BE 159: Signal Transduction and Mechanics in Morphogenesis

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3 Wnt signaling

In this lecture, we will discuss methods for modeling biochemical networks using mass action kinetics with the example of **Wnt signaling** as our motivation.

3.1 Introduction to Wnt signaling

The Wnt (pronounced "wint") signaling pathway is central in many developmental processes. To see how central it is, you might want to visit the Wnt homepage, run by Roel Nusse's lab at Stanford, which details the components of the pathway as well as a wealth of links to other information.

The history of the discovery of the Wnt family of proteins highlights its importance in development. In their Nobel Prize-winning work published in 1980, Nüsslein-Volhard and Wieschaus discovered several genes that are central to development in *Drosophila*. One of these was a segment polarity gene Wingless (Wg). The gene was so named because of its phenotype: wingless adult flies, so the gene has downstream effects past regulation of segment polarity. A couple years later, Nusse and Varmus discovered a gene in mice where mutations caused breast cancer, which they named integration 1, or int1. It was later discovered that int1 was highly conserved across species, including *Drosophila*, and that it was part of the same family as Wg. Going forward, this family of genes was referred to as Wnt, a combination of Wg and int.

During development, as we have mentioned in class, neighboring cells need to communicate to each other for differentiation. Beyond that, they need to sense their environment; e.g., they need to make changes to gene expression levels depending on external morphogen concentrations. In order to accomplish this, the "signal" must cross the cell membrane.

The Wnt pathway, shown in Fig. 2 is one major signaling pathway for accomplishing this. The transmembrane proteins Frizzled and LRP (lipoprotein receptor-related protein) are Wnt's binding partners. When unbound to Wnt, these proteins do not interfere with the destruction cycle of β-catenin, an important transcription factor (more on β-catenin soon). At the center of this destruction cycle is a complex of axin and APC (commonly referred to as the axin complex), which recruit casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK-3), which phosphorylate β-catenin. The phosphorylated β-catenin is then targeted by β-TrCP, which promotes polyubiquitinization of the phosphorylated β-catenin, which is then degraded by the proteasome.

When Wnt is present outside of the cell membrane, it binds to Fizzled and LRP, bringing them together as a heterodimer. In this configuration, Frizzled mediates the phosphorlyation and activation of Dishevelled (DVL, a.k.a. Dsh), which then

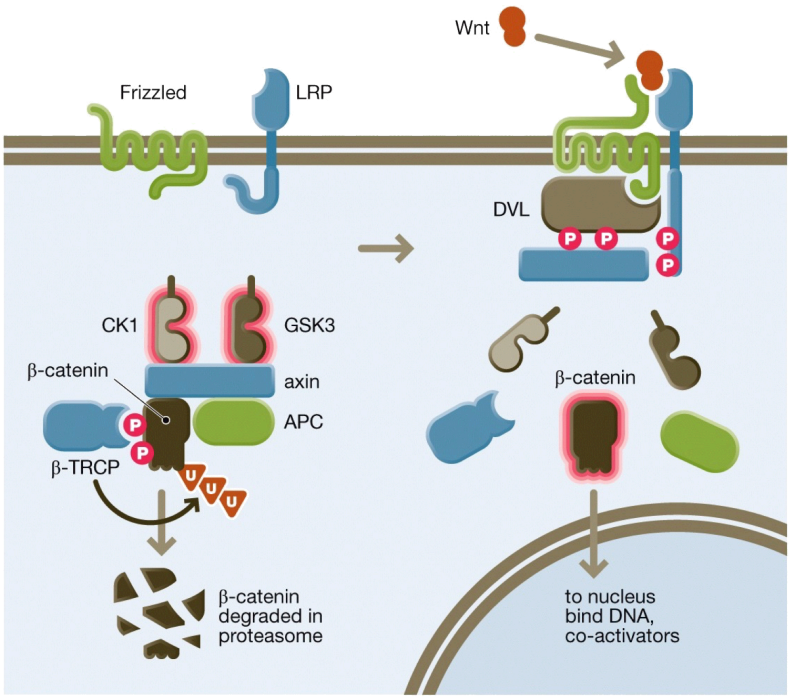

Figure 2: Schematic of the Wnt signaling pathway. Taken from Fig. 8.15 of Lim, Mayer, and Pawson, *Cell Signaling*, Garland Science, 2015.

