Supplement

Text S1 : Mathematical models of molecular, cellular and mechanical mechanisms

To model different types of periodic patterning mechanisms (as schematized in figure 1 of the main text), we adapt several partial differential equations (PDE) from the literature. We parameterize our models in terms of logarithmic sensitivities to facilitate parameter interpretation. To formulate this mathematically, consider when patterning components $\phi_i$ respond to signals $\{S_j\}$, and are limited by constant linear degradation - for example cell death, or molecular turnover:

$$\frac{\partial \phi_i}{\partial t} = f_i(\{S_j\}) - \frac{\phi_i}{\tau_i}$$

The steady state of this equation occurs provided

$$\frac{\phi_i}{\tau_i} = f_i(\{S_j\}) \forall i,$$

where bars indicate steady state levels. We can write an equation for the fractional deviations about this steady state, using this equation, and the definition of logarithmic sensitivities (from [1])

$$h_{ij} = \frac{\partial f_i}{\partial S_j} = \frac{\partial \ln f_i}{\partial \ln S_j}$$

as:

$$\frac{\partial}{\partial t} \delta \phi_i = \sum_j h_{ij} \delta S_j \frac{S_j}{\tau_i} - \frac{1}{\tau_i} \delta \phi_i$$

As an example, $h_{12} = 2$ means that the production rate of species 1 increases by 2% when the amount of signal 2 increases by 1%. These sensitivities are naturally dimensionless, and allow a consistent parameter interpretation. We now apply these parameterization to molecular, cellular and mechanical mechanisms of periodic patterning. We use each model to make two conclusions: 1) what are parameter constraints on the model such that periodic patterning occurs; and 2) how does the final pattern spacing scale with the underlying model parameters.

A. Reaction-diffusion models

Model

A generic reaction-diffusion model, consisting of an activator $A$ and inhibitor, $I$, is described by the following PDEs [2,3] :

$$\frac{\partial A}{\partial t} = f_A(A, I) - \frac{A}{\tau_A} + D_A \nabla^2 A$$

$$\frac{\partial I}{\partial t} = f_I(A, I) - \frac{I}{\tau_I} + D_I \nabla^2 I$$

and can be approximated about the homogeneous steady state as :

$$\frac{\partial}{\partial t} \left( \begin{array}{c} a \\ i \end{array} \right) = \left( \begin{array}{cc} \frac{h_{AA}}{\tau_A} & \frac{h_{AI}}{\tau_A} \\ \frac{h_{IA}}{\tau_I} & \frac{h_{II}}{\tau_I} \end{array} \right) \left( \begin{array}{c} a \\ i \end{array} \right) + \left( \begin{array}{cc} D_A & 0 \\ 0 & D_I \end{array} \right) \nabla^2 \left( \begin{array}{c} a \\ i \end{array} \right) - \left( \begin{array}{cc} \frac{\tau_A^{-1}} & 0 \\ 0 & \frac{\tau_I^{-1}} \end{array} \right) \left( \begin{array}{c} a \\ i \end{array} \right)$$

where $a, i$ represent fractional deviations from steady state. (In Box 1 in the main text, we have relabelled $(a i)$ as $(A I)$ for notational convenience). As in Box 1 of the main text, by looking for periodic solutions of the form $\phi_q(t) = \phi_q(0)\exp[\mu_q t]$ ($\phi$ denotes the vector $(a i)^T$), we obtain a quadratic equation for $\mu_q$.

Rewriting $\alpha \equiv q^2; D_A \equiv 1; D_I \equiv d; \frac{h_{AA} - 1}{\tau_A} \equiv a; \frac{h_{II} - 1}{\tau_I} \equiv b; \frac{h_{AB} h_{BA}}{\tau_A \tau_I} \equiv -c$ gives :

$$\left( \mu_\alpha + \alpha - a \right) \left( \mu_\alpha + d\alpha - b \right) = c$$

(6)
Parameter constraints

Firstly, we require that the homogeneous steady state is stable in the absence of diffusion. This requires that, when $\alpha = 0$, $\mu_\alpha < 0$, or:

\[ a + b < 0 \]  
\[ ab + c > 0 \]  

When diffusion is added:

\[ \mu_\alpha^2 + \mu_\alpha[\alpha(1 + d) - (a + b)] + [c + (\alpha - a)(da - b)] = 0 \]  

Since $\alpha(1 + d) - (a + b) > 0$ (from 7), this means that, for $\mu_\alpha$ to have a positive (real) value, we need, for some $\alpha$

\[ c + (\alpha - a)(da - b) < 0 \]  

Expanding 10 gives:

\[ da^2 + (ab + c) - \alpha(ad + b) < 0 \]  

Since $ab + c > 0$, we need $ad + b > 0$. Further, we find the condition for 10 by considering the minimum of the quadratic, which occurs at $\alpha = \frac{ad + b}{2d}$, giving a condition $(ad - b)^2 > 4cd$.

