BE 159: Signal Transduction and Mechanics in Morphogenesis

Justin Bois

Caltech

Winter, 2016

2 Turing patterns

This class is about signal transduction and mechanics in morphogenesis. Signal transduction usually refers to the mechanism by which external cues (the signals) are imported into a cell, followed by a chain of biochemical and transport events that result in altered gene expression (that's the transduction part). We will discuss a few specific examples of signal transduction later in the class (focusing primarily on Wnt and Delta-Notch signaling) and discuss the mechanisms.

To start, though, we will think about signal transduction a bit more abstractly. Specifically, we will first describe ways in which the signals, or biochemical cues, are themselves distributed in a spatially inhomogeneous way.[1] This is necessary to give the shape of the features. It is not difficult to imaging that the biochemical cues would operate in a concentration-dependent manner; higher concentrations result in stronger signals than lower concentrations. Such chemical species, which determine cell fate in a developmental context in a concentration-dependent way, are called **morphogens**.

Secondly, we will discuss the **information** present in morphogen patterns. I put the word "information" in bold because we will discuss a precise definition of information. For now, we will think about this in an intuitive sense: Can cell fate be determined with enough spatial refinement from the concentrations of a set of morphogens? The next step after that, which we will explore in the student presentations, is how the of information contained in the concentration of signaling molecules is transmitted through signaling circuits.

For this lecture, we will discuss **reaction-diffusion** mechanisms for generating spatial distributions of morphogens. This is important both practically, and historically.

2.1 Turing's thoughts on reaction-diffusion mechanisms for morphogenesis

In my favorite paper of all time, Alan Turing (yes, *that* Alan Turing) laid out a prescription for morphogenesis. He described what should be considered when studying the "changes of state" of a developing organism. Turing said,

> In determining the changes of state one should take into account:
>
> (i) the changes of position and velocity as given by Newton's laws of motion;

[1] Note that these cues could even have trivial signaling pathways; they can be transcription factors themselves.

(ii) the stresses as given by the elasticities and motions, also taking into account the osmotic pressures as given from the chemical data;

(iii) the chemical reactions;

(iv) the diffusion of the chemical substances (the region in which this diffusion is possible is given from the mechanical data).

He proceeded to state, a few lines later, "The interdependence of the chemical and mechanical data adds enormously to the difficulty, and attention will therefore be confined, so far as is possible, to cases where these can be separated."

In this class, we will attempt this enormously difficult task of bringing together the chemical and the mechanical. To start with, though, we will consider only chemical reactions and diffusion, and we will see that these together can produce patterns useful in development.

2.2 Reaction-diffusion equations for a single component

The reaction-diffusion equations are just statements of conservation of mass. We will get into conservation laws in more depth later in the course, but for today we will take the equation describing the continuum conservation law as given.

Consider a chemical species i with diffusion coefficient D_i. Its concentration, a function of position x and time t, is $c_i(x,t)$. Then, the dynamics of the concentration under control of diffusion is described by

$$\frac{\partial c_i}{\partial t} = D_i \frac{\partial^2 c_i}{\partial x^2}. \tag{2.1}$$

This functional form makes sense intuitively. Imagine there is a local area of high concentration. By diffusion, the concentration at this point will drop, and it will rise away form the high concentration region. The second derivative of the concentration profile at the peak is negative, so the time derivative is also negative, which means that the concentration decreases there. The second derivative is positive away from the peak, so the concentration will rise in those regions.

Now, recall your general chemistry class in which you learned about kinetics. Let $r_i(c_i)$ be the rate of production of species by chemical reaction. Then, the rate of chance of c_i due to chemical reaction is

$$\frac{\partial c_i}{\partial t} = r_i(c_i). \tag{2.2}$$

Now, if we couple the chemical reactions with diffusion, we get

$$\frac{\partial c_i}{\partial t} = D_i \frac{\partial^2 c_i}{\partial x^2} + r_i(c_i). \tag{2.3}$$

This generalizes to two or three dimensions.

$$\frac{\partial c_i}{\partial t} = D_i \nabla^2 c_i, + r_i(c_i), \tag{2.4}$$

where

$$\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}, \tag{2.5}$$

in three dimensional Cartesian coordinates, for example.

2.3 Example: the Bicoid gradient

Bicoid was the first morphogen discovered. This morphogen can bind both DNA and RNA and is involved in transcriptional and translational regulation. It is present in high concentrations at the anterior regions of a *Drosophila* embryo and decays away as we move toward the posterior. It it thought that the gradient is set up by a reaction-diffusion process. The reactions are simple.