has a strong affinity for axin. Furthermore, the tail of LRP is available for phosphorylation by CK1 and GSK-3. Thus, this activated Frizzled/LRP complex attracts the components of the degradation complex, thereby making them less available for degrading β-catenin. As a result, stable, unphosphorylated β-catenin can enter the nucleus. It then binds its coactivators, e.g., the transcription factor LEF1, and turns on expression of target genes. There are many Wnt-controlled target genes; c-Myc, a multifunctional regulator gene with roles in cellular transformation, is an example.

3.2 A more detailed look at Wnt signaling

In the Goentoro, et al. paper we are reading this week, we take a more detailed look of Wnt signaling beyond the cartoon in Fig. 2. The model is based on the work in Lee, et al. from the very first issue of *PLoS Biology*. Their schematic of Wnt signaling is shown in Fig. 3. They have labeled protein-protein interactions with arrows, each one identified with a number, with dashed arrows meaning interactions that are mediated through other proteins. Importantly, they have labeled subprocesses within this spaghetti-looking network to give it clarity. The destruction core cycle of β-catenin cycles along, provided the equilibrium described by reactions 4 and 5 is unperturbed. The presence of a Wnt molecule affects this equilibrium by activating

Dishevelled, which affects the reaction 4/5 equilbrium by breaking down the inactive APC/Axis, GSK-3 complex.

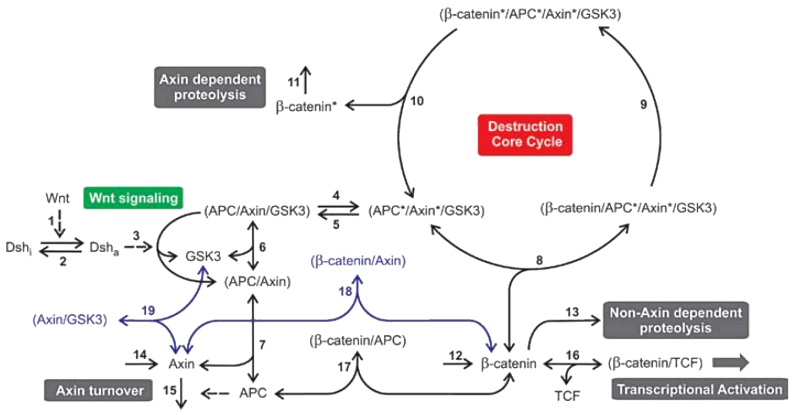

Figure 3: A more detailed model for Wnt signaling. Two headed arrows indicate reversible reactions and one-headed indicate irreversible reactions. Dashed arrows indicate reactions that have other mediators of the reactions. From Lee, et al., *PLoS Biology*, **1**, 116–132, 2003.

This is a complicated picture. Our goal is to mathematize this picture using the principle of mass action we talked about in the last lecture, get measured or estimated values for the parameters in the dynamical equations, and compute how changes in Wnt levels affect transcriptional activation.

3.3 Mathematizing the cartoon

As is often done in the study of signal transduction networks, **mass action kinetics** are used to model the dynamics. Recalling from last lecture, the rate of a chemical reaction is proportional to the product of the concentrations of the chemical species involved. The constant of proportionality is called the rate constant. Importantly, mass action kinetics do not consider individual reactant molecules, only concentrations of them. Bear in mind also when mass action is valid based on the assumptions we made when deriving it. When the number of reactants are small, or indeed their production is inherently stochastic, as in bursty gene expression, we should instead use stochastic simulation. Because we are not taking into account spatial arrangements of the molecules in our mass action treatment, are are implicitly making a **well-mixed assumption**, meaning that the concentrations are spatially homogeneous, or at least effectively so. Clearly, phosphorylated Dishevelled is not uniformly distributed in space, since it localizes to Frizzled/LRP on the membrane. Nonetheless, we assume that the dynamics of diffusion and spatial organization are fast compared to the chemical kinetics, so we neglect the spatial distribution of molecules. (In future studies, we will not neglect diffusion, to interesting consequences.)