To summarize, the conditions for patterning are:

\[ a + b < 0 \]  
\[ ab + c > 0 \]  
\[ ad + b > 0 \]  
\[ (ad - b)^2 > 4cd \]  

12 and 14 imply $d > 1$, $h_{AA} > 1$; and 15 implies $c > 0$.

B: Cellular mechanisms

Via cell movement

We consider two cases: firstly, cells move and interact with their direct neighbours to form a periodic pattern. We assume there are two cell types, whose densities are written as $\phi_1, \phi_2$. We write the PDE for the evolution of cell densities in a similar way to the reaction diffusion model, where now, diffusion constants have been changed into cell motilities, $\eta$, and sensitivities refer to the responses of cells to their neighbours - for example, by regulating cellular proliferation rate:

\[ \frac{\partial}{\partial t} \begin{pmatrix} \phi_1 \\ \phi_2 \end{pmatrix} = \begin{pmatrix} h_{11}/\tau_1 & h_{12}/\tau_1 \\ h_{21}/\tau_2 & h_{22}/\tau_2 \end{pmatrix} \begin{pmatrix} \phi_1 \\ \phi_2 \end{pmatrix} + \begin{pmatrix} \eta_{11} & \eta_{12} \\ \eta_{21} & \eta_{22} \end{pmatrix} \nabla^2 \begin{pmatrix} \phi_1 \\ \phi_2 \end{pmatrix} - \begin{pmatrix} \tau_1^{-1} & 0 \\ 0 & \tau_2^{-1} \end{pmatrix} \begin{pmatrix} \phi_1 \\ \phi_2 \end{pmatrix} \]  

The key difference between this model and a molecular model is the diffusion matrix. In a simple model, where the two cells move 'randomly' (i.e. their motion is undirected), then $\eta_{11} > 0, \eta_{22} > 0, \eta_{12} = \eta_{21} = 0$, and the equation that describes changes in cell density is equivalent to the reaction-diffusion model. Accordingly we can use the results from the previous section to say that, in this case, we require $\eta_{22} > \eta_{11}$ for patterning to occur (assuming cell type 2 is the 'inhibitor cell'), as given in Table 1 of the main text [Note, in the main text, we have written $\eta_{11} \equiv \eta_A, \eta_{22} \equiv \eta_B$ for simplicity]. However, cells can also move in more complicated
ways than molecules. For example, it is possible for cells to aggregate together (e.g. $\eta_{11} < 0$), or to migrate towards or away from other cells (e.g. $\eta_{12} \neq 0$). This greatly expands the possibility for pattern formation, and allows multiple different ways that this type of system can self-assemble periodic patterns.

We note that this model is based on cell-cell contact. More generally, there may be chemotactants/repellents in the system, as in previous studies [4–6]. If we denote a possible signal as $S$, we can write:

$$\frac{\partial \phi}{\partial t} = g_1(\nabla^2 \phi) - \nabla \cdot g_2(\phi \nabla S)$$  \hspace{1cm} (17)

($g_1, g_2$ are arbitrary functions). Linearizing, and redefining parameters, we write:

$$\frac{\partial \bar{\phi}}{\partial t} = D \nabla^2 \bar{\phi} - D_S \nabla^2 \bar{S}$$  \hspace{1cm} (18)

where $\bar{\phi}, \bar{S}$ correspond to spatial averages, $D$ is the effective diffusion coefficient of the cells, and $D_S$ is a normalized measure of how cell movement responds to a signal $S$. We define a sensitivity $h \equiv D_S / D$.

### Via direct cell contact

In the case that cells are static, the model we write is subtly different. For example, consider the case that cells are static, but proliferation is regulated by signaling between cells. We write the PDE for the evolution of cell density, $\phi$, which respond to signals, $S$, from its neighbors, via:

$$\frac{\partial \phi}{\partial t} = f(S, \phi) - \frac{\phi}{\tau}$$  \hspace{1cm} (19)

The first term represents growth - this could be $\phi(1 - \phi)g(S)$ for logistic growth, where the growth rate parameter is mediated by the signal $S$. The second term represents cell turnover.

