Lecture Notes in Systems Biology

Lecture 2 - Autoregulation and Negative Feedback

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1 Motivation

Autoregulation, especially negative autoregulation, is a common regulatory motif in the transcriptional network of E. coli. In the analysis of the transcriptional network, which had 424 nodes (operons) connected by 519 edges (transcriptional interactions), it appeared 40 times - much more than the 1-2 expected in the null model! Amongst these, 32 were negative autoregulation.

While we don't know the evolutionary pressures that shaped this network, it is unlikely that these interactions would be overrepresented to such a degree if there was no selective advantage. Promoter specificity can be easily adjusted by evolutionary forces, so we might hypothesize that autoregulation provides some benefit to the organism.

What could be the evolutionary advantage of this motif? To start addressing this question, we will consider the dynamics of protein abundance during bacterial growth.

2 Dynamics of protein abundance during steady growth

Let's consider a population of bacteria of size N growing at some rate γ:

$$\dot{N} = \gamma N \tag{1}$$

The population of bacteria grows as:

$$N(t) = N(0)e^{\gamma t} \tag{2}$$

And let's consider some protein of interest X, with an production rate α (unit - proteins):

$$\dot{X} = \alpha N \tag{3}$$

We will assume that X is stable and that it is not actively degraded by the bacteria. Then dynamics of the concentration of the protein $x = \frac{X}{N}$ is given by:

$$\frac{dx}{dt} = \frac{dX}{dt}\frac{1}{N} - \frac{X}{N^2}\frac{dN}{dt} = \alpha - \gamma\frac{X}{N} = \alpha - \gamma x \tag{4}$$

The dynamics of Eq. 7 can be solved:

$$x(t) = \frac{\alpha}{\gamma} + \left(x(0) - \frac{\alpha}{\gamma}\right)e^{-\gamma t} \tag{5}$$

We can see that **the initial conditions decay at rate $e^{-\gamma t}$, which corresponds to the growth rate of the bacteria**. Initial conditions will decay to half of their original level at time $t = \gamma^{-1}\log 2$. Thus, the inverse of the growth rate, γ^{-1}, provides the timescale for the convergence to the steady-state given by $x = \frac{\alpha}{\gamma}$. In bacteria such as E. coli, the doubling time is around an hour.

What happens if the bacteria need to respond and adjust their gene expression faster than this?

3 Negative autoregulation speeds up responses

The presence of transcriptional regulation adjusts α so that now is a function of x, that is, $\alpha = f(x)$. What would be a favorable $f(x)$? Intuitively, it is clear that as the removal rate is fixed, and for a fixed steady-state abundance, the response rate can be improved by having a negative dependence of the production rate on x (see Figure 1).

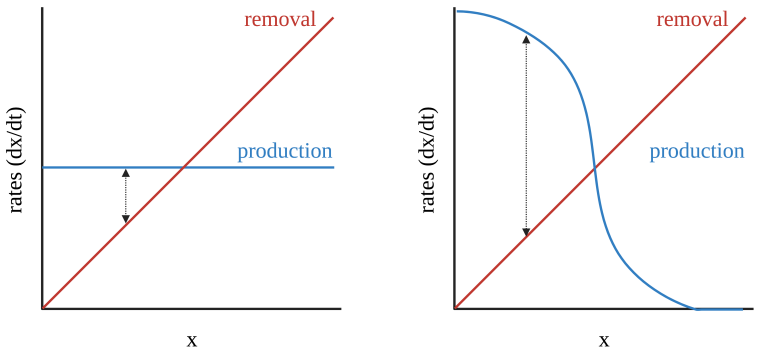

Figure 1

To make this more precise, we need to consider how this would be implemented in a transcriptional network. Transcriptional activation and repression are captured by hill functions: $f(x) = V\frac{x^n}{k^n+x^n}$ (activation) and $f(x) = V\frac{k^n}{k^n+x^n}$ (repression). Hill functions are sigmoidal functions that capture the fraction of time the promoter is bound by the transcription factor. The parameter V (proportional to) the maximal transcription rate. The parameter k captures the concentration of transcription factor needed to achieve half-maximal transcription. The parameter n corresponds to cooperativity (it is also known as the hill coefficient). It captures non-linearities in the activation/repression, which can happen, for example, when bound transcription factors increase the rate of further binding of other transcription factors. For negative autoregulation, we have the dynamics:

$$\dot{x} = V\frac{k^n}{k^n + x^n} - \gamma x \tag{6}$$

It is indeed clear that for a similar steady-state, negative autoregulation allows for much more rapid responses (Figure 1).

