BIOREPS Problem Set #12
Signal Transduction via Protein Kinase

1 Background

Cells communicate with each other in numerous ways, harnessing electricity and calling upon hormones and neurotransmitters to send signals. Once these chemical messages are received, there is still a long way to go before they can be transformed into a reaction. Within the cell there lies a separate network of chemical modification pathways that allow the signals to propagate, amplify, and carry out the intended action within the cell. These modifications are myriad, and include methylation, acetylation, and our focus here, phosphorylation.

Phosphorylation is a common modification in signaling pathways, particularly the MAP kinase pathway, playing a role in a variety of pathways initiated by everything from growth factors to chemical indicators of stress. The process involves a kinase enzyme covalently modifying its substrate through addition of a phosphate group, usually from some nucleotide triphosphate such as ATP or GTP.

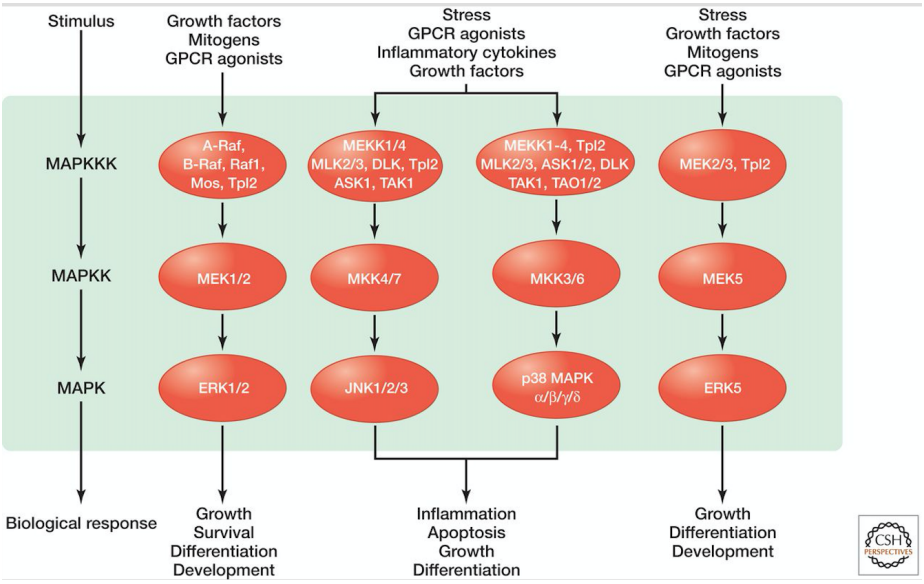

Figure 1: MAP kinase pathways. Image credit: http://cshperspectives.cshlp.org/content/4/11/a011254/F1.expansion.html

In the generic MAP kinase pathway, a stimulus activates some receptor protein, initiating a mechanism by which the first MAPK protein is phosphorylated. This kinase, in turn, phosphorylates the next MAP kinase protein in the pathway, and the chain of phosphorylation continues. Eventually the final MAPK protein phosphorylates another protein, activating or inactivating it to generate the appropriate biological response. Some pathways may include additional regulatory elements, including phosphatase enzymes, which can remove covalently bound phosphate groups. In this case, propagation of the signal depends upon the balance of addition and removal of phosphate groups on each subsequent kinase.

Enzyme-catalyzed reactions, such as the phosphorylation reactions occurring at each MAPK step, often follow similar kinetic schemes. Commonly found is Michaelis-Menten kinetics, in which the velocity of an enzymatic reaction varies hyperbolically with the concentration of the enzyme's ligand. The kinetics of signaling cascades, revealing the rates at which signals propagate (and how quickly one can expect to see a particular biological outcome) may be investigated by modeling each step in the pathway.

This problem set will explore the kinetics and propagation of a signal along its phosphorylation pathway, taking into account the possibilities of phosphorylation and dephosphorylation at each step.