For regulated cell survival, $f$ might take the form: $f(S, \phi) = \text{const.} - \phi g_{\text{kill}}(S)$, where the signal is a killing signal. Finally, for differentiation, $\phi$ represents how 'fated' a cell is e.g. concentration of the transcription factor encoding the cell fate. In this case, $f$ would correspond to regulated transcription; and $-\phi/\tau$ is transcription factor degradation. In each case, linearizing about the steady state gives an equation of the form:

$$\frac{\partial \delta \phi}{\partial t} = \frac{h_{\phi S} \delta S}{\tau} S + \frac{h_{\phi \phi} \delta \phi}{\tau} \frac{1}{\phi} - \frac{1}{\tau} \frac{\delta \phi}{\phi}$$  \hspace{1cm} (20)

For signaling via long cellular processes, we employ a phenomenological approach to describe the signal, $S$. We consider that cells, modelled by a cell density $\phi(x, t)$, interact with each other according to an interaction function, $K(x)$, where $x$ denotes the distance between the cell that is sending the signal, and the cell that is receiving the signal. We may write a simple model for the dynamics of cell density as:

$$\frac{\partial \phi}{\partial t} = K \star \phi$$  \hspace{1cm} (21)

where $\star$ is a convolution operator, and describes that the signal a given cell receives is the sum of all the signals it receives from its neighbors. By Fourier transforming, we identify: $\mu_q = K_q$.

We assume that there is one short range interaction (for example, by direct cell-cell contact); and one long-range interaction (for example, via longer cell protrusions). We use heaviside step functions, $H(x)$, to indicate that signaling via cellular contact has a distinct range (see figure 4.
of the main text). In addition, we can make this more realistic by smoothing the step function (for example $K = g_{\text{smooth}} * H$, where $g_{\text{smooth}}$ is Gaussian (i.e. Gaussian blurring)). In wavevector space, $K_q = g_{\text{smooth}}^q H_q$. Figure 1 shows that a simple local activation; long range inhibition logic can generate a peaked $K_q$ and so successfully pattern, even with a step function which is substantially smoothened. Note, in wavevector space the interaction functions take the form $\sin(qL)/qL$, where $L$ is the signalling protrusion lengthscale.

![Graph of K(|x|) and K(|x|) smooth]

**Figure 1** – Upper : Interaction functions for cellular processes, including an ideal case (left) and a smoothened case (right). Lower : $\mu_q$ for the two cases plotted above, showing that patterns form in both cases. Smoothing (right) decreases the height of the peak

### Parameter constraints

A closed form solution for the parameter constraints is difficult to obtain. Furthermore, the model is phenomenological, and so any parameter constraints are not going to be rigorously linked to the actual biology. One point to note, however, is that a well-defined signaling range - a Heaviside step function - naturally generates some periodicity, since its Fourier transform is $\sin(qL)/qL$. In fact, if we consider the long-range inhibition alone, this generates an interaction $K_q \sim -\sin(qL_1)/qL_1$, which has a maximum greater than zero at $qL_1 \sim 4.5$ i.e. this alone could generate some periodicity. Others have previously noted that step functions more easily generate spatial patterns than graded interactions [7, 8].

### C(i) Growth induced mechanical patterning

We refer the reader to [9] for a full description and calculation for the buckling geometry in figure 1, main text. The essence of the argument is as follows.

When tissue growth occurs, there are two possibilities : (1) strain is built up in the tissue, effectively compressing it. In this case, the elastic energy $F_{\text{elastic}} \sim g^2$, where $g$ is the strain induced by growth; (2) the tissue bends. For bending, we model the disturbance of the surface as $\phi = \phi_0 \cos(q_0x)$. $\phi_0$ can be related to $g$, since $g = \delta L/L_0$ is the fractional tissue growth. If this
is accompanied by bending, then \( L_0 + \delta L = \int_0^{L_0} \sqrt{1 + (dy/dx)^2} \, dx \simeq \int_0^{L_0} 1 + 0.5(dy/dx)^2 \, dx \) i.e.

\[
g = \frac{1}{4} \phi_0^2 q_0^2
\]  

(22)

The bending energy takes a form \( F \sim \phi_0^2 q_0^4 \) and the compression energy of the underlying substrate can be shown to be \( F \sim \phi_0^2 q_0^2 \). Minimizing the total energy w.r.t. \( |q| \), generates the pattern wavelength. When this minimal energy becomes lower than the elastic energy \( F_{\text{elastic}} \), then buckling of the tissue will occur, generating periodic deformations.

Parameter constraints

As calculated in [9], buckling occurs once growth exceeds a minimum value. This has been calculated as:

\[
g > \frac{1}{4} \left( \frac{3E}{E_{\text{sheet}}} \right)^{\frac{2}{3}}
\]  

(23)

C(ii) : Movement induced mechanical patterning

Model

We refer the reader to previous literature on mechanically generated patterns : [10–16], which we summarize below.

The essence of these models is to describe the extracellular matrix (ECM), via its density, \( \rho \), and displacement, \( u \); and its interactions with motile cells. There are two governing equations for the ECM.

