# BE 159: Signal Transduction and Mechanics in Morphogenesis

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# 5 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.

## 5.1 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 hilights its importance in development. In their Nobel Prize-winning work published in 1980, Nüssleín-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 differentation. 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 $\beta$-catenin, an important transcription factor (more on $\beta$-catenin soon). At the center of this destruction cycle is a complex of axin and APC, which recruit casein kinase 1 (CK1) and glycogen synthase kinase 3 (GSK-3), which phosphorylate $\beta$-catenin. The phosphorylated $\beta$-catenin is then targeted by $\beta$-TrCP, which promotes polyubiquitinization of the phosphorylated $\beta$-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 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 compo-

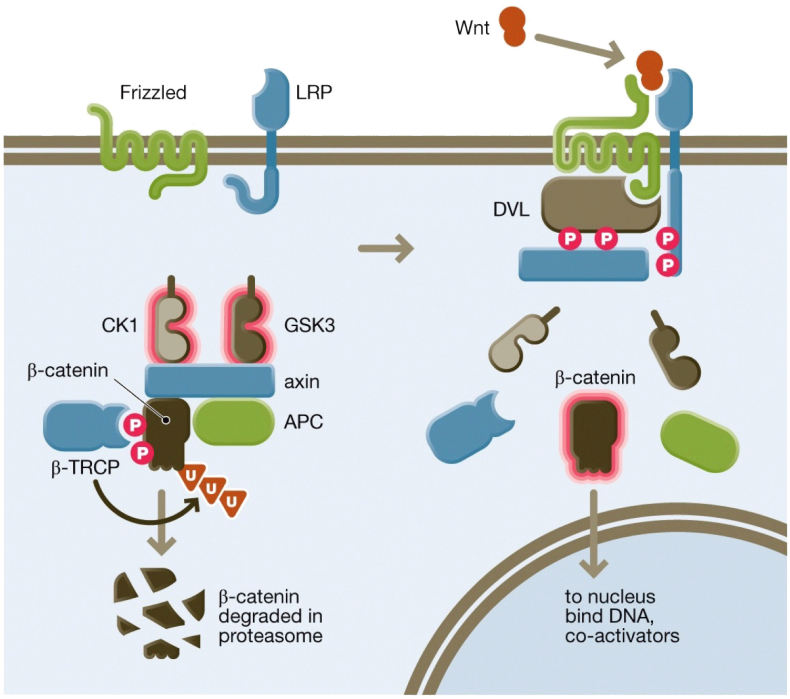


Figure 8.15 Cell Signaling (© Garland Science 2015)

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

nents of the degradation complex, thereby making them less available for degrading *beta*-catenin. As a result, stable, unphosphorylated $\beta$-catenin can enter the nucleus. It then binds its coactivators, e.g., the transcription factor LEF1, and turns on gene 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.

## 5.2 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 vert 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 $\beta$-catenin circles along, provided the equilibrium described by reactions 4 and 5 is unperturbed. The presense of a Wnt molecule affects this equilibrium by activating Dishevelled, which affects the reaction 4/5 equilbirium by breaking down the inactive APC/Axis, GSK-3 complex.

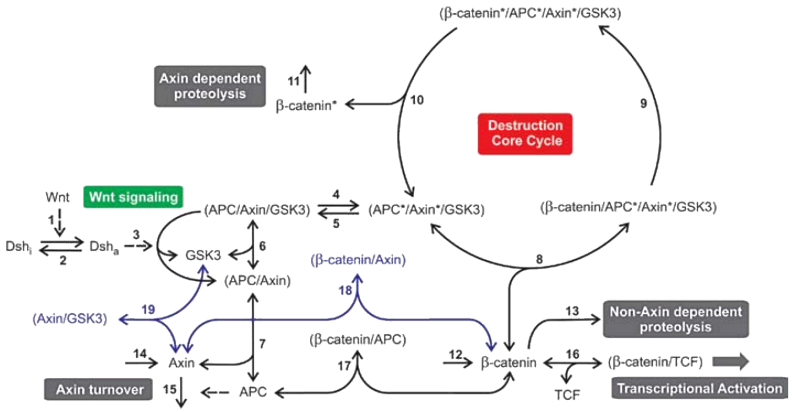


Figure 3: A more detailed model for Wnt signaling. Two headed arrows indicate reversible reactions and one-headed indicate irreversible reactions. Dashed arrows indicate reactions that have other mediators of the reactions. From Lee, et al., *PLoS Biology*, **1**, 116–132, 2003.

This is a complicated picture. Our goal is to mathematize this picture, get measured or estimated values for the parameters in the dynamical equations, and compute how changes in Wnt levels affect transcriptional activation.

## 5.3 Mathematizing the cartoon

As is often done in the study of signal transduction networks, **mass action kinetics** are used to model the dynamics. You may remember mass action kinetics from your general chemistry course. Specifically, 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. Furthermore, as was used in the Lee, et al. and Goentoro, et al. papers, we make 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 negelect the spatial distribution of molecules.