1. Like any protein, Bicoid degrades with some characteristic degradation rate, α.
2. *Bicoid* mRNA is tightly localized to the anterior of the embryo. Bicoid protein is continuously produced from this localized mRNA. To take into account the production and localization, we write this part of the chemical reaction as $q_0 f(x)$, where $f(x)$ is a dimensionless function describing the localization of the *bicoid* mRNA and therefore the Bicoid source.

Thus, $r(c,x) = -\alpha c + q_0 f(x)$. As already implied by our definition of Bicoid production, we will study this system in one dimension. The complete reaction diffusion equation is then

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - kc + q_0 f(x). \tag{2.6}$$

If we are interested in the steady state Bicoid concentration profile, we set $\partial c/\partial t = 0$, giving

$$\frac{\partial^2 c}{\partial x^2} - \frac{\alpha}{D} c = -\frac{q_0}{D} f(x). \tag{2.7}$$

Let $\lambda = \sqrt{D/\alpha}$ be the characteristic length scale and let $\tilde{x} = x/\lambda$. We then have

$$\frac{\partial^2 c}{\partial x^2} - c = -\frac{q_0}{\alpha} f(\tilde{x}). \tag{2.8}$$

Importantly, when we nondimensionalize this way, we see that q_0/α sets the scale of the concentration profile. We see further that, provided the source of Bicoid is sufficiently localized, λ is the only length scale in the problem and therefore must set the scale of the concentration gradient.

We can solve this equation in Fourier space as

$$\hat{c}(k) = \frac{q_0}{\alpha} \frac{\hat{f}(k)}{1 + k^2}. \tag{2.9}$$

We can then easily solve this numerically with FFTs. We have only to specify $f(\tilde{x})$. We will choose $f(\tilde{x}) = 1 - \theta(\tilde{x} - a)$, where $\theta(x)$ is the Heaviside step function. In other words, we assume that the *bicoid* mRNA is localized in a region of size a at the anterior, given a source of Bicoid protein of width a.

The result is shown in Fig. 1 with $a = 0.1$ (remember, this is in units of λ.). The code used to generate the figure follows.

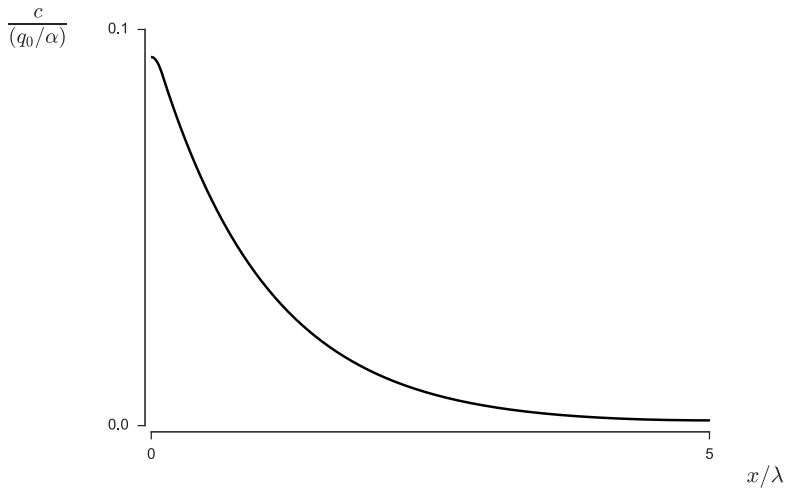

Figure 1: The gradient of Bcd. Note the style of the plot. This is useful for presentations. The Python code will help you generate such figures for your own talks.

```
import numpy as np
import matplotlib.pyplot as plt
import seaborn as sns

# Size of step in units of lambda
a = 0.1

# Length of embryo in units of lambda
L = 10

# Set up grid
```

```
n = 1024
x = np.linspace(-L/2, L/2, n)

# Wave numbers
k = np.fft.fftfreq(n, L / (2 * np.pi * n))

# Solve ODE
f = (abs(x) < a).astype(float)
f_hat = np.fft.fft(f)
c_hat = f_hat / (1  + k**2)
c = np.fft.ifft(c_hat).real

# Generate plot
sns.set_style('ticks')
fig, ax = plt.subplots(1, 1)
ax.plot(x, c, color='black', linewidth=2)
ax.set_xlim([0, 5])
ax.set_xticks([0, 5])
ax.set_yticks([0, 0.1])
ax.tick_params(labelsize=12)
ax.set_xlabel(r'$x/\lambda$', fontsize=18)
ax.set_ylabel(r'$\frac{c}{(q_0/\alpha)}$', fontsize=24, rotation=0)
ax.xaxis.set_label_coords(1.1, -0.1)
ax.yaxis.set_label_coords(-0.2, 0.95)
sns.despine(offset=5, trim=True);

fig.savefig('bcd_gradient.pdf', bbox_inches='tight', transparent=True)
```