1. Conservation of matrix

\[
\frac{\partial \rho}{\partial t} + \nabla \cdot (\rho \frac{\partial u}{\partial t}) = 0
\]  

(24)

2. Force balance. Here, we take three contributions to the force : (i) local elasticity, described by Lame constants \( \lambda, \mu \), assuming a linear, homogeneous, isotropic elastic material; (ii) 'global elasticity', where we assume the patterning ECM is anchored to some (more rigid) underlying substrate/ECM - so that cell movement effectively shears the patterning tissue from its equilibrium position (the shear force is parameterized by the shear modulus, \( G \), and tissue thickness, \( h \), yielding an effective "spring constant" \( G/h^2 \)); and (iii) forces exerted by the cells on the ECM as they move. We write this stress as \( \sigma_{\text{traction}} \equiv \sigma n \rho / n_{\text{sat}} \rho_{\text{sat}} \), where \( \sigma \) is the maximal stress a single cell can exert, and increases as the local cell and ECM density increases. Putting this together, and using the relation between forces and stresses \( \mathbf{F} = \nabla \cdot \mathbf{\sigma} \) gives:

\[
(\lambda + \mu) \nabla (\nabla \cdot \mathbf{u}) + \mu \nabla^2 \mathbf{u} + \sigma \frac{\nabla (n \rho)}{n_{\text{sat}} \rho_{\text{sat}}} - \frac{G \mathbf{u}}{h^2} = 0
\]  

(25)

3. Cell movement. [16] have considered many alternatives for how cells move, either by random motion, passive advection on the ECM, biased motion, or ECM-dependent motion (e.g. "haptotaxis").
Parameter constraints

[16] calculated a condition for periodic patterning, using a minimal mechanical model (see equation 21 in [16]). In our parameterization, this gives:

\[ \sigma_{\text{traction}} > \frac{1}{2} E \]  \hspace{1cm} (26)

**Text S2: General features of periodic patterning**

2A: Many different PDEs can generate periodic patterns

Each model we have considered is a form of PDE. If we linearize the PDE about a homogeneous steady state, [17,18] demonstrated that a sufficient condition for a set of PDEs to form periodic patterns is that some small periodic disturbances grow over time. Mathematically, if we define \( \mathbf{q} \) as the wave-vector of a Fourier transform, this requires that for the Fourier-transformed, linearized PDEs, there is some eigenvalue in Fourier space with positive real part [17].

[17] have obtained a phenomenological model of periodic patterning by considering only a single eigenvalue - the one with largest real part, which we assume, for now, to be real, and denote by \( \mu_q \). In terms of a component which describes the pattern, \( \phi \), the early time evolution evolves as \( \phi(q,t) = \phi(q,0) \exp[\mu_q t] \). The condition for pattern is then that \( \mu_q > 0 \) for some \( q \). We can make a leading order, generalized model for \( \mu_q \) by considering: (1) \( \mu_q \) must be peaked; (2) \( \mu_q \) is a function of \( |q|^2 \) in a translationally invariant system; and (3) \( \mu_q \) is dominated by a single mode, identified by the one with largest \( \text{Re}(\mu_q) \). [Note, assumption (1) is not strictly required for the existence of some stable fluctuations, but it is commonly found in dispersion relations and selects a single wavelength]

We choose a parameterization:

\[ \mu_q = a \left( 1 - \kappa \left( \frac{q^2 - q_0^2}{q_0^2} \right)^2 \right) \]  \hspace{1cm} (27)

where \( a \) denotes peak height; \( q_0 \) peak position; and \( \kappa \) (normalized) peak width.

(Here, we have implicitly assumed \( \mu_q \) to be real. In fact, \( \mu_q \) can have non-zero imaginary parts - indeed many reaction-diffusion systems can exhibit temporal oscillations, corresponding to imaginary parts of \( \mu_q \))
Transforming to real-space, and adding non-linear terms to ensure bounded solutions, we arrive at the equation:

\[
\frac{\partial \phi}{\partial t} = a(1 - \kappa)\phi - \frac{2ak}{q_0^2} \nabla^2 \phi - \frac{ak}{q_0^4} \nabla^4 \phi - c\phi^2 - d\phi^3
\]  

(28)

(In general, \(c, d\) could also include differential operators, but we consider them as scalars here).

Equation 28 is a generalized model of periodic patterning, which will capture most of the qualitative features associated with patterning, and reflects "local activation, long range inhibition" [19, 20]. It is known as the generalized Swift-Hohenberg equation, and has been used to interpret periodic patterning in a number of contexts [17].