2 Model

The focus of the analysis in this problem set will be an isolated 1D chain of protein kinases preceeded by a singal receptor. Stimulation of a receptor results in the activation of the kinases. Phosphorylation is the signal output of this system and can be terminated by phosphatases through dephosphorylation of the kinases [2]. Through the creation of a network and solving for the necessary conditions of the rates of phosphorylation and dephosphorylation, we can observe some of the dynames.

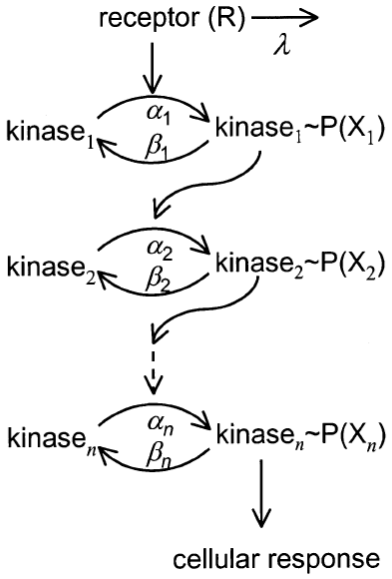

Figure 2: Model Network [2]

For this model, we describe the signaling cascade as a series of reactions between the phosphorylated form of a kinase, say kinase *i*-1, and the non-phosphorylated form of the next kinase, kinase *i*. If kinase *i*-1 does not experience phosphorylation, then the signal is terminated. All of these events are predicated on the stimulation of the original receptor. α_i and β_i are the rates of phosphorylation and dephosphorylation respectively. λ is the inverse characteristic time during which the receptor is activated. $X_i(t)$ and $\bar{X}_i(t)$ are the concentrations of phosphorylated and dephosphorylated kinases respectively.

Multiple assumptions must be made for this model to be practical. First, it will be assumed that the concentration of each form of kinase will be small compared to the total concentration of reaction partners. The total concentration of both forms of kinase must be held constant and this total concentration will be defined as $C_i = X_i + \bar{X}_i$ [2].

Finally, the concentration of all activated kinase *i* can be defined by the following differential equation.

$$\frac{dX_i}{dt} = \bar{\alpha}_i X_{i-1} \bar{X}_i - \beta_i X_i \qquad (i > 1) \tag{1}$$

The rate of phosphorylation is given by the difference of two quantities. The first is the product of the rate of phosphorylation by the *i*th kinase, the concentration of activated kinases in the *i*th position, and the concentration of inactivated *i* kinases. The second is the product of the rate of dephosphorylation by the *i*th phosphatase and the concentration of actived kinase form at the *i*th position.

References

[1] Day, E. K., Sosale, N. G., & Lazzara, M. J. Cell signaling regulation by protein phosphorylation: a multivariate, heterogeneous, and context-dependent process. *Current opinion in biotechnology*, **40**, 185-192 (2016).

[2] Heinrich, R., Neel, B. G., & Rapoport, T. A. Mathematical models of protein kinase signal transduction. *Molecular cell*, **9(5)**, 957-970 (2002).

[3] Morrison, D. K. MAP kinase pathways. *Cold Spring Harbor perspectives in biology*, **4(11)**, a011254 (2012).

3 Problems

Part A: Linear Signaling

i) Rewrite equation (1) in terms of α_i, β_i, C_i, X_{i-1}, and X_i. To do this, you will need to know that $\alpha_i = \bar{\alpha}_i C_i$. When i = 1, X_{i-1} is replaced by R(t) = $e^{-\lambda t}$ because it is preceeded by the receptor rather than another kinase.

ii) Now take advantage of our assumption that the concentration of each form of kinase is very small compared to the total concentration ($X_i << C_i$). Rewrite your answer from the previous question. Your answer should be the following.

$$\frac{dX_i}{dt} = \alpha_i X_{i-1} - \beta_i X_i \tag{2}$$

Part B: Weakly Activated Pathways

In this section, the conditions necessary for signal amplification and, therefore, signal propogation will be determined for this simple model from Part A.

