10

Linear Pathways

10.1 Basic Properties

Linear pathways represent the simplest network motif and are a good starting point to begin to gain insight into how cellular networks operate. The simplest linear pathway is one where the kinetics are simple mass-action. Consider the following linear pathway:

$$X_o \rightarrow S_1 \rightarrow S_2 \rightarrow \ldots S_m \rightarrow X_1$$

This pathway has m floating species and n reactions ($n = m + 1$). X_o and X_1 are are fixed species representing the source and sink pools respectively. To make matters simpler, we can assume that each reaction obeys the following simple reversible mass-action kinetic law:

$$v_i = k_i S_{i-1} - k_{-i} S_i$$

Recall that the equilibrium constant for such as simple reaction is given by

$$K_{eq} = q = \frac{k_i}{k_{-i}} = \frac{S_i}{S_{i-1}}$$

which means we can replace the reverse rate constant and rewrite the rate law as

$$v_i = k_i \left(S_{i-1} - \frac{S_i}{q_i} \right)$$

This model is simple enough that we can derive the analytical equation for the steady state flux through the pathway. One way to do this is to first start with a two step pathway:

$$X_o \rightarrow S_1 \rightarrow X_1$$

where the rates for the two steps are given by:

$$v_1 = k_1 \left(X_0 - \frac{S_1}{q_1} \right) \qquad v_2 = k_2 \left(S_1 - \frac{X_1}{q_2} \right)$$

By setting $v_1 = v_2$ we can solve for the steady state concentration of S_1 and then insert this solution into one of the rate laws. This leads to the steady state flux:

$$J = \frac{X_o q_1 q_2 - X_1}{\frac{1}{k_2} q_1 q_2 + \frac{1}{k_1} q_2}$$

The same can be done for a three step pathway and by comparing the two solutions we can induce that the solution for a pathway of arbitrary length will be given by:

$$J = \frac{X_o \prod_{i=1}^{n} q_i - X_1}{\sum_{i=1}^{n} \frac{1}{k_i} \left(\prod_{j=i}^{n} q_j \right)}$$

For example if the pathway has four steps then the steady state flux is given by

$$J = \frac{X_o\, q_1 q_2 q_3 q_4 - X_1}{\frac{1}{k_1} q_1 q_2 q_3 q_4 + \frac{1}{k_2} q_2 q_3 q_4 + \frac{1}{k_3} q_3 q_4 + \frac{1}{k_4} q_4}$$

and so on. The first thing to note about the flux relationship is that the flux is a function of all kinetic and thermodynamic parameters. There is no single parameter that determines the flux completely. This means that a pathway with randomly assigned parameters is extremely unlikely to have the first step as the rate limiting step, that is a control coefficient of one.

From the flux expression we can also compute the corresponding flux control coefficients. For this we need to differentiate the flux equation with respect to an enzyme activity-like parameter. One way to do this is to add an e_i term to each rate law, such as:

$$v_i = e_i k_i \left(S_{i-1} - \frac{S_i}{q_i} \right)$$

We can eliminate the e_i terms afterwards by setting them to one. The result of this yields the following expression for the flux control coefficient of the ith step:

$$C_i^J = \frac{1/k_i \prod_{j=1}^{n} q_j}{\sum_{j=1}^{n} 1/k_j \prod_{k=j}^{n} q_k}$$

For a three step pathway the flux control coefficients for each step will be given by:

$$D = \frac{1}{k_1}q_1q_2q_3 + \frac{1}{k_2}q_2q_3 + \frac{1}{k_3}q_3$$

$$C_1^J = \frac{1}{k_1}q_1q_2q_3/D$$

$$C_2^J = \frac{1}{k_2}q_2q_3/D$$

$$C_3^J = \frac{1}{k_3}q_3/D$$

10.2 Irreversibility and Fast Reactions

From the flux control coefficient equation we can make some general statements. Let us assume for example that each equilibrium constant, q_i is greater than one, $q_i > 1$ and also that all forward rate constants are equal to each other and all reverse rate constants are equal to each other. This also means that all equilibrium constants are the same. If we now take the ratio of two adjacent steps, for example the i^{th} and $i + 1^{\text{th}}$ step, then we find:

$$\frac{C_i^J}{C_{i+1}^J} = \frac{1/k_i \prod_{j=i}^{n} q_j}{1/k_{i+1} \prod_{j=i+1}^{n} q_j} = \frac{k_{i+1}}{k_i}q_i = q$$