Despite all of these caveats, mass action kinetics seem to be unreasonably effective at describing measured dynamics and making testable predictions. We will therefore employ them in mathematizing the cartoon of the Wnt signaling pathway.

Lee and coworkers write dynamical equations for the entirety of the cartoon, making simplifying assumptions along the way. For demonstration purposes, we will mathematize only the β-catenin destruction core cycle with β-catenin input and phosphorylated β-catenin output. I.e., will will disconnect it from the reversible phosphorylation of APC (reactions 4 and 5 in Fig. 3). Note that reactions 4 and 5 are obviously crucial for getting the full dynamics of Wnt signaling.

In writing the dynamical equations, we do as Lee, et al. and assign numbers for the complexes, since "(β-catenin*/APC*/Axin*/GSK3)" is a bit big for a subscript!

number	**species**
3	APC*/Axin*/GSK-3
8	β-catenin/APC*/Axin*/GSK-3
9	β-catenin*/APC*/Axin*/GSK-3
10	β-catenin*
11	β-catenin

Now we can write down the differential equations using mass action.

$$\frac{\mathrm{d}c_3}{\mathrm{d}t} = -k_8 c_3 c_{11} + k_{-8} c_8 + k_{10} c_9, \tag{3.1}$$

$$\frac{\mathrm{d}c_8}{\mathrm{d}t} = k_8 c_3 c_{11} - k_{-8} c_8 - k_9 c_8, \tag{3.2}$$

$$\frac{\mathrm{d}c_9}{\mathrm{d}t} = k_9 c_8 - k_{10} c_9, \tag{3.3}$$

$$\frac{\mathrm{d}c_{10}}{\mathrm{d}t} = k_{10} c_9 - k_{11} c_{10}, \tag{3.4}$$

$$\frac{\mathrm{d}c_{11}}{\mathrm{d}t} = k_{12} - k_8 c_3 c_{11} + k_{-8} c_8. \tag{3.5}$$

We see that

$$\frac{\mathrm{d}c_3}{\mathrm{d}t} + \frac{\mathrm{d}c_8}{\mathrm{d}t} + \frac{\mathrm{d}c_9}{\mathrm{d}t} = 0, \tag{3.6}$$

which implies that the quantity $c_3 + c_8 + c_9$ is conserved. This makes sense, since this is the total amount of APC/Axin/GSK-3 present. We will call this conserved quantity c_A.

3.3.1 The unique steady state

We can solve for the steady state of this system of ODEs by setting the time derivatives equal to zero and solving. We can subtract equation (3.1) from equation (3.5) and solve to get that $c_9 = k_{12}/k_{10}$ at steady state. Then, using equations (3.2) and (3.4), we get $c_8 = k_{12}/k_9$ and $c_{10} = k_{12}/k_{11}$ at steady state. We then find that at steady state

$$c_3 = c_A - c_8 - c_9 = c_A - \frac{k_{12}}{k_9} - \frac{k_{12}}{k_8}. \tag{3.7}$$

We finally can solve for c_{11} at steady state to get

$$c_{11} = \frac{k_{12}}{k_8}\left(1 - \frac{k_{-8}}{k_9}\right)\left(c_A - \frac{k_{12}}{k_9} - \frac{k_{12}}{k_8}\right)^{-1}. \tag{3.8}$$

So, we have found a unique steady state. That the steady state exists and is unique is a useful piece of information in and of itself. We have also found that the steady state values of all species depend on the production rate of β-catenin, k_{12}.