2.4 Reaction-diffusion equations for multiple components

The equations for reaction-diffusion dynamics generalize to multiple components. Let $\mathbf{c} = \{c_1, c_2, \ldots\}$ be the concentrations of each of n total species. Then, we can write

$$\frac{\partial c_i}{\partial t} = D_i \nabla^2 c_i, + r_i(\mathbf{c}). \tag{2.10}$$

Here, we have assumed that the diffusion of each species is independent of that of all others. The chemical reaction rates, though, may depend on other species.

In the study of many signaling studies, authors make the **well-mixed approximation**, and neglect the diffusion term and any spatial dependence on the chemical components.

In the next section, we will see what beautiful patterns emerge from reaction-diffusion with two chemical species. These patterns are called **Turing patterns**.

2.5 Turing patterns two-component R-D systems

Consider two chemical species that can undergo diffusion in one dimension (for simplicity). Then, the reaction-diffusion equations for these species are

$$\frac{\partial a}{\partial t} = D_a \frac{\partial^2 a}{\partial x^2} + r_a(a,s), \tag{2.11}$$

$$\frac{\partial h}{\partial t} = D_s \frac{\partial^2 s}{\partial x^2} + r_s(a,s), \tag{2.12}$$

where we have denoted the concentration of the two species to be a and s. To have a concrete example in mind, since this often helps understanding, we will take

$$r_a = \rho a^2 s - \alpha a \tag{2.13}$$

$$r_s = \beta - \rho a^2 s \tag{2.14}$$

This means that a serves as an activator and s is an inhibitor. We can see this but looking at each term. The $\rho a^2 s$ term means that a is catalyzes its own production, but needs a substrate enzyme to do so. The appearance of the $-\rho a^2 s$ term means that the substrate is consumed in this process. The activator undergoes autodegradation (the $-\alpha a$ term), and the substrate is produced at a constant rate β. We could include autodegradation of the substrate, but we assume that that process is very slow compared to the other processes at play and neglect it for simplicity.

This model is called the activator-substrate depletion model, or ASDM.

2.5.1 Nondimensionalization

In studying dynamical systems, it is almost always a good idea to **nondimensionalize** them. To do this, we manipulate the equations such that every term is dimensionless, i.e., has no units. This often results in a reduction in the number of parameters.

In general, we can choose a units of time to be τ so that we can nondimensionalize time, $\tilde{t} = t/\tau$. We nondimensionalize position x as $\tilde{x} = \sqrt{D_s \tau}$ (a similar length scale that appeared in the Bicoid example). Then, the reaction diffusion system can be written as

$$\frac{\partial a}{\partial \tilde{t}} = d \frac{\partial^2 a}{\partial \tilde{x}^2} + \tau r_a(a,s), \tag{2.15}$$

$$\frac{\partial s}{\partial \tilde{t}} = \frac{\partial^2 s}{\partial \tilde{x}^2} + \tau r_s(a,s), \tag{2.16}$$

where the tildes represent dimensionless quantities and $d \equiv D_a/D_s$ is the ratio of the diffusive rates of the activator and substrate. This already shows us that the *ratio* of the diffusion coefficients will be an important parameter.

We are free to choose how we nondimensionalize the concentrations of the activator and substrate to get fully nondimensional dynamical equations. It is convenient to nondimenionalize using $\tau = 1/\alpha$, $a = \beta\tilde{a}/\alpha$, and $s = \alpha^2\tilde{s}/\beta\rho$.

Thus, we can write the reaction-diffusion equations as

$$\frac{\partial a}{\partial t} = d\frac{\partial^2 a}{\partial x^2} + a^2 s - a \tag{2.17}$$

$$\frac{\partial h}{\partial t} = \frac{\partial^2 s}{\partial x^2} + \mu(1 - a^2 s), \tag{2.18}$$

where $\mu = \beta^2\rho/k^3$ and we have dropped the tildes for notational convenience, knowing that all variables and parameters are dimensionless. Conveniently, we have gone from five parameters down to two.[2] So, the dynamics are governed by only two parameters, the ratio of the diffusion coefficients, d, and the ratio of production to degradation rates μ.