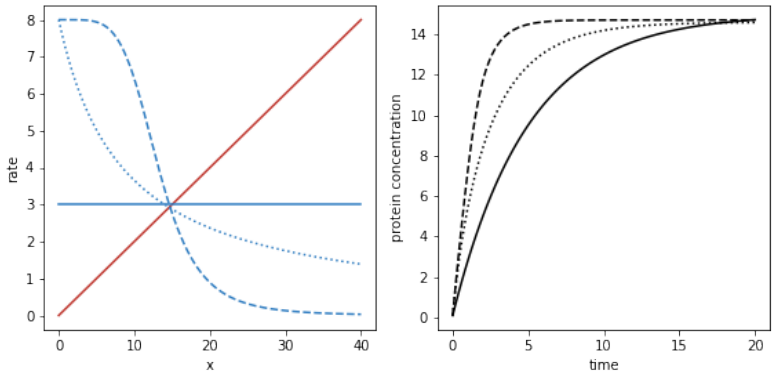

Figure 2

How much more rapid are the responses? Taken to an extreme, we can consider (for a very large n) a step-like repressive function, given by $f(x) = V$ when $x < k$ and $f(x) = 0$ when $x > k$. This function locks the steady-state of x at around $x = k$, with the dynamics (from $x = 0$) given by:

$$\frac{dx}{dt} = V - \gamma X \tag{7}$$

Now, let's assume the promoter is quite strong, so $V \gg \gamma k$. In this case, we can simply take $\frac{dx}{dt} \approx V$. There would be an approximately linear accumulation of protein until $x = k$ at rate V. The timescale to reach the steady state at $x = k$ would therefore be around $\frac{k}{V}$, compared with the timescale of γ^{-1} of the original system. A strong promoter, combined with negative autoregulation, can provide a substantial speed-up of these responses.

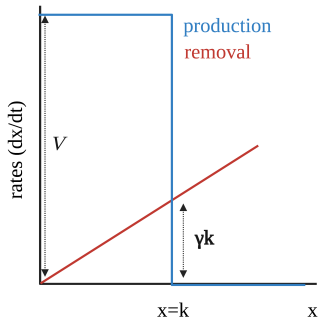

Figure 3

So a strong promoter and strong auto-repression allow for rapid responses at a rate that is effectively decoupled from the growth rate of the cell. Are there other beneficial aspects to such regulation?

Let's consider the effect of negative autoregulation on the steady-state. Without autoregulation, the steady state would depend on both the production rate α and the growth rate γ. These parameters are likely to have some variability or uncertainty. The growth rate may change, depending on the growth conditions. The production rate may fluctuate, for example, due to changes in the resources available for transcription. There would therefore be high uncertainty in the steady state of x.

For negative autoregulation, especially when there is strong cooperativity (large n), the steady-state only depends on k and is almost independent of the other parameters. k is a biochemical parameter that depends on the properties of the promoter and we do not expect that it would fluctuate over time. Negative autoregulation, therefore, allows the system to have a robust steady-state. Robustness is a key aspect of systems biology, and we will discuss it extensively in the first half of this course.

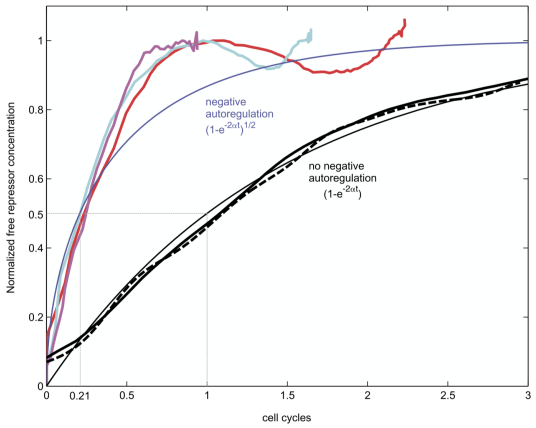

Figure 4: Negative autoregulation in a synthetic biological circuit, from Rosenfeld et al.

3.1 Negative feedback can result in oscillatory dynamics

Is there any downside to negative feedback? One important aspect is that negative feedback may cause responses to overshoot, undershoot, or even destabilize the dynamics. These interesting phenomena cannot be captured in one-dimensional systems, so we will consider systems with more than one component. As a simple example, consider the following two-component negative feedback circuit:

$$\frac{dx}{dt} = \gamma_1(y - x) \tag{8}$$

$$\frac{dy}{dt} = \gamma_2(V\frac{k^n}{k^n + x^n} - y) \tag{9}$$

For large cooperativity, there would be a fixed point (a solution to the equations) around $y = x \approx k$. Recall that to analyze the stability around a fixed point, we need to linearize the system and study the eigenvalues of the Jacobian.