3.3.2 Numerical solution

A system of linear ODEs is easily solved numerically using `scipy.intergrate.odeint()`. In solving the ODEs, we take an initial condition of no β-catenin at all in the system, starting only with Axin complex. The total concentration of Axin complex is conserved, with a level of 50 nM, as given in the Lee, et al. paper. We take all other parameters as those reported in the paper as well. The two parameters that are not reported there are k_{-8} and k_{12}. (Actually, k_8 is not reported either, but $K_{d,8} = k_{-8}/k_8$ is reported.)

It is easiest to see the effects of varying k_{12} and k_{-8} using interactive plotting. To do this, I used the Python code below, which can be downloaded here. To get the interactive plot, input

```
bokeh serve betacat_destruction_cycle.py
```

at the command line. Then, open a web browser and go to the address

```
http://localhost:5006/betacat_destruction_cycle.
```

You will need to have a working Python 3 distribution with NumPy, SciPy, Pandas, and Bokeh (v. 0.12.3 or higher) installed.

A sample of the plot is shown in Fig. 4. In moving the sliders in the interactive plot, we see that k_{12} serves to set the scale of β-cat and β-cat* concentrations. Varying $k_{\text{-}8}$ sets the total amount of β-catenin. Interestingly, for these parameter values, the concentrations of all Axin-associated complexes is essentially constant. We could make this approximation in the dynamics and get simplified equations for the kinetics.

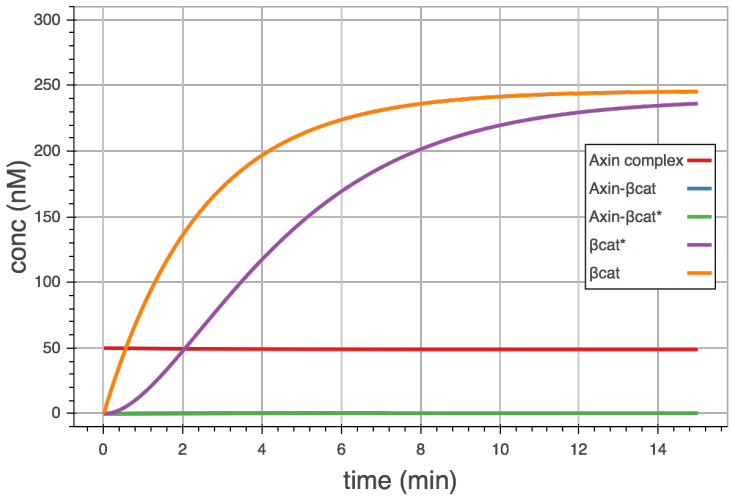

Figure 4: The dynamics of the major species in the β-catenin destruction cycle with all parameters as given in Lee, et al., and $k_{\text{-}8} = 1$ min^{-1} and $k_{12} = 100$ (nM-min)$^{-1}$.

```
"""
To do interactive plotting, run:

    bokeh serve betacat_destruction_cycle.py

on the command line.  Then, point a web browser to
http://localhost:5006/betacat_destruction_cycle.
"""

import numpy as np
import pandas as pd
import scipy.integrate

import bokeh.core.properties
import bokeh.io
import bokeh.models.widgets
import bokeh.palettes
import bokeh.plotting

def dcdt(c, t, k8, km8, k9, k10, k11, k12):
    """
```

```
    Time derivative of concentrations.
    c = (c3, c8, c9, c10, c11)
    """
    # Unpack concentrations and parameters
    c3, c8, c9, c10, c11 = c

    # Build derivatives
    deriv = np.empty(5)
    deriv[0] = -k8*c3*c11 + km8*c8 + k10*c9
    deriv[1] = k8*c3*c11 - (km8 + k9)*c8
    deriv[2] = k9*c8 - k10*c9
    deriv[3] = k10*c9 - k11*c10
    deriv[4] = -k8*c3*c11 + km8*c8 + k12

    return deriv

# Key for names
names = ['Axin complex', 'Axinβ-cat', 'Axinβ-cat*', 'βcat*', 'βcat']

# Specify colors
colors = ['#e41a1c','#377eb8','#4daf4a','#984ea3','#ff7f00']

# Define known parameters from Lee, et al, PLoS Biology, 2003
c_A = 50          # nM (given by fixed GSK-3 concentration)
k9 = 206          # 1/min
k10 = 206         # 1/min
k11 = 0.417       # 1/min
Kd8 = 120         # nM

# Unknown parameters
log10_km8 = 0     # log10(1/min)
k12 = 100         # nM/min

# k8 determined form Kd8 and km8
km8 = 10**log10_km8
k8 = km8 / Kd8    # 1/nM-min

# Initial conditions
c0 = np.array([c_A, 0, 0, 0, 0])

# Set up time points and solve
t = np.linspace(0, 15, 400)
c = scipy.integrate.odeint(dcdt, c0, t, args=(k8, km8, k9, k10, k11,
    k12))

# Store in a DataFrame for convenience in plotting
df = pd.DataFrame(data=c, columns=names)
df['t'] = t

# Data source
source = bokeh.models.ColumnDataSource(data=df)