2B : Alternatives to the activation-inhibition logic

Whilst many "local-activation, long-range inhibition" models can be phenomenologically described by a Swift-Hohenberg equation, it does not follow that all Swift-Hohenberg models involve "local activation, long-range inhibition". In fact, other mechanisms can give rise to periodic patterns. The substrate depletion model is one such alternative [21]. Referring to the notation in section S1A, an activator/inhibitor system has \(h_{AI} < 0, h_{IA} > 0\); whereas in the substrate-depletion model, \(h_{AI} > 0, h_{IA} < 0\), i.e. the interaction logic is reversed. Similarly, [4] proposed a model with local diffusion of cells coupled to long-range chemotraction that could generate periodic patterns. Thus, the "local activation, long-range inhibition" principle encompasses a large variety, but not all, periodic patterning mechanisms.

2C : A phenomenological model captures many features associated with periodic patterning

Analysing equation 28 in Fourier space representation generates the following results, taken from previous literature [17, 18]:

1. \(a > 0\) for periodic pattern formation, to guarantee instability of the homogeneous steady state, considering \(\mu_q \leq a\). (Others have analysed parameters corresponding to \(a < 0\), where localized states can emerge, see [22])
2. If the wavelength of the pattern of the final pattern is \(\lambda\), then \(\mu_2\pi/\lambda > 0\) guarantees that patterns of this wavelength can form.
3. The timescale for the fastest growing mode is given by \(\frac{\partial \phi_q}{\partial t} = a\phi_q\), i.e. a doubling time of \(\ln 2/a\). We refer to a "typical time" for pattern to form as \(a^{-1}\) [23].
4. The complex, nonlinear equation 28 admits several stable patterns in 2D. These types of equations have been studied extensively in the literature, and the transition between stable patterns - spots, stripes, labyrinthine stripes, zigzags, hexagonal spots - are shown to depend both on boundary conditions and parameters [24] [18]. We consider a single case as an illustration, following the analysis of Ermentout, which determines whether spots or stripes are produced in a large domain [25]. The analysis uses a bifurcation approximation to identify a stable periodic disturbance as either (i) stripes, \(\phi = \phi_0 \cos(q_0 x)\); or (2) spots, \(\phi = \phi_1 \cos(q_1 x) + \phi_1 \cos(q_1 y)\). Ermentout identified a transition between these two states by examining the stability of the solutions. Applying this analysis to our system (equation 28) gives the result that high quadratic terms favor spots, with the transition occurring at \(27dak = 70c^3\). [17] have systematically analyzed the stripes, spots, hexagonal spots and zigzag transitions for the Swift-Hohenberg model, in each case depending on the underlying parameters in the model.
5. Patterns can be oriented by initial conditions. Consider the case of a striped pattern, and an initial condition as shown in figure 2B of the main text. Assuming the $x$ axis is horizontal, and the $y$ axis is vertical, this may be described as $\phi(x, t=0) = \delta(y)$, or in Fourier space as $\phi_{q,t=0} = \delta(q_x)$. Consider the time evolution of the initial state, $\phi_{q,t} = \phi_{q,t=0}\exp[\mu|q|t] = \delta(q_x)\exp[\mu|q|t]$. In this case, we can see that only the $q_y$ term contributes to the pattern, and stripes grow parallel to the $x$-axis. [26]

6. In the case that the size of the tissue is not much larger than the wavelength of the pattern, then boundary conditions can affect the final pattern which forms. This was considered in detail for periodic patterns in [18].

Text S3 : Dimensional analysis predicts scaling of final pattern wavelength

Forming dimensionless groups has been used in a variety of fields to obtain simple scaling relationships for complicated models, including PDEs [27]. For each of the models considered above, we form dimensionless groups and then write an equation for the scaling of the pattern spacing, to give the results in Table 2. The key point is that pattern spacing has units of length, so that we look for combinations of parameters which also have units of length.

For reaction-diffusion models, the set of parameters is $\{D_A, D_I, \tau_A, \tau_I\}$ (plus dimensionless logarithmic sensitivities, which we consider later). We write the output we consider, the pattern spacing, as $\lambda$. We can form three dimensionless groups : $\{\lambda^2/D_A\tau_A, D_I/D_A, \tau_I/\tau_A\}$. By the Buckingham Pi theorem [28], we can then write

$$\lambda = \sqrt{D_A\tau_A}f\left(\frac{D_I}{D_A}, \frac{\tau_I}{\tau_A}\right)$$

(29)

Where $f$ is an arbitrary function, and can include the effects of the logarithmic sensitivities.

In the case that boundary conditions are important, we introduce the sizes of the tissue (which has units of length) into this expression, which complicates interpretations. For example, on a square domain of size $L$, we would have :

$$\lambda = \sqrt{D_A\tau_A}f\left(\frac{D_I}{D_A}, \frac{\tau_I}{\tau_A}, \frac{L^2}{D_A\tau_A}\right)$$

(30)

We can use exactly the same approach for the other models we have considered, and the results are summarized in Table 2. Note, for mechanical buckling, additional scaling laws may be obtained by using dimensional analysis on the constituent energy terms which govern the phenomenon. [9] showed that the pattern spacing scales as $\lambda \propto h(E_{\text{sheet}}/E)^{1/3}$.