Given that $q > 1$, then $C_i^J > C_{i+1}^J$, that is earlier steps will have more flux control. This pattern applies across the entire pathway such that steps near the beginning of a pathway will have more control than steps near the end. We call this effect **front loading** of control and gives some credence to the traditional idea that the first or committed step is the most important step in a pathway. However, front loading only applies to unregulated pathways, the moment we add regulation to the pathway this picture changes. We will consider front loading again in a little but more detail later on.

Another way to look at a linear pathway is via the mass-action ratio:

$$\Gamma = \frac{S_2}{S_1}$$

where the species concentrations are measured at steady state. We define the disequilibrium ratio, ρ to be equal to:

$$\rho = \frac{\Gamma}{K_{eq}}$$

If a step is near equilibrium, then $\rho \simeq 1$ whereas if a step is far from equilibrium then $\rho \ll 1$.

Consider the following linear pathway, where X_o and X_1 are fixed species:

$$X_o \xrightarrow[v_1]{\varepsilon_1} S_1 \xrightarrow[v_2]{\varepsilon_2} S_2 \xrightarrow[v_3]{\varepsilon_3} S_3 \xrightarrow[v_4]{\varepsilon_4} X_4$$

The elasticities have been labeled 1 to 6, for example ε_1 represents $\varepsilon_{S_1}^{v_1}$, ε_2 represents $\varepsilon_{S_1}^{v_2}$ etc. If we give an arbitrary value of one to the first flux control coefficient for the linear pathway, then by considering the connectivity theorem for each metabolite, the ratios of all the flux control coefficients can be shown to be:

$$C_1^J : C_2^J : C_3^J : C_4^J = 1 : -\frac{\varepsilon_1}{\varepsilon_2} : -\frac{\varepsilon_1}{\varepsilon_2}\left(-\frac{\varepsilon_3}{\varepsilon_4}\right) : -\frac{\varepsilon_1}{\varepsilon_2}\left(-\frac{\varepsilon_3}{\varepsilon_4}\right)\left(-\frac{\varepsilon_5}{\varepsilon_6}\right)$$

or for a pathway of arbitrary length, the n^{th} term will equal:

$$\prod_{i=1}^{n-1}\left(-\frac{\varepsilon_i}{\varepsilon_{i+1}}\right)$$

If we assume that the enzymes are operating below saturation so that they are governed by the rate law, $v_i = \mathrm{Vm}_i/\mathrm{Km}_i(S_{i-1} - S_i/\mathrm{Keq\_i})$, then we can replace the substrate elasticities by $1/(1-\rho_i)$ and the product

elasticities by $-\rho_i/(1-\rho_i)$. If we do these substitutions, the ratios of flux control coefficients become:

$$C_1^J : C_2^J : C_3^J : C_4^J = \\ (1-\rho_1) : \rho_1(1-\rho_2) : \rho_1\rho_2(1-\rho_3) : \rho_1\rho_2\rho_3(1-\rho_4) \quad (10.1)$$

or for an arbitrary length pathway, the n^{th} term is equal to:

$$\left(\prod_{i=2}^{n-1} \rho_i\right)(1-p_n)$$

We can draw some interesting conclusions from this relation. Let us make one of the steps irreversible, say step i, so that the disequilibrium ratio for that step is zero, ($\rho_i = 0$), then we can see that since ρ_i appears as a multiplier in the ratio terms down-stream of the irreversible step, all the flux control coefficients for steps beyond will be zero. Thus steps beyond an irreversible reaction have no control over the flux. However, steps up-stream of the irreversible step may still have control. Therefore, provided the irreversible step is not the first step of the pathway, an irreversible step will not necessarily carry a control coefficient of one.