```

```
# Set up the figure
p = bokeh.plotting.Figure(plot_width=650, plot_height=450,
                          x_axis_label='time (min)',
                          y_axis_label='conc (nM)',
                          y_range=[-10,310],
                          border_fill_alpha=0, background_fill_alpha
    =0)

# Add glyphs
for i, name in enumerate(names):
    p.line(x='t', y=name, source=source, line_width=3, color=colors[i
    ],
    legend=bokeh.core.properties.value(name))

# Place legend
p.legend.location = 'top_left'

# Set up widgets
k12_val = bokeh.models.Slider(title='k12 [1/nm-min]', value=100,
                              start=20, end=100)
log10_km8_val = bokeh.models.Slider(title='log10 km8 [log10(1/min)]',
     value=0,
                                    start=-2, end=4)

# Set up callbacks
def update_data(attrname, old, new):

    # Compute k8
    log10_km8 = log10_km8_val.value
    km8 = 10**log10_km8
    k8 = km8 / Kd8

    # Generate the new curve
    c = scipy.integrate.odeint(dcdt, c0, t,
                 args=(k8, km8, k9, k10, k11, k12_val.value))
    df = pd.DataFrame(data=c, columns=names)
    df['t'] = t

    # Re-source
    source.data = dict(df)

# Change values upon activating slider
for widget in [k12_val, log10_km8_val]:
    widget.on_change('value', update_data)

# Set up layouts and add to document
inputs = bokeh.models.layouts.WidgetBox(children=[k12_val,
    log10_km8_val])
bokeh.io.curdoc().add_root(bokeh.models.HBox(children=[inputs, p],
    width=800))
```

betacat_destruction_cycle.py

6 Reaction-diffusion based patterns

We have talked about the specifics of a Wnt and Delta-Notch signaling, and the paper we read this week deals with bone morphogenic protein (BMP) signaling. In order for biochemical signals to shape an organism, the signaling molecules themselves[4] need to be distributed in a spatially inhomogeneous way. It is not difficult to imagine 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**.

For this lecture, we will discuss **reaction-diffusion** mechanisms for generating spatial distributions of morphogens. This is important both practically, and historically. Before we proceed in this lecture, I will highlight a couple things we will not cover. First, we will more carefully derive the reaction-diffusion equations when we get to our lectures on continuum mechanics, so we will more or less state them without proof here. Second, we will focus on a specific type of pattern, called *Turing patterns*, that arise from reaction-diffusion mechanisms. We will not talk much about a scaled morphogen gradient that is the subject of the Ben-Zvi et al. paper, but the fundamental mechanism, simply having diffusing and reacting species, is the same.

6.1 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;
> (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."

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

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

6.2 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. Recall that the diffusion coefficient has dimension of L^2/T, or length squared over time. Its concentration, a function of position x and time t, is $c_i(x,t)$. Then, the **flux** of species i in the x-direction due to diffusion, j_i is given by Fick's First Law,

$$j_i = -D_i \frac{\partial c_i}{\partial x}. \tag{6.1}$$

In investigating this equation, we see that flux has units of number of particles per area per time, N/L^2T. So, a flux, sometimes referred to as a *current*, is the number of particles that pass through a unit cross sectional area per unit time.

