Solution of Exercise 1:

1.1 *A change in production rate*. A gene Y with simple regulation is produced at a constant rate β_1. The production rate suddenly shifts to a different rate β_2.

(a) Calculate and plot Y(t).

(b) What is the response time (time to reach halfway between the steady-states)?

Solution:

(a): Lets mark the time when the shift occurs as $t = 0$. Before the shift, Y reaches steady state at a level $Y(t = 0) = Y_{St} = \beta 1 \,/\, \alpha$. After the shift,

$$\frac{dY}{dt} = \beta_2 - \alpha\, Y.$$

The solution of such an equation is generally $Y = \beta_2 \,/\, \alpha + C_1\, exp(-\alpha\, t)$, where the constant C_1 need to be determined so that $Y(t = 0) = \beta_1 \,/\, \alpha$. This yields the following solution

$$Y(t) = \frac{\beta_1}{\alpha}\, exp(-\alpha t) + \frac{\beta_2}{\alpha}\,(1 - exp(-\alpha t))$$

One can see that the initial condition β_1 /α decays exponentially at the same rate as the new steady state β_2 /α increases.

(b) To find the response time we have to solve the equation :

$$\frac{\beta_1}{\alpha}\, exp(-\alpha\, \tau_{1/2}) + \frac{\beta_2}{\alpha}\,(1 - exp(-\alpha\, \tau_{1/2})) = \frac{1}{2}\,(\frac{\beta_1}{\alpha} + \frac{\beta_2}{\alpha})$$

After some algebra the response time is found to be $log(2)/\,\alpha$.

1.2 *Cascades*. Consider a cascade of three activators, X → Y → Z. Protein X is initially present in the cell in its inactive from. The input signal of X, Sx, appears at time t = 0. As a result, X rapidly becomes active and binds the promoter of gene Y, so that protein Y starts to be produced at rate β. When Y levels exceed a threshold Ky, gene Z begins to be transcribed. All proteins have the same degradation/dilution rate α. What is the concentration of protein Z as a function of time? What is its response time with respect to the time of addition of Sx? What about a cascade of three repressors? Compare your solution to the experiments shown in Rosenfeld and Alon, 2003.

Solution:

We will assume all proteins have the same α. After induction, Y is produced at rate β_y and degraded/diluted at rate α:

$$\frac{dY}{dt} = \beta_y - \alpha Y$$

yielding the familiar exponential approach to steady-state:

$$Y(t) = \frac{\beta_y}{\alpha}\,(1 - exp(-\alpha t))$$

Assuming a step function for the activation of gene Z by Y (logic input function), transcription of gene Z starts at time τ_{yz} when $Y(\tau_{yz}) = K_y$:

$$Y(\tau_{yz}) = \frac{\beta_y}{\alpha}\left(1 - \exp(-\alpha\tau_{yz})\right) = K_y \Rightarrow \tau_{yz} = \frac{1}{\alpha}\log\left(\frac{Y_{st}}{Y_{st} - K_y}\right)$$

where $Y_{st} = \beta_y/\alpha$. Just for extra clarity, let's consider the limits of this equation to see if this makes sense. When $K_y \ll Y_{st}$, $Y_{st} - K_y \to Y_{st}$ and $\tau_{yz} \to 0$. In this case the threshold for Z activation is low, and Y levels cross it very fast. Conversely, if the activation threshold K_y is very high, approaching Y_{st}, Z is never activated because $Y_{st} - K_y \to 0$ and $\tau_{yz} \to \infty$.

Production of Z starts after time $t = \tau_{yz}$ at a constant rate of β_z:

$$\frac{dZ}{dt} = \begin{cases} 0 & t < \tau_{yz} \\ \beta_z - \alpha Z & t > \tau_{yz} \end{cases}$$

Solving we get

$$Z(t) = \begin{cases} 0 & t < \tau_{yz} \\ \frac{\beta_z}{\alpha}\left(1 - \exp\left(-\alpha(t - \tau_{yz})\right)\right) & t > \tau_{yz} \end{cases}$$

Solving for the response time, the time to reach half of the steady state of Z:

$$\frac{\beta_z}{\alpha}\left(1-\exp\left(-\alpha\left(t_{1/2}-\tau_{yz}\right)\right)\right)=\frac{1}{2}\frac{\beta_z}{\alpha}\Rightarrow t_{1/2}=\tau_{yz}+\log(2)/\alpha$$

Hence, there is an extra delay of τ_{yz} in the response time of gene Z relative to simple regulation with no cascade. If Z activates a third gene W when it crosses a threshold K_Z, this will occur at a time of τ_{zw} found from:

$$\frac{\beta_z}{\alpha}\left(1-e^{-a(\tau_{zw}-\tau_{yz})}\right)=Z_{st}\left(1-e^{-a(\tau_{zw}-\tau_{yz})}\right)=K_Z$$

solving for τ_{zw} we obtain:

$$\tau_{zw}=\tau_{yz}+\frac{1}{\alpha}\log\left(\frac{Z_{st}}{Z_{st}-K_z}\right)$$