Text S4 : Different mechanisms have different interaction functions, $K(x)$

At the core of the periodic patterning mechanisms we have considered is "local activation, long-range inhibition." For each of the PDE equations we considered in Text S1, we can compute the functional form of this "local-activation, long-range inhibition," $K(x)$. For concreteness, we consider the case when cells form the pattern, and that cells signal to each other in one of three ways : molecular signals; cell-contact signals; or mechanical signals. We can compute $K(x)$ by
considering a cell at a single location (the sender cell) and determining how much of each of the
signals is received at a distance $x$ away from the sender cell.

**Molecular signals**

Here, a short-ranged molecule, A, achieves local activation; and a long-ranged molecule, I,
achieves long-range inhibition. If we describe cell density by $\phi$, and assume that cells produce
both A and I, then we can write a PDE for the evolution of A as:

$$\frac{\partial A}{\partial t} = D_A \nabla^2 A - \frac{A}{\tau_A} + f_A \phi,$$

and an equivalent equation for I. To calculate the interaction function, we consider a single
sender cell, i.e. $\phi \sim \delta(x)$, and arrive at an interaction of the form (for simplicity, in a 1D tissue):

$$K(x) = K_A e^{-\frac{|x|}{\sqrt{D_A \tau_A}}} - K_I e^{-\frac{|x|}{\sqrt{D_I \tau_I}}}$$

$K_A, K_I$ are constants describing the effect of each of the signals on cell behaviours. This function
is plotted using Mathematica in figure 4B. Note that this can be generalized to include a time
component, noting that the molecular signal is not instantaneous.

**Cell-contact signals**

We have previously defined the effect of cell contact signals in section Text S1B. We directly
plot this distance dependence using Mathematica in figure 4B.

**Mechanical signals**

Intuitively, signals generated by mechanical buckling are long-ranged, instant and generate
periodic disturbances over a long length range. For example, near the buckling point, growth at a
particular location in the tissue can cause the tissue to buckle, generating an interaction which is
periodic and felt at long distances (like that plotted in figure 4B). For migratory cells, [16] showed
that many models had dispersion relations, $\mu_q$, that contained singularities. Transforming the
dispersion relation back to real space gives an effective interaction for moving cells interacting
at different points in space - $\mu_q = K_q$, as discussed in Box 2 of the main text. For singularities
in the dispersion relation, we expect sinusoidal real-space representation - for example, a simple
singularity :

$$\mu_q \propto \frac{1}{A - Bq^2}$$

(where $A, B$ are constants) gives a periodic interaction function which is long ranged. In a 2D
tissue, this corresponds to a zeroth order Bessel function, $J_0$, which we have plotted in figure 4B
of the main text.

**Text S5 : Using timelapse imaging to estimate $K(x)$**

Our analysis has showed that the interaction function, $K(x)$ has qualitatively different forms
depending on the type of signal :

1. Molecular signals decay in a smooth fashion (often assumed exponentially) and have a
timescale dominated by diffusion and degradation
2. Cellular processes are instant interactions, with a well-defined lengthscale. Mechanical forces transmit signals instantly and over a long range. In addition, the signal is transmitted by local material stresses i.e. heterogeneities. If we can estimate \( K(x) \), we can: first, find the qualitative logic of the interaction; and (2) compare the experimental result to our theoretical models.

In the main text, we considered cell movement generated pattern. In this description, the interaction becomes discrete - with cells at positions \( \{x_i\} \) corresponding to \( \phi = \sum \delta(x - x_i) \). In terms of interactions, this gives the velocity response as:

\[
v_i = \sum K(|x_i - x_j|) \frac{x_i - x_j}{|x_i - x_j|}
\]

If we then take many measurements of \( \{x_i\} \) and \( \{v_i\} \), we can estimate the form of \( K(x) \). Algorithmically, we discretize space to transform 34 into a linear equation, where the observed velocity is a linear sum of \( K \) values. By combining many observations, this gives many linear equations for \( K \) i.e. a matrix equation (forming a generalized linear model). We can then invert this matrix observations to generate an estimate for \( K(x) \) (a maximum likelihood estimation), as plotted in the main text.