As we will derive in our discussions on continuum mechanics, the rate of change of concentration per unit time due to diffusion is given by the derivative[5] of the flux, as given by Fick's Second Law.

$$\frac{\partial c_i}{\partial t} = D_i \frac{\partial^2 c_i}{\partial x^2}. \tag{6.2}$$

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.

Let $r_i(c_i)$ be the rate of production of species i by chemical reaction. Then, the rate of change of c_i due to chemical reaction is

$$\frac{\partial c_i}{\partial t} = r_i(c_i). \tag{6.3}$$

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

$$\frac{\partial c_i}{\partial t} = D_i \frac{\partial^2 c_i}{\partial x^2} + r_i(c_i). \tag{6.4}$$

[5] Actually, in 3D, the divergence.

This generalizes to two or three dimensions and multiple species.

$$\frac{\partial c_i}{\partial t} = D_i \nabla^2 c_i, + r_i(\mathbf{c}), \tag{6.5}$$

where

$$\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2}, \tag{6.6}$$

in three dimensional Cartesian coordinates, for example, and **c** is an array of the concentrations of all biochemical species.

6.3 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. In the most commonly used model, the reactions are simple.

1. 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

$$\frac{\partial c}{\partial t} = D \frac{\partial^2 c}{\partial x^2} - \alpha c + q_0 f(x). \tag{6.7}$$

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

$$\frac{\partial^2 c}{\partial x^2} - \frac{\alpha}{D} c = -\frac{q_0}{D} f(x). \tag{6.8}$$

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

$$\frac{\partial^2 c}{\partial x^2} - c = -\frac{q_0}{\alpha} f(\tilde{x}). \tag{6.9}$$

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

$$\hat{c}(k) = \frac{q_0}{\alpha} \frac{\hat{f}(k)}{1+k^2}. \tag{6.10}$$

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

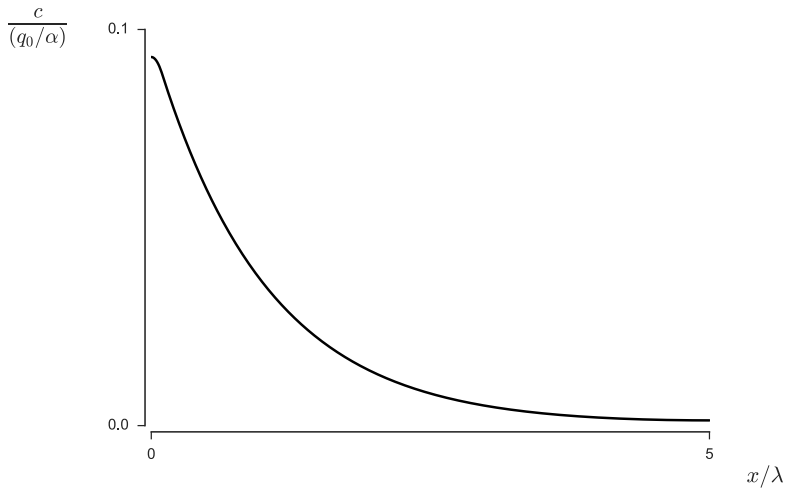

Figure 12: The gradient of Bcd. Note the style of the plot. This is useful for presentations. The Python code will help you generate such figures for your own talks.

```
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
    )
```

This method of modeling the source of Bcd is useful, but another commonly used method is to consider a constant flux of Bcd at the anterior. In this case, the dynamical equations for the profile are

$$\frac{\partial c}{\partial t} = D\frac{\partial^2 c}{\partial x^2} - \alpha c, \tag{6.11}$$

$$j(x=0) = -D\frac{\partial c}{\partial x} = j_0. \tag{6.12}$$

At steady state, we have

$$D\frac{\partial^2 c}{\partial x^2} - \alpha c = 0, \tag{6.13}$$

which has solution

$$c(x) = c_1 e^{-x/\lambda} + c_2 e^{x/\lambda}, \tag{6.14}$$

with again $\lambda = \sqrt{D/\alpha}$, and c_1 and c_2 being constants of integration. Since in a very large system the concentration must be bounded, $c_2 = 0$. We use the specified flux boundary condition to find c_1.