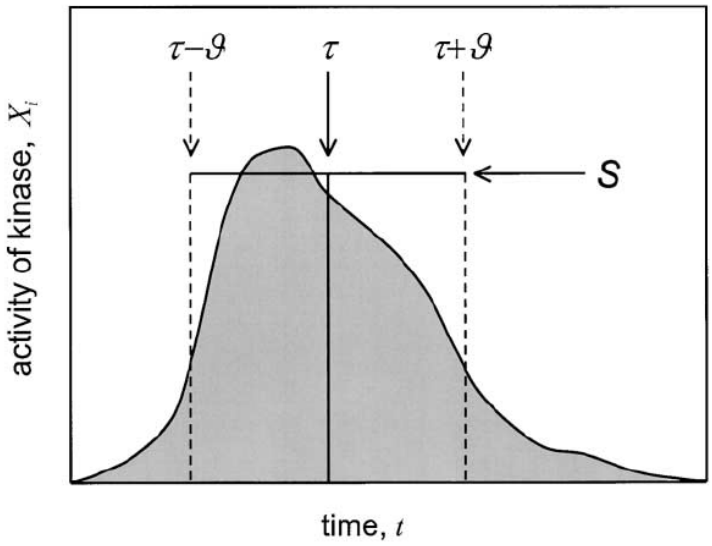

Figure 3: Depiction of a signal and qunatities needed to analyze signal propogation [2]

Before we proceed, some things shown in Figure 3 will have to be defined. First, we define the signaling time, τ_i, as the average time to activate kinase *i*. A signal can be imagined as depicted in Figure 3 below.

$$\begin{aligned} < \tau_i > &= \frac{T_i}{I_i} \\ T_i &= \int_0^\infty t X_i(t) dt \\ I_i &= \int_0^\infty X_i(t) dt \end{aligned} \tag{3}$$

T_i and I_i are the average time to activate the kinases and the total number of activated kinases up to the *i*th kinase respectively. The average time to activate one kinase is given by the ratio of the two quantities. The standard deviation of the mean activation time is given by Equation (4).

$$\begin{aligned} \theta_i &= \sqrt{< \tau_i^2 > - < \tau_i >^2} \\ < \tau_i^2 > &= \frac{\int_0^\infty t^2 X_i(t) dt}{I_i} \end{aligned} \tag{4}$$

Now it is apparent from Figure 3 that θ_i is one half the width of the signal. We can use this to define the signal amplitude, S.

$$S_i = \frac{I_i}{2\theta_i} \tag{5}$$

Now that the properties of a signal can be calculated, the goal is to solve for the conditions of the network that allow for signal propagation.

i) Start with Equation (2) and solve for X_1, X_2, and X_3. **Hint**: Mathematica is highly recommended (Specifically DSolve) and remember to substitute R(t) for X_{i-1} when i = 1.

ii) Calculate S_2 and S_3 by using Equations (3,4,5). **Hint**: Mathematica is highly recommended. If you used Mathematica or solved the differential equation three separate times correctly, just plug the solutions into the equations above and perform the calculations.

You should find that S_i has the following form (and that I_i and θ_i are the numerator and denominator respectively and A_o is a collection of a couple constants) from the pattern of S_2 and S_3.

$$S_n = \frac{A_o \prod_{k=1}^{n} \frac{\alpha_k}{\beta_k}}{\sqrt{1 + \lambda^2 \sum_{k=1}^{n} \beta_k^{-2}}} \tag{6}$$

iii) We want to solve for constraints on the model parameters α_i and β_i such that the signal amplitude remains the same or is amplified. To do this, subtract S_2 from S_3 and set this to be ≥ 0 and solve for β_3 in terms of α_3 and θ_2. **Hints**: The algebra can be a little taxing here. First, factor out as many things as you can and treat θ as a variable rather than plugging in its relation seen in Equation (6) to save some steps at first. Then solve for β_3 such that the amplitude will remain the same and plug in for θ_i and solve for the value of β_3 using Mathematica.