In a linear pathway governed by linear kinetics and without regulation, all steps downstream of an irreversible step have no flux control.

If any of the steps is near equilibrium then the disequilibrium ratio for that step will be nearly equal to one. i.e. for step i close to equilibrium, $\rho_i \approx 1$. Under these conditions, the term, $(1-\rho_i)$ will equal approximately zero and therefore the flux control coefficient for that step will also be near zero. In addition, steps other than step i, act as if step i is not part of the pathway and the pathway appears effectively shortened.

In a linear pathway governed by linear kinetics and without regulation, any step that is very close to equilibrium will have a control coefficient close to zero.

The relationship also supports the notion that in an unregulated pathway, flux control is biased towards the front of the pathway (front loaded). It is possible to show that the disequilibrium ratio, ρ is equal to the ratio of the reverse and forward rates for a given reaction:

$$\rho = \frac{v_r}{v_f}$$

Since the forward rate will always be greater than the reverse rate for a pathway showing a positive net rate, the disequilibrium ratio will always be less than one:

$$\rho \leq 1$$

Since ρ is always less than one, the tendency is for flux control to be higher near the front of the pathway since downstream steps have greater multiples of ρ values that are less than one.

10.3 Front Loading

In a previous section an effect called front loading was introduced. This is where in a linear pathway with linear reversible kinetics on each step, given two adjacent flux control coefficients, the upstream coefficient will always be equal or larger than the downstream coefficient, that is for the i^{th} step the following is true:

$$C_i^J \geq C_{i+1}^J$$

This means that in a linear pathway control will be concentrated upstream. To understand what this should be the case we must consider the elasticities and control equations for a linear pathway.

Using the flux summation and connectivity theorems it is straight forward to derive the flux control equations. For example for the three step pathway:

$$X_o \xrightarrow{v_1} S_1 \xrightarrow{v_2} S_2 \xrightarrow{v_3} X_1$$

one can derive the following flux control coefficient equations:

$$C^J_{E_1} = \varepsilon^2_1 \varepsilon^3_2 / D$$

$$C^J_{E_2} = -\varepsilon^1_1 \varepsilon^3_2 / D$$

$$C^J_{E_3} = \varepsilon^1_1 \varepsilon^2_2 / D$$

where D the denominator is given by:

$$D = \varepsilon^2_1 \varepsilon^3_2 - \varepsilon^1_1 \varepsilon^3_2 + \varepsilon^1_1 \varepsilon^2_2$$

It is possible to do this for pathways with additional steps from which a clear pattern emerges in the equations. For a pathway with n steps where n is even, we have the following equations:

$$C^J_1 = \varepsilon^2_1 \, \varepsilon^3_2 \, \varepsilon^4_3 \, \varepsilon^5_4 \cdots \varepsilon^{n+1}_n / D$$

$$\vdots$$

$$C^J_m = \prod_{k=m}^{n} \varepsilon^{k+1}_k \prod_{k=m-1}^{1} \varepsilon^k_k / D$$

$$\vdots$$

$$C^J_n = \varepsilon^1_1 \, \varepsilon^2_2 \, \varepsilon^3_3 \, \varepsilon^4_4 \cdots \varepsilon^{n+1}_{n+1} / D$$

If we look carefully at C^J_1 we see that the numerator is the product of all the substrate elasticities. This tells us that the perturbation 'hops' from one enzyme to the next until it reaches the end of the pathway. Conversely, the

control coefficient of the last enzyme includes all the product elasticities, that is the perturbation 'hops' from one enzyme to the next until it reaches the beginning of the pathway.

If we looked at any intermediate enzyme step we would find two groups of elasticities, one group representing the perturbation traveling downstream via the substrate elasticities and the other representing the perturbation traveling upstream via product elasticities.