$$j_0 = -D \left.\frac{\partial c}{\partial x}\right|_{x=0} = \frac{c_1 D}{\lambda}, \tag{6.15}$$

which gives $c_1 = j_0/\sqrt{D\alpha}$. Thus,

$$c(x) = \frac{j_0}{\sqrt{D\alpha}} \, \mathrm{e}^{-x/\lambda}, \tag{6.16}$$

the same form as before.

6.4 Scaling of the Bcd gradient

Note that the reaction-diffusion mechanism we considered for the Bicoid gradient does not allow for **scaling**. A system exhibits scaling, or is scale invariant, if the pattern does not change if the overall system size changes. Think of it like this: imagine a two-dimensional square zebra and another one twice its size. If the mechanism that generates stripes exhibits scaling, these two zebras will have the same number of stripes. Similarly, flags scale; a tiny American flag has the same pattern as a giant one.

Mathematically, if we non-dimensionalized x by the total length of the system (organism), then the length would not appear at all in the system. Clearly this is not the case for the proposed model of Bicoid, since the natural length scale is λ. Indeed, defining $\tilde{x} = x/L$, we have

$$c(\tilde{x}) = \frac{j_0}{\sqrt{D\alpha}} \, \mathrm{e}^{-\tilde{x}L/\lambda}, \tag{6.17}$$

with L appearing explicitly in the concentration profile.

In the Ben-Zvi paper, the authors discuss a mechanism for scaling of a similar gradient, that of BMP in dorsal-ventral patterning in a *Xenopus* embryo. In your homework, you will explore other means of scaling.

6.5 Reaction-diffusion equations for multiple components

As mentioned before, 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

$$\frac{\partial c_i}{\partial t} = D_i \nabla^2 c_i, + r_i(\mathbf{c}). \tag{6.18}$$

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 often 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**.

6.6 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

$$\frac{\partial a}{\partial t} = D_a \frac{\partial^2 a}{\partial x^2} + r_a(a,s), \tag{6.19}$$

$$\frac{\partial h}{\partial t} = D_s \frac{\partial^2 s}{\partial x^2} + r_s(a,s), \tag{6.20}$$

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

$$r_a = \rho a^2 s - \alpha a \tag{6.21}$$

$$r_s = \beta - \rho a^2 s \tag{6.22}$$

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.

6.6.1 Nondimensionalization

In studying dynamical systems, it is almost always a good idea to **nondimensionalize** them. 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

$$\frac{\partial a}{\partial \tilde{t}} = d \frac{\partial^2 a}{\partial \tilde{x}^2} + \tau r_a(a,s), \tag{6.23}$$

$$\frac{\partial s}{\partial \tilde{t}} = \frac{\partial^2 s}{\partial \tilde{x}^2} + \tau r_s(a,s), \tag{6.24}$$

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

$$\frac{\partial a}{\partial t} = d\frac{\partial^2 a}{\partial x^2} + a^2 s - a \tag{6.25}$$

$$\frac{\partial h}{\partial t} = \frac{\partial^2 s}{\partial x^2} + \mu(1 - a^2 s), \tag{6.26}$$

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.[6] 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

$$\frac{\partial a}{\partial t} = d\frac{\partial^2 a}{\partial x^2} + r_a(a,s), \tag{6.27}$$

$$\frac{\partial s}{\partial t} = \frac{\partial^2 s}{\partial x^2} + r_s(a,s). \tag{6.28}$$

6.6.2 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.

[6] 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.

6.6.3 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 addressed using **linear stability analysis**.