This generalizes for all β_i and the solution has the following form.

$$\beta_i \leq \alpha_i \sqrt{1 - \frac{1}{\alpha_i^2 \theta_{i-1}^2}} \tag{7}$$

If our network satisfies this criteria a signal will not be diminished through the chain.

Part C: Strongly Activated Pathways

Now we will consider pathways that are strongly activated. Pathways are considered strongly actived if kinases are essentially permanently in the phosphorylated state. Substantial stimulation of the receptor or $\alpha_i >> \beta_i$ can cause a pathway to be strongly activated. These are important to consider because the previous approximation characterized by $X_i << C_i$ can no longer be applied.

i) First, a permanently strongly activated system will be considered. Since kinases will be permanently activated, there will be no change in X_i. Go back to your solution to **Part A i)** and set $\frac{dX_i}{dt} = 0$ and solve for X_i algebraically.

ii) The solution to the previous part shows that amplification, in this case, occurs when $X_i > X_{i-1}$. Solve for the condition on X_{i-1} which allows for amplification. The solution should be of the form of Equation (8).

$$X_{i-1} < C_i(1 - \frac{\beta_i}{\alpha_i}) \tag{8}$$

iii) We want to find a general form of X_i that does not depend on the recursive relationship of X_i and X_{i-1} until we reach the receptor and, instead, depends on the rates α_i and β_i. Write out X_1 and X_2 and show that X_2 can be written in the form of Equation (9).

$$\frac{1}{X_i} = \sum_{j=1}^{i} \frac{1}{C_j} \prod_{k=j+1}^{i} \frac{\beta_k}{\alpha_k} + \frac{1}{R} \prod_{k=1}^{i} \frac{\beta_k}{\alpha_k} \tag{9}$$

As described above, Michaelis-Menten kinetics are often used in modeling enzyme-catalyzed reactions. The general form of a Michaelis-Menten modeled equation is of the form:

$$X_i = \frac{X_i^{max} R}{K_{M,i} + R} \tag{10}$$

Here, X_i^{max} is the maximum possible concentration of X_i and $K_{M,i}$ is the Michaelis-Menten, or half saturation, constant.

iv) Using $X_i^{max} = C_i$, show Equation 9 can be written in Michaelis-Menten form (Equation 10). **Hint:** Write out $\frac{1}{X_1}$ and $\frac{1}{X_2}$ from equation 9 and show that X_2 follows this relationship. Generalize this for all X_i. Use $K_{M,1} = C_1 \frac{\beta_1}{\alpha_1}$ and $K_{M,2} = C_2 \frac{\beta_2}{\alpha_2}(\frac{1}{C_1} + \frac{\beta_1}{\alpha_1})$.

For weakly activated pathways, we saw that we needed $\beta_i < \alpha_i$ (Equation 7) in order for gradual amplification of the signal to be observed. In contrast, we can see here that amplification depends heavily on R (the concentration of the receptor) instead. These models provide a starting point to continue to model more complex systems, including time-dependent signaling, cross-talk between different pathways, as well as their stability in biological systems.

v) Plot your solution to part i vs R where $\alpha_1 = 1$, $\beta_1 = .3$, and $C_i = 1$ for i = 1,2,3. Also, plot a line such that $X_i = x$. **Hints:** Since $\lambda = 0$, just use R as the x variable. This is also easy to do with a for loop in Mathematica.

Notice that longer chains reach the maximum for activated kinase concentrations at lower R. Longer chains require less stimuli because amplification can perpetuate the signal. Also, note that at $R \geq C_i(1 - \frac{\beta_i}{\alpha_i})$, signals begin to dampen. This is consistent with our findings in Equation (8).