Exercises, chapter 2

2.1 *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): Let's 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,

(2.1.1) $dY/dt= \beta_2 - \alpha Y$.

The solution of such an equation is generally $Y = C_1 + C_2 \exp(-\alpha t)$, where the constants C_1 and C_2 need to be determined so that $Y(t=0)= \beta_1 / \alpha$, and Y at long times reaches its new steady state β_2 / α. This yields the following sum of an exponential and a constant

(2.1.2) $Y(t) = \beta_1 / \alpha + (\beta_2 / \alpha - \beta_1 / \alpha)(1-e^{-\alpha t}) = \beta_2 / \alpha + (\beta_1 / \alpha - \beta_2 / \alpha) e^{-\alpha t}$

Take the derivative with respect to time, dY/dt, and verify that Eq. 2.1.1 is fulfilled.
(b) The response time, which is the time to reach half way between the two steady states, is $\log(2)/\alpha$.

2.2 *mRNA dynamics*. In the main text, we considered the activation of transcription of a gene (mRNA production), and used a dynamical equation to describe the changes in the concentration of the gene product, the concentration of protein Y: $dY/dt= \beta - \alpha Y$, in which β describes the rate of protein production. In reality, mRNA needs to be translated to form the protein, and mRNA itself is also degraded by specific enzymes.
(a) Derive dynamical equations for the rate of change of mRNA and the rate of change of the protein product, assuming that mRNA is produced at rate β_m and degraded at rate α_m, and that each mRNA produces on average p protein molecules per second. The protein product is degraded/diluted at rate α.
(b) Note that mRNA is often degraded much faster than the protein product $\alpha_m >> \alpha$ (in bacteria, mRNA lifetime is usually on the order of minutes (Bernstein et al., 2002) whereas most proteins are stable for hours). Can this be used to form a quasi-steady-state assumption that mRNA levels are at steady-state with respect to slower processes? What is the effective protein production rate β in terms of β_m, α_m and p?

What would be the response time if the mRNA lifetime were much longer than the protein lifetime?

Solution:

(a) The dynamic equation for the concentration of mRNA of gene Y, Y_m, is

(2.2.1) $dY_m / dt = \beta_m - \alpha_m Y_m$.

The dynamical equation for the protein product is due to production of p copies per mRNA and degradation/dilution at rate α:

(2.2.2) $dY / dt = p Y_m - \alpha Y$

(b) In the typical case that mRNA degradation is faster than the degradation/dilution of the protein product, we can assume that Y_m reaches steady-state quickly in comparison to the protein levels. The reason is that the typical time for the mRNA to reach steady state is the response time $\log(2)/ \alpha_m$, which is much shorter than the protein response time $\log(2)/ \alpha$ because $\alpha_m >> \alpha$. The steady-state mRNA level is found by setting $dY_m / dt=0$ in Eq. 2.2.1 , yielding

(2.2.3) $Y_{m, st} = \beta_m / \alpha_m$

Using this for Y_m in Eq 2.2.2 yields the following equation for the protein production rate

(2.2.4) $dY / dt = p \beta_m / \alpha_m - \alpha Y$

In other words, the effective protein production rate, which is the first term on the right hand side of the equation, is equal to the steady state mRNA level times the number of proteins translated from each mRNA

(2.2.5) $\beta = p \beta_m / \alpha_m$

In cases where $\alpha_m << \alpha$, that is when mRNA is much more stable than the protein, the response time is governed by the slower process, accumulation of mRNA. For each level of mRNA, $Y_m(t)$, protein level 'instantly' reaches its momentary steady state $pY_m(t)/\alpha$. In such a case the response time is $\log(2)/\alpha_m$ according to the same reasoning as in the text for the protein response time. For advanced students, a full solution of the dynamics is given at the end (not necessary for the solution required in this exercise).

2.3 *Time-dependent production and decay.* A gene Y with simple regulation has a time-dependent production rate $\beta(t)$ and a time-dependent degradation rate $\alpha(t)$. Solve for its concentration as a function of time.

Solution:

Verify by taking the time derivative that the following is correct:

(2.3.1) $$Y(t) = e^{-\int \alpha(t')dt'}[Y(0) + \int \beta(t')e^{\int \alpha(t'')dt''}dt']$$

where all integrals are between 0 and t.

For example, for the case of problem 2.1, $\alpha(t)$ is constant over time so that $exp(-\int \alpha(t')\,dt') = \exp(-\alpha t)$, and $\beta(t')$ is constant after t=0 and equal to β_2 so that:

$$\int \beta(t')e^{\int \alpha(t'')dt''}dt' = \int \beta_2 e^{\alpha t'}dt' = \frac{\beta_2}{\alpha}(e^{\alpha t} - 1) ,$$

and we obtain the desired result $Y(t)=\beta_2 / \alpha + (\beta_1 / \alpha - \beta_2 / \alpha) \exp(-\alpha t)$.