Linear stability analysis. Consider a one-dimensional system $\dot{x} = f(x)$. The system describes a flow on a line (the x-axis), where for every point there is either a flow to the right $f(x) > 0$, to the left $f(x) < 0$, or no change $f(x) = 0$. Let x_0 be a fixed of f (so $f(x_0) = 0$), and consider the Taylor expansion of f around x_0. When $f'(x_0) \neq 0$ we will ignore higher order terms:

$$\dot{x} = f(x) = \cancel{f(x_0)} + (x - x_0)f'(x_0) + ... \approx (x - x_0)f'(x_0) \tag{10}$$

Small perturbations around $x = x_0$ grow or decay like $e^{f'(x_0)}$. Therefore, when $f'(x_0) < 0$ the fixed point is stable, while when $f'(x_0) > 0$ it is unstable. When $f'(x_0) = 0$, on the other hand, the point *critical* - in this case, we cannot ignore the higher order terms of the Taylor expansion. More generally, consider a high dimensional ODE system, given by a collection of variables $\mathbf{x} = (x_1, \ldots, x_n)$ and time-evolution rules $\mathbf{f} = (f_1, \ldots, f_n)$. Let $\mathbf{x}^*$ be a fixed point of the system, and let's denote $\delta_i = x_i - x_i^*$ as a small perturbation away from the fixed point ($\delta = \mathbf{x} - \mathbf{x}^*$). To get the time-evolution of the perturbation we can linearize each of the dynamical equations:

$$\dot{\delta} = \mathbf{J}^* \delta \tag{11}$$

Where J is the Jacobian estimated around the critical point:

$$\mathbf{J}^* = \begin{bmatrix} \frac{\partial f_1}{\partial x_1}(\mathbf{x}^*) & \ldots & \frac{\partial f_1}{\partial x_n}(\mathbf{x}^*) \\ \vdots & \ddots & \vdots \\ \frac{\partial f_n}{\partial x_1}(\mathbf{x}^*) & \ldots & \frac{\partial f_n}{\partial x_n}(\mathbf{x}^*) \end{bmatrix} \tag{12}$$

Each solution of Eq. 11 can be written as a linear combination $e^{\lambda_i t}$, where λ_i are the eigenvalues of $\mathbf{J}^*$. In general, each λ_i can be a complex number. The contribution of its imaginary part $b = \mathrm{Im}(\lambda_i)$ is an oscillating component $e^{bti} = \cos bt + i \sin bt$. The real part $b = \mathrm{Re}(\lambda_i)$, however, contributes a component that increases exponentially with $b > 0$ or decreases with $b < 0$. Therefore, if the eigenvalues of $\mathbf{J}^*$ have negative real parts then $\mathbf{x}^*$ is stable; if at least one has a positive real part, the fixed point is unstable. The case of eigenvalues which are zeros will again be considered as special and we will have to consider the contribution of the nonlinear terms.

The Jacobian of the system is given by:

$$\mathbf{J} = \begin{bmatrix} \frac{\partial}{\partial x}(\gamma_1(y - x)) & \frac{\partial}{\partial y}(\gamma_1(y - x)) \\ \frac{\partial}{\partial x}(\gamma_2(V\frac{k^n}{k^n + x^n} - y)) & \frac{\partial}{\partial y}(\gamma_2(V\frac{k^n}{k^n + x^n} - y)) \end{bmatrix} = \begin{bmatrix} -\gamma_1 & \gamma_1 \\ -\frac{\gamma_2 k^n V n x^{n-1}}{(k^n + x^n)^2} & -\gamma_2 \end{bmatrix} \tag{13}$$

$$\mathbf{J}^* = \begin{bmatrix} -\gamma_1 & \gamma_1 \\ -\frac{\nu}{4}\gamma_2 & -\gamma_2 \end{bmatrix} \tag{14}$$

where $\nu = \frac{nV}{k}$ corresponds to the strength of the feedback. The eigenvalues of the system are $\lambda_1 = \frac{1}{2}\left(-\sqrt{(\gamma_1 - \gamma_2)^2 - \gamma_1\gamma_2\nu} - \gamma_1 - \gamma_2\right)$, $\lambda_2 = \frac{1}{2}\left(\sqrt{(\gamma_1 - \gamma_2)^2 - \gamma_1\gamma_2\nu} - \gamma_1 - \gamma_2\right)$. We can make the following observations:

- When the timescales γ_1, γ_2 are very different, and feedback strength is moderate, the eigenvalues will be real (and the dynamics overdamped), with the slow timescale dominating the response to perturbations.
- When they are similar, e.g. $\gamma_1 = \gamma_2 = \gamma$, the eigenvalues are $\lambda_{1,2} = -\gamma\left(1 \pm \frac{\sqrt{-\nu}}{2}\right)$. In this case the dynamics would be damped oscillatory for any feedback strength ($\nu > 0$), with the frequency of the oscillations increasing with feedback strength. In this case, we expect the response to overshoot.