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

$$\frac{\partial}{\partial t}(a_0 + \delta a) = d\,\frac{\partial^2}{\partial x^2}(a_0 + \delta a) + r_a(a_0, s_0) + r_{a,a}\,\delta a + r_{a,s}\,\delta s + \cdots, \tag{6.29}$$

$$\frac{\partial}{\partial t}(s_0 + \delta s) = \frac{\partial^2}{\partial x^2}(s_0 + \delta a) + r_s(a_0, s_0) + r_{s,a}\,\delta a + r_{s,s}\,\delta s + \cdots, \tag{6.30}$$

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

$$r_{a,s} = \left.\frac{\partial r_a}{\partial a}\right|_{a_0, s_0}, \tag{6.31}$$

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

$$\frac{\partial\,\delta a}{\partial t} = d\,\frac{\partial^2\,\delta a}{\partial x^2} + r_{a,a}\,\delta a + r_{a,s}\,\delta s, \tag{6.32}$$

$$\frac{\partial\,\delta s}{\partial t} = \frac{\partial^2\,\delta s}{\partial x^2} + r_{s,a}\,\delta a + r_{s,s}\,\delta s. \tag{6.33}$$

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

$$\frac{\mathrm{d}\,\delta a_k}{\mathrm{d}t} = -dk^2\,\delta a_k + r_{a,a}\,\delta a_k + r_{a,s}\,\delta s_k, \tag{6.34}$$

$$\frac{\mathrm{d}\,\delta s_k}{\mathrm{d}t} = -k^2 + r_{s,a}\,\delta a_k + r_{s,s}\,\delta s_k. \tag{6.35}$$

This can be written in matrix form as

$$\frac{\mathrm{d}}{\mathrm{d}t}\begin{pmatrix}\delta a_k \\ \delta s_k\end{pmatrix} = \mathsf{A}\cdot\begin{pmatrix}\delta a_k \\ \delta s_k\end{pmatrix}, \tag{6.36}$$

where

$$\mathsf{A} = \begin{pmatrix}-dk^2 + r_{a,a} & r_{a,s} \\ r_{s,a} & -k^2 + r_{s,s}\end{pmatrix} \tag{6.37}$$

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

$$\begin{pmatrix} \delta a_k \\ \delta s_k \end{pmatrix} = c_1 \mathbf{v}_1 e^{\sigma_1 t} + c_2 \mathbf{v}_2 e^{\sigma_2 t}, \tag{6.38}$$

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

$$\sigma = \frac{1}{2}\left(\text{tr A} \pm \sqrt{\text{tr}^2\text{A} - 4\text{det A}}\right). \tag{6.39}$$

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.

6.6.4 Consequences of linear stability analysis

We can write the trace and determinant explicitly.

$$\text{tr A} = -(1+d)k^2 + r_{a,a} + r_{s,s}, \tag{6.40}$$

$$\text{det A} = dk^4 - (r_{a,a} + dr_{s,s})k^2 + r_{a,a}r_{s,s} - r_{a,s}r_{s,a}. \tag{6.41}$$

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 want a Hopf bifurcation in development, 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

and quadratic in k^2, so it has a minimum when

$$\frac{\partial^2}{\partial k^2} \det \mathsf{A} = 2dk^2 - r_{a,a} - dr_{s,s} = 0. \tag{6.42}$$

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

$$k_0^2 = \frac{r_{a,a} + dr_{s,s}}{2d}. \tag{6.43}$$

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").

6.6.5 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

$$\mathsf{A} = \begin{pmatrix} 1 - dk^2 & 1 \\ -2\mu & -\mu - k^2 \end{pmatrix}. \tag{6.44}$$

The trace and determinant are

$$\operatorname{tr} \mathsf{A} = -(1+d)k^2 + 1 - \mu \tag{6.45}$$

$$\det \mathsf{A} = (dk^2 - 1)(\mu + k^2) + 2\mu = dk^4 - (1 - d\mu)k^2 + \mu. \tag{6.46}$$

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

$$k_0^2 = \frac{1 - d\mu}{2d}. \tag{6.47}$$

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.

6.6.6 Turing patterns do not scale

Turing patterns, such as those generated by the ASDM, do not scale because the wavelength of the pattern, given by the fastest growing mode, k, is independent of system size. So, if a system is twice as large, it would have twice as many peaks and valleys in the pattern.