate full complexity of the problem. We make the following simplifying assumptions: (*i*) only one morphogen is needed to stimulate growth in the disc and it is secreted with a constant rate by a single cell in the middle of the tissue, (*ii*) the spatial dynamics of the morphogen can be described by diffusion and constant degradation rate,

and (*iii*) the time needed to establish the spatial morphogen profile is short compared to the $1/\kappa$ or $1/\gamma$. Assumptions *ii* and *iii* yield a morphogen profile that decays exponentially with the distance to the source.

Simulation of the Discrete Model of Tissue Growth

The numerical simulation of the discrete model defined above was implemented via a custom written C-program. Minimization of the energy given by the Hamiltonian (**S1-1**) with respect to vertex coordinates and ξ variables was carried out at each step after cell division using the conjugate gradients method (9). The simulation was "event driven" similar to the Gillespie algorithm: cells were chosen for division with the probability reflecting their relative rate of growth. In addition, whenever two vertices got within 0.1% of the average edge length of each other, a T1 topological exchange move was implemented followed by another energy minimization step.

Model Parameters

Hamiltonian in Eq. **S1-1** is phenomenological in its nature and is intended to represent mechanical interactions between the cells with a small number of parameters. Specifically we parameterize mechanical energy via bulk (volume), surface (perimeter) and cell height terms. In the Hamiltonian above, the balance between these terms is controlled by the parameters *a*, *b*, and *c*. The volume term (*a*) specifies a preferred volume for the cell and together with the cell height term (*b*) determines the area of a cell. For a given cell area, the perimeter term (the first term in Eq. **S1-1**) imposes an energy penalty for a not circular cell shape. The perimeter term is the simplest representation of the contractile effect of apical actin cortices and has a form of surface tension. On length scales large compared to individual cells mechanical deformations would be described by the continuum elasticity, independent of the details of the "microscopic" Hamiltonian (10).

We have checked that our conclusions are not affected by changes in the Hamiltonian. For example, we also used length-scale independent form $\rho_\alpha/\sqrt{A_\alpha}$ as a perimeter term and $b\sum_\beta(\xi_\alpha-\xi_\beta)^2/(\xi_\alpha+\xi_\beta)^2$ as a coupling term between the neighboring cell heights. Furthermore, for the Hamiltonian given in Eq. **S1-1,** we have verified that our results are insensitive to parameters by changing key parameters by >20%. The results are summarized in SI Table 1. As expected, our proposed integral feedback mechanism for size determination is insensitive to specific model details and parameter values.

Continuum Model of Mechanical Stress in Nonuniformly Growing Tissue

On length scales large compared with the size of single cell, *a,* and the thickness of the cell layer, *w,* the deformation of the tissue that results from non-uniform growth may be described in a continuum approximation. Let vector $\Delta u_a(r)$ denote the incremental displacement of a tissue patch (initially at position *r*) after small time interval Δt. It is determined by minimization of the elastic strain energy given by

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

[S2-1]

where $\Delta u_{ab}(r) \equiv \frac{1}{2}[\frac{\partial \Delta u_b(r)}{\partial r_a} + \frac{\partial \Delta u_a(r)}{\partial r_b}]$ is the strain tensor quantifying spatial nonuniformity of $\Delta u(r)$ (and we are using the convention that repeated indices are summed). $\Delta\xi$ is the incremental strain along the axis perpendicular to the cell layer, corresponding to the modulation of layer thickness (cell height). Tissue displacement is driven by the action of by nonuniform tissue growth with a spatially nonuniform rate $\gamma(r)$ acting over the time interval Δt. Δs remind us that we are looking at incremental displacements and strains generated over a short time interval Δt. Parameter μ is the shear modulus for in-plane deformations, β couples the in-plane and transverse strains, and K and K_ξ are the in-plane and transverse "bulk" moduli. This elastic energy minimization is appropriate as long as growth is slow compared to elastic response. According to Eq. **S2-1** elastic distortion energy would be minimized by the incremental displacement vector Δu_a and $\Delta\xi$ obeying:

$$(K+\mu)\nabla^2 \frac{\partial \Delta u_c(r)}{\partial r_c} + \beta\nabla^2\Delta\xi = \Delta t K \nabla^2 \gamma(r) \qquad \textbf{[S2-2]}$$

$$-w^2\nabla^2\Delta\xi + K_\xi\Delta\xi = \Delta t\beta(\gamma - \frac{\partial \Delta u_c}{\partial r_c}) \qquad \textbf{[S2-3]}$$