It is important to note that mass action kinetics are valid for dilute solutions with large numbers of reactants. 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.

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 $\beta$-catenin destruction core cycle with $\beta$-catenin input and phosphorylated $\beta$-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 "($\beta$-catenin$^*$/APC$^*$/Axin$^*$/GSK3)" is a bit big for a subscript!

| **number** | **species** |
|---|---|
| 3 | APC$^*$/Axin$^*$/GSK-3 |
| 8 | $\beta$-catenin/APC$^*$/Axin$^*$/GSK-3 |
| 9 | $\beta$-catenin$^*$/APC$^*$/Axin$^*$/GSK-3 |
| 10 | $\beta$-catenin$^*$ |
| 11 | $\beta$-catenin |

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

$$\frac{\mathrm{d}c_3}{\mathrm{d}t} = -k_8 c_3 c_{11} + k_{-8} c_8 + k_{10} c_9, \tag{5.1}$$

$$\frac{\mathrm{d}c_8}{\mathrm{d}t} = k_8 c_3 c_{11} - k_{-8} c_8 - k_9 c_8, \tag{5.2}$$

$$\frac{\mathrm{d}c_9}{\mathrm{d}t} = k_9 c_8 - k_{10} c_9, \tag{5.3}$$

$$\frac{\mathrm{d}c_{10}}{\mathrm{d}t} = k_{10} c_9 - k_{11} c_{10}, \tag{5.4}$$

$$\frac{\mathrm{d}c_{11}}{\mathrm{d}t} = k_{12} - k_8 c_3 c_{11} + k_{-8} c_8. \tag{5.5}$$

We see that

$$\frac{\mathrm{d}c_3}{\mathrm{d}t} + \frac{\mathrm{d}c_8}{\mathrm{d}t} + \frac{\mathrm{d}c_9}{\mathrm{d}t} = 0, \tag{5.6}$$

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 $c_A$.

### 5.3.1 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 (5.1) from equation (5.5) and solve to get that $c_9 = k_{12}/k_{10}$ at steady state. Then, using equations (5.2) and (5.4), 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

$$c_3 = c_A - c_8 - c_9 = c_A - \frac{k_{12}}{k_9} - \frac{k_{12}}{k_8}. \tag{5.7}$$

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

$$c_{11} = \frac{k_{12}}{k_8}\left(1 - \frac{k_{-8}}{k_9}\right)\left(c_A - \frac{k_{12}}{k_9} - \frac{k_{12}}{k_8}\right)^{-1}. \tag{5.8}$$

So, we have found a unique steady state. This 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 $\beta$-catenin, $k_{12}$.

### 5.3.2 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 $\beta$-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. 0.11 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 $\beta$-cat and $\beta$-cat$^*$ concentrations. Varying $k_{\text{-}8}$ sets the total amount of $\beta$-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.

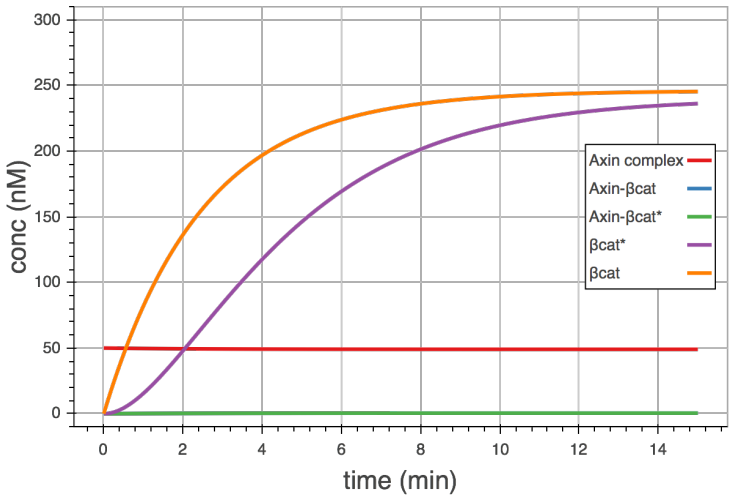


Figure 4: The dynamics of the major species in the $\beta$-catenin destruction cycle with all parameters as given in Lee, et al., and $k_{\text{-}8} = 1$ min$^{-1}$ and $k_{12} = 100$ (nM-min)$^{-1}$.

```
"""
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.io
import bokeh.models.widgets
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', 'Axin -cat', 'Axin -cat*', ' cat*', ' cat']

# 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('t', name, source=source, line_width=3, color=colors[i],
           legend=names[i])

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

# 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.VBoxForm(children=[k12_val, log10_km8_val])
bokeh.io.curdoc().add_root(bokeh.models.HBox(children=[inputs, p], width=800))
```

betacat_destruction_cycle.py