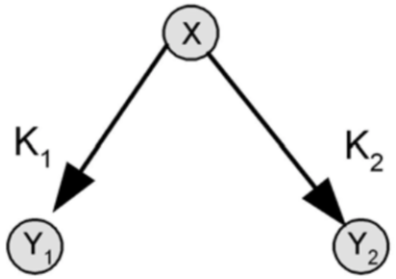
We can generalize this result: each step in a cascade, where a gene X activates a downstream gene after crossing a threshold K_X adds a delay of :

$$\tau_{delay}=\frac{1}{\alpha}\log\left(\frac{X_{st}}{X_{st}-K_x}\right)$$

In the special case in which the activation threshold is half the steady-state level (this can be shown to be in some cases an optimal value), the delay is $\tau_{delay}=log(2)/\alpha$. In summary, since $1/\alpha$ is often on the scale of a cell generation, a transcriptional cascade can be a slow process.

1.3 *Fan-out*: Transcription factor X regulates two genes Y_1 and Y_2. Draw the resulting network, termed a fan-out with two target genes. The activation thresholds for these genes are K_1 and K_2. The activator X begins to be produced at time t=0 at rate β, and is degraded/diluted at rate α, and its signal S_X is present throughout. What are the times at which Y_1 and Y_2 reach halfway to their maximal expression? Design a fan-out with three target genes in which the genes are activated with equal temporal spacing.

Solution:



Based on the previous problem:

$$\tau_1 = \frac{1}{\alpha}\log\left(\frac{X_{st}}{X_{st}-K_1}\right)$$

$$\tau_2 = \frac{1}{\alpha}\log\left(\frac{X_{st}}{X_{st}-K_2}\right)$$

After the corresponding delays in gene activation, denoted τ_1 and τ_2, production of Y_1 and Y_2 starts at a constant rate reaching half the steady state after $log(2)/\alpha$. The time to reach half maximum is therefore: $\tau_{1/2} = t_i + log(2)/\alpha$ (i=1,2), where i=1,2 for Y_1 and Y_2 respectively.

For three target genes, we require $\tau_2 - \tau_1 = \tau_3 - \tau_2$, or $\tau_2 = \frac{1}{2}(\tau_1 + \tau_3)$. This amounts to the following requirements on the thresholds,

$$\frac{1}{\alpha}\log\left(\frac{X_{st}}{X_{st}-K_2}\right) = \frac{1}{2}\left(\frac{1}{\alpha}\log\left(\frac{X_{st}}{X_{st}-K_1}\right) + \frac{1}{\alpha}\log\left(\frac{X_{st}}{X_{st}-K_3}\right)\right) \Rightarrow$$

$$X_{st} - K_2 = \sqrt{(X_{st}-K_1)(X_{st}-K_3)}$$

namely that the difference between X_{st} and the thresholds are arranged according to a geometric mean.

1.4. *Positive feedback*. What is the effect of positive auto-regulation on the response time? Use as a model the following linear equation:

$$dX/dt = \beta + \beta_1 X - \alpha X$$

Explain each term and solve for the response time. When might such a design be biologically useful?

Solution: The basal production rate is β, the positive effect of X on its own production (positive auto-regulation - PAR) is described in this model by the linear term $\beta_1 X$, and degradation/dilution is represented as usual by $-\alpha X$. Let's group the terms that multiply X in this linear model:

$$\frac{dX}{dt} = \beta - (\alpha - \beta_1) X$$

We see that the degradation/dilution rate is effectively reduced by positive auto-regulation, to an effective rate $\alpha' = \alpha - \beta_1$. Assuming that the auto-regulation is not too strong, that is that $\beta_1 < \alpha$, the term multiplying X is negative and we get an approach to a stable steady-state:

$$X(t) = X_{st} (1 - exp(-\alpha' t)).$$

Where $\alpha' = \alpha - \beta_1$. The response time is defined as the time to reach half of the steady state: $T_{1/2}(PAR) = log(2)/\alpha'$. The response time is longer than that for simple regulation due to the reduced effective degradation/dilution rate

$$T_{1/2}(PAR) = log(2)/(\alpha - \beta_1) > log(2)/\alpha = T_{1/2}(simple).$$

Thus positive auto-regulation has an effect that is opposite to that of negative auto-regulation. The former slows response time, whereas the latter speeds response times. Note that strong auto-regulation, in which $\beta_1 > \alpha$, can lead to instability and unchecked growth of X in the model. In real systems, this instability will be limited by other factors (such as saturation of the input function), locking Y in an ON state of high expression even after its activating input β vanishes. Hence, strong positive feedback creates a bi-stable system, in which X is either at a low or at a high fixed point. This is useful for commitment-type biological decisions, such as those made in development. Positive feedback characterizes developmental systems that make a switch that is either OFF or is locked ON (e.g., a cell commits to become a muscle cell rather than, say , a blood cell, by means of positive feedback loops on key transcription factors).

A different biological example is found in some regulatory systems that govern the transcription of protein parts of multi-protein structures that are assembled slowly. An example is the bacterial flagellum described in Chapter 6 that can take two cell generations to be completed. Such slow processes can benefit from weak positive auto-regulation to slow down responses and prolong delays(Kalir et al., 2005)