We must now recall that given a reversible mass-action rate law, such as $k_1 S - k_2 P$, the elasticities are given by:

$$\varepsilon_S^v = \frac{1}{1-\rho}$$

$$\varepsilon_P^v = -\frac{\rho}{1-\rho}$$

From these equations it follows that $\varepsilon_S^v + \varepsilon_P^v = 1$, that is:

$$\| \varepsilon_S^v \| \geq \| \varepsilon_P^v \|$$

That is the absolute value of the substrate elasticity is always greater than the product elasticity. Given that an upstream enzyme will have more substrate elasticities than product elasticities, it follows that the numerator will be larger when compared to an enzyme further downstream which will have more of the small value product elasticities. The origins of the asymmetry between the substrate and product elasticities is a thermodynamic one. If the thermodynamic gradient were to be reversed so that the pathway flux travel 'upstream', the elasticity values exchange so that now the front loading occurs downstream, although ''downstream' is now 'upstream' because the flux has reversed.

In a linear pathway governed by linear kinetics and without regulation, flux control is biased towards the start of the pathway, an effect called **front loading**.

10.4 Optimal Allocation of Protein

Protein synthesis constitutes a significant drain on resources in a cell. For example, protein synthesis consumes approximately 7.5 ATP equivalents per peptide bond compared to one glucose molecule yielding roughly 36 molecules of ATP. If the average number of peptide bonds in a protein is 300, then it takes roughly 62 molecules of glucose to make just one protein molecule, not including the cost of the amino acids. In some cultured mammalian cells, protein synthesis consumes 35% to 50% of all ATP production. In addition to the energetic cost, proteins also occupy a significant proportion of cell volume at around 20 to 30% of the cell. This high level approaches the solubility limit of proteins and also limits the diffusion of other smaller molecules. These and other issues effectively put an upper limit on the total amount of protein in a cell. It would seem logical to assume that the distribution of a fixed amount of protein is not evenly distributed because some processes may requires higher levels of protein compared to others suggesting competition for protein between different processes. Such distributions are likely to be under evolutionary selection so that there exists an optimal allocation of the fixed amount of protein to all process in the cell. The optimal allocation is also likely to shift as environmental conditions change.

In this section we will consider what is the optimal allocation of a fixed amount of protein in a metabolic pathway such that the steady state pathway flux is maximized.

Let us consider a very simple two step metabolic scheme shown below:

$$X_o \rightarrow S_1 \rightarrow X_1$$

Assume that the first step is catalyzed by an enzyme E_1 and the second step by an enzyme E_2. Let us reduce the amount of enzyme E_1 by a small amount, δE_1, such that the pathway flux is reduced by an amount δJ. We can now increase the level of E_2 by δE_2 so that the pathway flux is returned to the original state. The net change in protein is therefore $\delta E_1 + \delta E_2$.

Let us also assume that the levels of E_1 and E_2 had previously being adjusted so that for a given flux, the total $E_1 + E_2$ was at a minimum, that

is the distribution of protein was optimal. In other words it would not be possible to reduce the total amount of protein and at the same time adjust the protein distribution such that the flux is unchanged. Then it must be true that:

$$\delta E_1 + \delta E_2 = 0$$

Given these changes in E_i and the fact that the flux does not change, we can write the following:

$$C^J_{E_1}\frac{\delta E_1}{E_1} + C^J_{E_2}\frac{\delta E_2}{E_2} = \frac{\delta J}{J} = 0$$

Submitting $\delta E_1 + \delta E_2 = 0$ into the above relation yields:

$$C^J_{E_1}\frac{1}{E_1} = C^J_{E_2}\frac{1}{E_2}$$

We can now invoke the flux summation theorem to eliminate one of the control coefficients to yield:

$$C^J_{E_1}\frac{1}{E_1} = \left(1 - C^J_{E_1}\right)\frac{1}{E_2}$$

Rearranging this to solve for $C^J_{E_1}$ yields:

$$C^J_{E_1} = \frac{E_1}{E_1 + E_2}$$

This result can be generalized to any length pathway so that for a given total amount of protein and a given flux, the optimal allocation of protein at a particular step, i, is given by:

$$C^J_{E_i} = \frac{E_i}{\sum E_i}$$

Exercises

1. Prove equation 10.1 in the main text.