Going forward, in the general treatment of the two-component system, we will assume everything is properly nondimensionalized and write our dynamical equations as

$$\frac{\partial a}{\partial t} = d\frac{\partial^2 a}{\partial x^2} + r_a(a, s), \tag{2.19}$$

$$\frac{\partial s}{\partial t} = \frac{\partial^2 s}{\partial x^2} + r_s(a, s). \tag{2.20}$$

2.5.2 Homogeneous steady state

The reaction-diffusion system is at **steady state** when the time derivatives are zero. A steady state is **homogeneous** when the spatial derivatives are also zero. This just means that the concentration of all chemical species are spatially uniform. A homogeneous steady state (a_0, s_0) then satisfies $r_a(a_0, s_0) = r_s(a_0, s_0) = 0$. For the ASDM, the homogeneous steady state is $a_0 = s_0 = 1$ and is unique.

2.5.3 Linear stability analysis

Imagine the system is in the homogeneous steady state. What happens to this system if it experiences a small perturbation? This question can be address using **linear stability analysis**.

[2] We could actually arrive at the same dimensionless equations if we had a different ρ values, say ρ_a and ρ_s, for production of activator and depletion of substrate, bringing the parameter count from six down to two.

Let use expand both sides of our dynamical equations in a Taylor series about $(a, s) = (a_0, s_0)$.

$$\frac{\partial}{\partial t}(a_0 + \delta a) = d\,\frac{\partial^2}{\partial x^2}(a_0 + \delta a) + r_a(a_0, s_0) + r_{a,a}\,\delta a + r_{a,s}\,\delta s + \cdots, \tag{2.21}$$

$$\frac{\partial}{\partial t}(s_0 + \delta s) = \frac{\partial^2}{\partial x^2}(s_0 + \delta a) + r_s(a_0, s_0) + r_{s,a}\,\delta a + r_{s,s}\,\delta s + \cdots, \tag{2.22}$$

where $\delta a = a - a_0$ and $\delta s = s - s_0$. We also defined

$$r_{a,s} = \left.\frac{\partial r_a}{\partial a}\right|_{a_0, s_0}, \tag{2.23}$$

with other parameters similarly defined. Now, $r_a(a_0, s_0) = r_s(a_0, s_0) = 0$, since (a_0, s_0) is a homogeneous steady state, and all derivatives of a_0 and s_0 are also zero. Then, to linear order in the perturbation $(\delta a, \delta s)$, we have

$$\frac{\partial \delta a}{\partial t} = d\,\frac{\partial^2 \delta a}{\partial x^2} + r_{a,a}\,\delta a + r_{a,s}\,\delta s, \tag{2.24}$$

$$\frac{\partial \delta s}{\partial t} = \frac{\partial^2 \delta s}{\partial x^2} + r_{s,a}\,\delta a + r_{s,s}\,\delta s. \tag{2.25}$$

We can write the spatial variation in the perturbation as a Fourier series, with mode k being $\delta a_k(t)\mathrm{e}^{ikx}$. Then the dynamical equation for mode k is

$$\frac{\mathrm{d}\delta a}{\mathrm{d}t} = -dk^2\,\delta a_k + r_{a,a}\,\delta a + r_{a,s}\,\delta s, \tag{2.26}$$

$$\frac{\mathrm{d}\delta s}{\mathrm{d}t} = -k^2 + r_{s,a}\,\delta a + r_{s,s}\,\delta s. \tag{2.27}$$

This can be written in matrix form as

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}\delta a \\ \delta s\end{pmatrix} = \mathsf{A}\cdot\begin{pmatrix}\delta a \\ \delta s\end{pmatrix}, \tag{2.28}$$

where

$$\mathsf{A} = \begin{pmatrix} -dk^2 + r_{a,a} & r_{a,s} \\ \\ r_{s,a} & -k^2 + r_{s,s}\end{pmatrix} \tag{2.29}$$

is the **linear stability matrix**. This is now a linear system of equations and the solution is

$$\begin{pmatrix}\delta a \\ \delta s\end{pmatrix} = c_1\mathbf{v}_1\mathrm{e}^{\sigma_1 t} + c_2\mathbf{v}_2\mathrm{e}^{\sigma_2 t}, \tag{2.30}$$

where σ_1 and σ_2 are the eigenvalues of A and $\mathbf{v}_1$ and $\mathbf{v}_2$ are the eigenvectors. So, if the real part of one of the σ's is positive, the kth mode of the perturbation will grow over time.