Eq. **S2-2** can be integrated leading to

$$(K+\mu)\frac{\partial \Delta u_c(r)}{\partial r_c} + \beta\xi = \Delta t K\gamma(r) + \Delta t\chi(r) \qquad \textbf{[S2-4]}$$

with a scalar function $\chi(r)$ satisfying $\partial_c^2\chi = 0$ and determined by the boundary conditions. Eq. **S2-4** can be rewritten as

$$\frac{\partial \Delta u_c(r)}{\partial r_c} - \Delta t\gamma(r) = -\frac{\beta}{\mu+K}\Delta\xi - \Delta t\frac{\mu}{\mu+K}(\gamma - \bar{\gamma}) \ , \qquad \textbf{[S2-5]}$$

where we have eliminated the scalar function $\chi(r)$ by imposing the condition that in the case of free boundaries uniform growth, where $\gamma(r) = \bar{\gamma}$, its average over the tissue, generates no stress and a displacement satisfying $\partial \Delta u_c(r) / \partial r_c = \Delta t\bar{\gamma}$.

On the other hand, defining an in-plane pressure increment $\Delta p = -\frac{\delta H}{\delta \Delta u_{cc}}$ we have

$$\Delta p = -K[\frac{\partial \Delta u_c(r)}{\partial r_c} - \Delta t\gamma(r)] - \beta\Delta\xi \ , \qquad \textbf{[S2-6]}$$

which when combined with Eq. **S2-5** gives

$$\Delta p = -\frac{\beta\mu}{\mu+K}\Delta\xi + \Delta t\frac{\mu K}{\mu+K}(\gamma - \bar{\gamma}) \qquad \textbf{[S2-7]}$$

which we rewrite in terms of time derivatives

$$\frac{dp}{dt} = -\frac{\beta\mu}{\mu+K}\frac{d\xi}{dt} + \frac{\mu K}{\mu+K}(\gamma - \bar{\gamma}) \ . \qquad \textbf{[S2-8]}$$

This equation generalizes the continuum description of nonuniform growth (1) to include the effect of tissue deformation in the direction normal to the layer, i.e. modulation of cell height. The latter reduces the accumulation of stress by allowing cells to change their shape, i.e. to get taller in response to lateral compression. This effect is contained in the first term on the right side of Eq. **S2-8**.

To complete the description we need an equation governing the height deformation. It is derived by combining **S2-3** and **S2-6** to eliminate $\partial \Delta u_c(r) / \partial r_c$. This leads to

$$-w^2 \nabla^2 \Delta\xi + K_\xi (1 - \frac{\beta^2}{K K_\xi}) \Delta\xi = \beta K^{-1} \Delta p \ , \qquad \textbf{[S2-9]}$$

which upon time integration (which replaces $\Delta\xi$ by ξ and Δp by p) and rescaling yields

$$-w^2 \nabla^2 \xi(r,t) + \xi(r,t) = \alpha p(r,t) \ , \qquad \textbf{[S2-10]}$$

which states that deformation of the layer height $\xi(r,t)$ is proportional to the local stress (with the coefficient of proportionality α) with the first term on the left side of Eq. **S2-10** expressing the fact that $\xi(r,t)$ can not vary too rapidly with position and has a characteristic length w, below which its variation is suppressed. The latter effect generates a nonlocal response to any deviation of $\gamma(r,t)$ from its spatial average.

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