2.4 *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, S_x, 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 K_y, gene Z begins to be transcribed. What is the concentration of gene product Z as a function of time? What is its response-time with respect to addition of S_x? What about a cascade of three repressors? Compare your solution to the experiments shown in Fig 2.7.

Solution:

We will assume all proteins have the same dilution/degredation rate α. After induction, Y is produced at rate

β_y and degraded/diluted at rate α:

(2.4.1) $$dY/dt=\beta_y-\alpha Y$$

yielding the familiar exponential approach to steady-state:

$Y(t)=\beta_y/\alpha\,(1-\exp(-\alpha t))$

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 $Y(\tau_{yz})=K_y$:

(2.4.2) $$Y(\tau_{yz})=\beta_y/\alpha\,(1-\exp(-\alpha\tau_{yz}))=K_y \Longrightarrow \tau_{yz}=1/\alpha \log(Y_{st}/(Y_{st}-K_y))$$

where $Y_{st}=\beta_y/\alpha$. Just for extra clarity, let's consider the limits of (2.4.2) to see if this makes sense. When $K_y<<Y_{st}$, $Y_{st}-K_y \longrightarrow Y_{st}$ and $\tau_{yz} \longrightarrow 0$. 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 $Y_{st}-K_y \longrightarrow 0$ and $\tau_{yz} \longrightarrow \infty$.

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

(2.4.3) $dZ/dt = 0 \quad (t<\tau_{yz})$

$\beta_z - \alpha Z \quad (t>\tau_{yz})$

Solving this we get:

$Z(t) = 0 \quad (t<\tau_{yz})$

$\beta_z/\alpha\,(1-\exp(-\alpha(t-\tau_{yz}))) \quad (t>\tau_{yz})$

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

(2.4.4) $\beta_z/\alpha\,(1-\exp(-\alpha(t_{1/2}-\tau_{yz}))) = 1/2\,\beta_z/\alpha \Longrightarrow$

$t_{1/2}=\tau_{yz}+\log(2)/\alpha$

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 τ_{zw} found from:

(2.4.5) $\beta_z/\alpha\,(1-\exp(-\alpha(\tau_{zw}-\tau_{yz})))= Z_{st}\,(1-\exp(-\alpha(\tau_{zw}-\tau_{yz}))) = K_z$

solving for τ_{zw} we obtain:

(2.4.6) $t_{zw}=t_{yz}+1/\alpha\,\log(Z_{st}/(Z_{st}-K_z))$

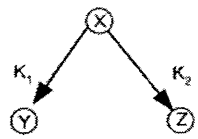
We can generalize this result: each step in a cascade, where a gene X activates a downstream gene after crossing a threshold K_x adds a delay of :

(2.4.7) $\tau_{delay}=1/\alpha\,\log(X_{st}/(X_{st}-K_x))$

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.

2.5 *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.



Based on problem 2.4:

(2.5.1) $\tau_1=1/\alpha \log(X_{st}/(X_{st}-K_1))$, $\tau_2=1/\alpha \log(X_{st}/(X_{st}-K_2))$

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: $t_{1/2}=\tau_i+\log(2)/\alpha$ (i=1,2), where i=1,2 for Y_1 and Y_2 respectively.

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

(2.5.2) $1/\alpha \log(X_{st}/(X_{st}-K_2))=1/2\ (1/\alpha \log(X_{st}/(X_{st}-K_1))+ 1/\alpha \log(X_{st}/(X_{st}-K_3)))$

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

2.6 Pulse of activation: Consider the cascade of exercise 2.4. The input signal S_x appears at time t=0 for a pulse of duration D, and then vanishes.

(a) What is the concentration Y(t)?

(b) What is the minimal pulse duration needed for activation of gene Z?

(c) Plot the maximal level reached by the gene product Z as a function of the pulse duration D.

Solution:

a) Protein X*, the active conformation of protein X bound to its inducer, binds the promoter of Y commencing its production according to (2.4.1) yielding the familiar exponential approach to steady-state:

(2.6.1) $Y(t)=\beta_y/\alpha\,(1-\exp(-\alpha t))$

b) Protein Z will be activated when Y levels cross the threshold K_{YZ}:

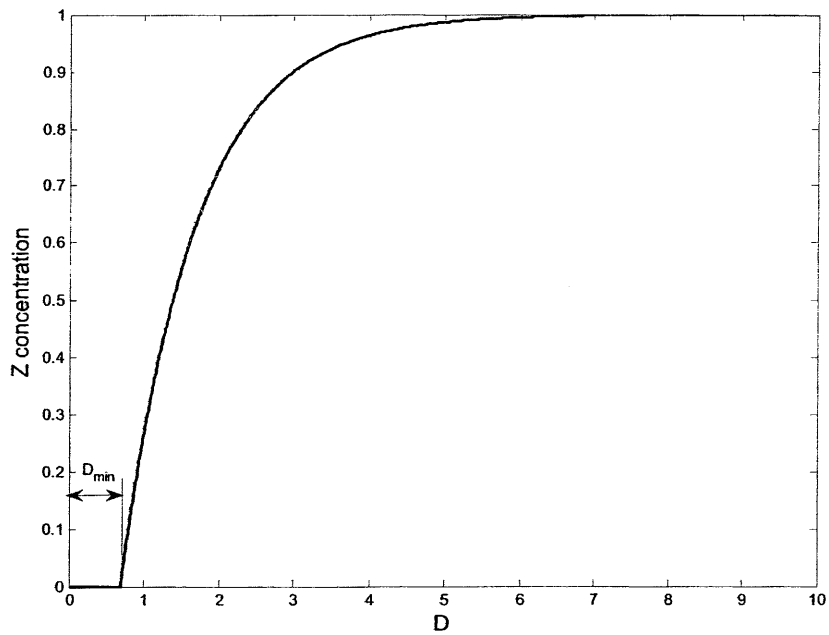
(2.6.2) $Y(t)=\beta_y/\alpha\,(1-\exp(-\alpha D_{min}))= Y_{st}\,(1-\exp(-\alpha D_{min}))=K_{YZ}$

Solving for D:

(2.6.3) $D_{min}=1/\alpha\,\log\,(1/(1-K_{YZ}/Y_{st}))$

Let's consider the limits of (2.6.3). If K_{YZ} is very small D approaches 0, and a very short duration of the input S_x suffices to activate Z. When K_{YZ} approaches Y_{st} D approaches infinity.

c)



The schematic plot uses $K_{YZ}=0.5*Y_{st}$, and $\alpha=1$. After the delay of D_{min} Z concentration follows the familiar exponential rise to steady state. In summary, the 3-gene cascade results in a delay in the activation of the Z gene following an input to the X gene.

Appendix

Full solution of the dynamics of problem 2.2

The full solution of this problem can be obtained by inserting the time-dependent solution of equations 2.2.1 into equation 2.2.2 to obtain:

(2.A.1) $dY/dt=p\ Y_m-\alpha Y=p\beta_m/\alpha_m\ (1-\exp(-\alpha_m t))-\alpha Y$

Using the method of problem 2.3 (below) the full solution for Y(t) is:

(2.A.2) $$Y(t)=\frac{p\beta_m}{\alpha_m(\alpha-\alpha_m)}(1-\exp(-\alpha_m t))-\frac{p\beta_m}{\alpha(\alpha-\alpha_m)}(1-\exp(-\alpha t))$$

In the limit where mRNA degradation is much higher than protein degradation and for $1/\alpha_m<<t$, we can approximate $(\alpha-\alpha_m)\sim-\alpha_m$ and $\exp(-\alpha_m t)\sim 0$. Equation (2.A.2) then reduces to:

(2.A.3) $$Y(t)=\frac{p\beta_m}{\alpha\,\alpha_m}(1-\exp(-\alpha t))-\frac{p\beta_m}{\alpha_m^2}\sim\frac{p\beta_m}{\alpha\,\alpha_m}(1-\exp(-\alpha t))$$

In the limit where mRNA degradation is much smaller than protein degradation and for $1/\alpha<<t$, we can approximate $(\alpha-\alpha_m)\sim\alpha$ and $\exp(-\alpha t)\sim 0$. Equation (2.A.2) then reduces to:

(2.A.4) $$Y(t)=\frac{p\beta_m}{\alpha_m\alpha}(1-\exp(-\alpha_m t))-\frac{p\beta_m}{\alpha^2}\sim\frac{p\beta_m}{\alpha_m\alpha}(1-\exp(-\alpha_m t))$$

The steady state solution for Y is the same in both of these limiting cases : $Y_{st}=\ p\ \beta_m\ /\ \alpha\alpha_m$, but the response time is governed by the slower process of the two degradation processes – if mRNA degradation is much higher than protein degradation, the response time is governed by protein degradation: $t_{1/2}=\log(2)/\alpha$. If mRNA degradation is much smaller than protein degradation, the response time is governed by mRNA degradation $t_{1/2}=\log(2)/\alpha_m$.

Problem 2.3 – integration factors

A general solution of a first order linear differential equation of the following form:

(2.A.5) $dY/dt+\alpha(t)Y=\beta(t)$

can be obtained by first multiplying both sides by an integration factor $\mu(t)$ defined as:

(2.A.6) $\mu(t)=exp(\int\alpha(t')dt')$

This gives us:

(2.A.7) $\mu(dY/dt)+\alpha\mu Y=\beta\mu$

The left side can be replaced by:

(2.A.8) $d(\mu Y)/dt=\beta\mu$

Integrating this equation:

(2.A.9) $\mu Y = \int \beta \mu dt$

which leads to

(2.A.10) $Y(t) = 1/\mu \, [Y(0) + \int \beta \mu dt \,]$

Finally, inserting μ from 2.A.6 we get equation 2.3.1

(2.A.11) $Y(t) = exp(-\int \alpha(t')dt') \, [Y(0) + \int \beta(t') \, exp(\int \alpha(t'')dt'') \, dt' \,]$