Remember that for a 2×2 matrix, the eigenvalues are

$$\sigma = \frac{1}{2}\left(\text{tr A} \pm \sqrt{\text{tr}^2\text{A} - 4\text{det A}}\right). \tag{2.31}$$

So, the real part of the largest eigenvalue is negative if the trace of the linear stability matrix is negative and its determinant is positive. Otherwise, the largest eigenvalue has a positive real part and the homogeneous steady state is not stable and patterns or oscillations can spontaneously emerge.

2.5.4 Consequences of linear stability analysis

We can write the trace and determinant explicitly.

$$\text{tr A} = -(1+d)k^2 + r_{a,a} + r_{s,s}, \tag{2.32}$$

$$\text{det A} = dk^4 - (r_{a,a} + dr_{s,s})k^2 + r_{a,a}r_{s,s} - r_{a,s}r_{s,a}. \tag{2.33}$$

In the absence of spatial information (and therefore diffusion), the trace is negative if and only if at least one of $r_{a,a}$ and $r_{s,s}$ is negative. This means that chemical reaction system by itself is stable. Interestingly, the trace is maximal for the zeroth mode, which means that an instability arising from the trace becoming positive has the zeroth mode as its fastest growing. If the determinant is positive at the onset of the instability (when the trace crosses zero), the eigenvalues are imaginary, which means that the zeroth mode is oscillatory. This is called a Hopf bifurcation.

For patterning in a developmental context, we want stable chemical reaction systems, and we would like patterns to be emergent as the organism grows. Note that the size of the embryo sets which values of k are allowed; the organism has to be big enough to fit the modes. So, an organism grows long enough to fit a mode for which the eigenvalue is positive, and then patterns spontaneously emerge. So, we generally do not a Hopf bifurcation, which means that a necessary condition is that at least one of $r_{a,a}$ or $r_{s,s}$ is negative.

Now, the requirement that the chemical reaction system is stable in the absence of spatial information implies that $r_{a,a}r_{s,s} - r_{a,s}r_{s,a} > 0$. The determinant is convex quadratic in k^2, so it has a minimum when

$$\frac{\partial^2}{\partial k^2}\,\text{det A} = 2dk^2 - r_{a,a} - dr_{s,s} = 0. \tag{2.34}$$

Therefore, the fastest growing mode in the instability is given by

$$k_0^2 = \frac{r_{a,a} + dr_{s,s}}{2d}. \tag{2.35}$$

This minimum occurs for real k_0 only in the presence of positive feedback, or, in chemical terms, if at least one of the species is autocatalytic, meaning that either $r_{a,a} > 0$ or $r_{s,s} > 0$ or both. We determined earlier that the condition of stable chemical reactions implies that at least one of these terms is negative, so we now have that exactly one must be positive and one must be negative. We arbitrarily pick $r_{a,a}$ to be autocatalyic (hence the name, "activator").

2.5.5 Linear stability analysis for the ASDM

For the ASDM, we have $r_{a,a} = r_{a,s} = 0$, $r_{s,a} = -2\mu$, and $r_{s,s} = -\mu$, giving

$$\mathsf{A} = \begin{pmatrix} 1 - dk^2 & 1 \\ -2\mu & -\mu - k^2 \end{pmatrix}. \tag{2.36}$$

The trace and determinant are

$$\operatorname{tr} \mathsf{A} = -(1+d)k^2 + 1 - \mu \tag{2.37}$$

$$\det \mathsf{A} = (dk^2 - 1)(\mu + k^2) + 2\mu = dk^4 - (1 - d\mu)k^2 + \mu. \tag{2.38}$$

So, in order to avoid the Hopf bifurcation, we need $\mu > 1$. The fastest growing mode is

$$k_0^2 = \frac{1 - d\mu}{2d}. \tag{2.39}$$

For k_0 to be real, we must have $d/\mu < 1$. Since $\mu > 1$, the condition for a Turing instability is that $d < 1$. This can be shown to be the case in general, not just for the ASDM. So, we have summarized the requirements for a Turing instability.

1. One species is autocatalytic ($r_{a,a} > 0$) and one is inhibitory ($r_{s,s} < 0$).
2. The inhibitory species (in the ASDM model, this is the substrate) must diffuse more rapidly than the activating species.

The intuition here is that the activator starts producing more of itself locally. The local peak starts to spread, but the inhibitor diffuses more quickly that pins the peak of activator in so that it cannot spread. This gives a set wavelength of the pattern of peaks.