We have simulated a one-dimensional system where cells interact using molecular signals, in which case the interaction function is: \( K = K_A \exp[-x/L_A] - K_I \exp[-x/L_I] \). To this, we added some noise to the system to represent random cell motion. The MATLAB code used to generate this simulation is found in Appendix A. (As stated in the main text, we also simulated a cellular interaction, modelled as \( K = K_A(1 - H(x - L_A)) - K_I(1 - H(x - L_I)) \), where \( H \) denotes the Heaviside step function.) We note that, in both cases, the simulations are very simplistic - they do not, for example, take into account the effects of volume exclusion when cells occupy the same space; furthermore the simulations are one-dimensional. (These effects become most important towards the later stages of patterning, a stage we do not use in our analysis.) However, we hope that the simulations illustrate our approach.

We took a number of timepoints and repeats and measured the \( \{x_i, v_i\} \) pairs from the simulated data. Using the matrix inversion approach, we generated an estimate for each \( K(x) \). As shown in the figure below, we found that this estimation closely resembled the synthetic interactions we began with (using data from 200 cells at 100 timepoints for 10 independent repeats), and could distinguish between the two types of mechanism (see Appendix A for MATLAB code). This suggests that, even with noisy data, the form of the interaction is accessible using timelapse microscopy.

The cell density plots in figure 4C of the main text were generated by smoothing the discrete cell density formula \( \phi = \sum \delta(x - x_i) \) and convolving it with a gaussian blurring filter \( \exp(-x^2/2\sigma^2) \), using \( \sigma = 1.5 \). We plot three representative time points in figure 4C.

**Text S6 : Additional parameter constraints, as applied to reaction-diffusion systems**

In Table 1 of the main text, we list conditions for periodic patterning to occur - specifically, that \( \mu_q > 0 \) for some \( q \), using the notation from section S1A. One further condition is obtained if the pattern wavelength, \( \lambda \) can be measured (here, we write the observed wavevector as \( q_{obs} = 2\pi/\lambda \)). In order for a periodic pattern of wavelength \( \lambda \) to form (assuming homogeneous initial conditions and small fluctuations), we need

\[
\mu_{q_{obs}} > 0
\]
Figure 3 – The interaction function inferred from measuring dynamics closely resembles the synthetic interaction function.

(As in section S1A, we often have, in addition, $\mu_q = 0 < 0$). Equation 35 may be applied to each of the models described in the text. This is beyond the scope of the current paper, but we provide the results for reaction-diffusion mechanisms to illustrate the approach.

Imagine you observe $\alpha \equiv \dot{q}_{obs} = 4\pi^2/\lambda^2$, then equation 35 can be solved, together with the definitions in section S1A, to give:

$$2\mu_\alpha = (a + b) - \alpha(1 + d) + \sqrt{[\alpha(d - 1) + (a - b)]^2 - 4c}$$

(36)

We need $\mu_\alpha > 0$, i.e.

$$[\alpha(d - 1) + (a - b)]^2 - 4c > [(a + b) - \alpha(1 + d)]^2$$

(37)

Using $ab + c > 0$, and rearranging implies:

$$ad + b > d\alpha$$

(38)

Since $b < -a$, then $a > d\alpha/(d - 1)$.

Since $a < -b$, then $(-b) > d\alpha/(d - 1)$.

Since $ab + c > 0$, $b < -a$, then $\sqrt{c} > d\alpha/(d - 1)$.

For large $d$, these limits become:

$$h_{AA} - 1 > \frac{4\pi^2}{\lambda^2} D_A \tau_A$$

(39)

$$1 - h_{II} > \frac{4\pi^2}{\lambda^2} D_A \tau_I$$

(40)

$$\sqrt{-h_{AI} h_{IA}} > \frac{4\pi^2}{\lambda^2} D_A \sqrt{\tau_I \tau_A}$$

(41)
Text S7 : Interpretation of perturbations to nonlinear parameters

As discussed in the main text, perturbing dimensionless quantities in the equations in Table 2 generates results that are difficult to interpret. For example, activating (overexpressing, knocking down, or inhibiting) putative activator/inhibitor signaling molecules changes the nonlinear reaction terms in a reaction-diffusion system. Whilst these perturbations are challenging to interpret, they are sometimes the most feasible to perform and are currently the mostly commonly found in existing literature. In this case, what can our mathematical analysis tell us about interpreting these experiments?

Referring to section S6, we cannot accurately compute the pattern spacing for a given mechanism, but we can find the range of possible pattern spacings. Specifically, we rewrite the inequalities from the previous section as :

$$\lambda^2 > \frac{4\pi^2 D_A \tau_A}{h_{AA} - 1}$$ (42)

(and similarly for the other inequalities). Therefore, changing reaction sensitivities (i.e. activating or inhibiting pathways) changes the minimum allowed pattern spacing ($\lambda_{\text{min}} \rightarrow \tilde{\lambda}_{\text{min}}$), but cannot definitively tell you how the actual pattern spacing should change ($\lambda \rightarrow \tilde{\lambda}$). To show that a perturbation should affect a given periodic pattern therefore requires showing that this minimum bound is relevant i.e. $\tilde{\lambda} < \lambda_{\text{min}}$ - which would require measurements (or estimations) of $D_A$ and $\tau_A$.