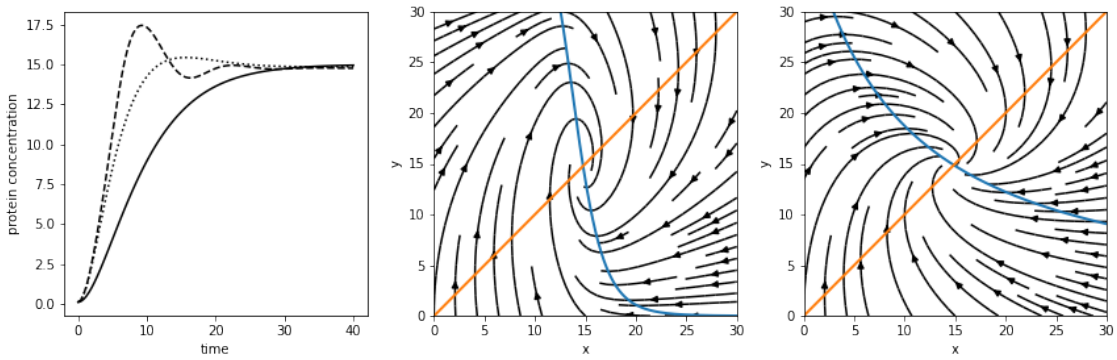

Figure 5

An example of damped oscillations appears in Figure 5, which demonstrates how stronger feedback (due to a steeper hill coefficient) results in both faster responses, and a larger overshoot of the dynamics.

Damped oscillations are a hallmark of negative feedback. They can be observed even in the simple implementation of negative auto-regulation by Rosenfeld et al. (Figure 4). Overshooting may be detrimental (this is clear in the glucose-insulin system, which we will discuss later on), yet the benefits may be offset by the importance of rapid responses. As an example, populations of cells in tissues are maintained in tight homeostasis. After an injury, they repopulate the tissue. The recovery, over weeks, bears the hallmarks of damped oscillations due to negative feedback (Figure 6).

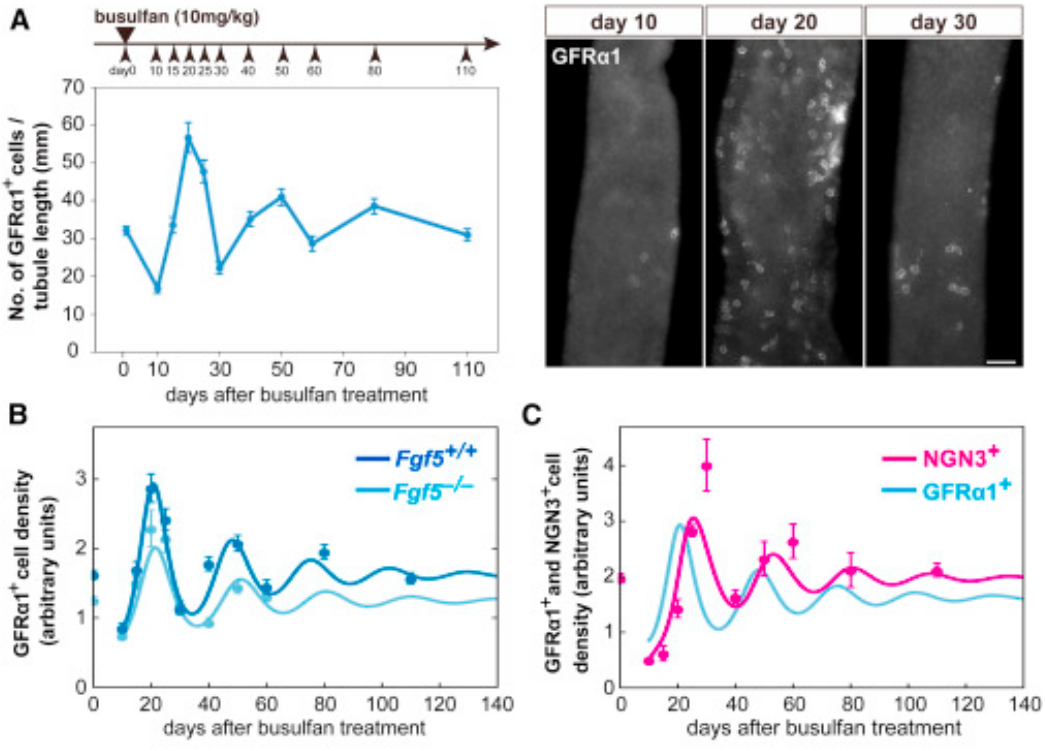

Figure 6: Damped oscillations in the recovery of stem cells in the mouse germline, from Kitadate et al.

4 Summary

Negative autoregulation allows systems to respond more quickly to perturbations. This is by implementing a strategy based on strong activation combined with a strong "brake". Delays cause

systems with negative feedback to have damped oscillations (spiral fixed point) which can be associated with under/overshoots in the system.