Appendix A : Code

We present code used to analyze cell movements and positions, in order to infer interaction types - an example is plotted in figure 4B in the main text.

Creation of synthetic data

%1D periodic pattern simulator%
Cell movement generates the pattern%

%Initialization
RandStream.setGlobalStream(RandStream('mt19937ar','seed',sum(100*clock)))
clear A; clear B; clear C;
N = 200; %Number of particles
L = 100; %Length of total space
T = 20; %Number of timesteps to simulate
dt = 0.001; %Time increment
eta=30; %Noise in the system

%Local activation
l0 = 7.5; %Lengthscale of interaction
j0 = 10; %Strength of interaction

%Long-range inhibition
l1 = 20; %Lengthscale of interaction
j1 = 20; %Strength of interaction
% Boundary conditions implemented by strong repelling boundaries
jb = 100;
lb = 3;

% Collect observations every NN timepoints
NN = 10; % How many timesteps store for
nn=0;
C=zeros(N,(T/(dt*NN)));

% Initialize randomly A = particle coordinates
A = L*(rand(N,1));
B = zeros(N,1);

% Increment
for i=1:(T/dt)
  % Store observation of particle positions
  if(mod(i,NN) == 0)
    nn = nn + 1;
    C(:,nn) = A;
  end

  % Cells move with a velocity determined by interactions with all other cells.
  % In this example, the interaction is molecular, given by two exponential functions.
  for j=1:N
    A = A - j0*dt.*(sign(A-A(j))).*(1/10).*{exp(-abs(A-A(j))/10)};
    A = A + j1*dt.*(sign(A-A(j))).*(1/11).*{exp(-abs(A-A(j))/11)};
  end

  % Aside:
  % Cellular mechanism:
  % Note parameters must be modified to get the same pattern spacing
  % A = A - j0*dt.*(sign(A-A(j))).*(1 - heaviside(abs(A-A(j)) - l0));
  % A = A + j1*dt.*(sign(A-A(j))).*(1 - heaviside(abs(A-A(j)) - l1));

  % Boundary concentration
  A = A + dt*jb*(1-heaviside(A+10));
  A = A - dt*jb*heaviside(A-l10);

  % Random noise component
  A = A + dt*eta*(-0.5+rand(N,1));

  % Plotting function (optional)
  if(mod(i,1000) == 0)
    scatter(A,B,'.');
    axis([0 100 -1 1]);
    pause(0.01);
  end
end
**Inference of \( K(x) \)**

This function takes in input observation matrix, \( C \), and infers the interaction function \( K(x) \), estimated by \( \text{Corr} \).

```matlab
function [\text{Corr} \ v \ F] = \text{Brute}(C);
%v are velocity measurements; \( F \) is observations written in linear form

\text{clear \ Corr; clear \ v; clear \ F;}

%Discretization of space
\text{Nx} = 50;
\text{x=(100/Nx)*}(1:1:Nx);

%Initialize
\text{dim = size(C);} \\
\text{F = zeros(dim(1)*dim(2),Nx);} \\
\text{dt = 0.001; \%Taken from Simulate.m}
\text{Corr = zeros(25,1);} \\
\text{step = 1;}

%Summarize the observations in matrix form, relating cell velocities to positions of all other cells
\text{for \ ti=2:dim(2)}

%infer velocities and positions, at each timepoint, from \( C \)
\text{V = (C(:,ti)-C(:,ti-1))/dt;} \\
\text{X = C(:,ti);} \\

\text{for \ i=1:dim(1)}

% v(step) = V(i);
\text{for \ j = 1:dim(1)}

% dist = abs(round(Nx/100*(X(i) - X(j))));
\text{if \ i ~= j \ && \ dist > 0 \ && \ dist < (Nx + 1)}
\text{F(step,dist) = F(step,dist) + sign(X(i)-X(j));}
\text{end}
\text{end}
\text{step = step + 1;}
\text{end}

%Truncate observations to remove zero terms (this is currently done manually)
\text{F2 = \text{F}(1:10000,:); \ v2 = \text{transpose(v); \ v2 = \text{v2}(1:10000);}

%compute correlation by matrix inversion
\text{Corr = F2 \ v2;}
\text{end}
```
Example script to use the two functions

NCounts = 10;
clear f;
Simulate;
f = Brute(C);
for indexCount=1:(NCounts−1)
    Simulate;
    f = f + Brute(C);
    indexCount
end

% plot((2:2:50),−f(1:25),'.')

Références


