IV. 2. Multistability in the lactose utilization network of Escherichia coli

Reference:

1. E. M. Ozbudak, M. Thattai, H. N. Lim, B. I. Shraiman, and A. van Oudenaarden. Multistability in the lactose utilization network of *Escherichia coli*. *Nature* **427**, 737-740 (2004).

Multistability, the capacity to achieve multiple internal states in response to a single set of external inputs, is the defining characteristic of a switch. The multistability of genetic networks has been attributed to positive feedback loops in underlying regulatory networks as was shown in the last chapter for lambda phage. However, feedback alone does not guarantee multistability. The phase diagram of a multistable system, a concise description of available internal states as key parameters are varied, reveals the precise requirements to obtain a functional switch. Here we will determine the phase diagram of the bistable lactose utilization network of *Escherichia coli*.

The *lacZYA* operon comprises three genes required for the uptake and metabolism of lactose and related sugars (Fig. 12): *lacZ* codes for β-galactosidase, an enzyme responsible for the conversion of lactose into allolactose and subsequent metabolic intermediates; *lacY* codes for the lactose permease (LacY), which facilitates the uptake of lactose and similar molecules, including thio-methylgalactoside (TMG), a non-metabolizable lactose analog; and *lacA* codes for an acetyltransferase involved in sugar metabolism. The operon has two transcriptional regulators, a repressor (LacI), and an activator, the cyclic AMP receptor protein (CRP). Inducers, among them allolactose and TMG, bind to and inhibit repression by LacI, while cyclic AMP (cAMP) binds to and triggers activation by CRP. The concentration of cAMP drops in response to the uptake of various carbon sources, including glucose and lactose; glucose uptake also interferes with LacY activity, leading to inducer exclusion. Together, these effects mediate catabolite repression, the ability of glucose to inhibit *lac* expression. Crucially, cAMP

levels are not affected by TMG uptake. Therefore, by varying the extracellular concentrations of TMG and glucose, we are able to independently regulate the activities of LacI and CRP, the two cis-regulatory inputs of the *lac* operon. However, the response of the operon must be considered within the broader context of the network. The uptake of TMG induces the synthesis of LacY, which in turn promotes further TMG uptake; the resulting positive feedback loop creates the potential for bistability.

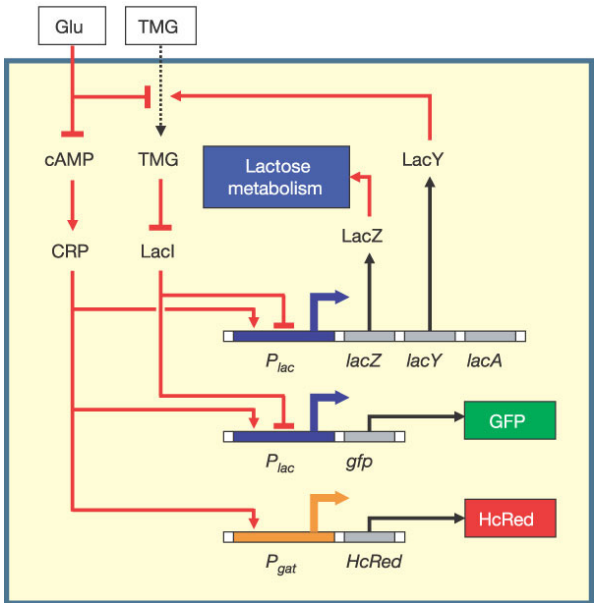

Figure 12. Red lines represent regulatory interactions, with pointed ends for activation and blunt ends for inhibition; black arrows represent protein creation through transcription and translation, and dotted arrows represent uptake across the cell membrane. In our experiments we vary two external inputs, the extracellular concentrations of glucose and TMG, and measure the resulting levels of two fluorescent reporter proteins: GFP, expressed at the lac promoter, and HcRed, expressed at the gat promoter. LacY catalyses the uptake of TMG, which induces further expression of LacY, resulting in a positive feedback loop.

We model the *lac* system using the following equations:

$$\frac{R}{R_T} = \frac{1}{1+(x/x_0)^n} \qquad \textbf{[IV.14]}$$

$$\tau_y \frac{dy}{dt} = \alpha \frac{1}{1+R/R_0} - y \qquad \textbf{[IV.15]}$$

$$\tau_x \frac{dx}{dt} = \beta y - x \qquad \textbf{[IV.16]}$$

Here, x is the intracellular TMG concentration, y is the concentration of LacY in green fluorescence units, R_T is the total concentration of LacI tetramers, and R is the concentration of active LacI. The active fraction of LacI is a decreasing sigmoidal function of the TMG concentration x, with half-saturation concentration x_0, and Hill coefficient n (Eq. [IV.14]). This sigmoidal behavior arises from the fact that the binding of TMG to any one of four possible sites on the LacI tetramer is sufficient to interfere with LacI activity, while higher TMG occupancies cause even further impairment. There is extensive experimental evidence showing that $n \approx 2$. The interaction of a single active LacI tetramer with multiple operator sites on the *lac* promoter generates a DNA loop which blocks transcription. The rate of generation of LacY is therefore a decreasing hyperbolic function of R, with maximal value α, half-saturation concentration R_0, and minimal value α/ρ achieved at $R = R_T$. The repression factor $\rho = 1 + R_T/R_0$ describes how tightly LacI is able to regulate *lac* expression. LacY is depleted in a first-order reaction with time constant τ_y, due to a combination of degradation and dilution (Eq. [IV.15]). TMG enters the cell at a rate proportional to y, and is similarly depleted in a first-order reaction with time constant τ_x (Eq. [IV.16]). The parameter β measures the TMG uptake rate per LacY molecule. Since we cannot directly measure x, we are free to choose its units so that $x_0 = 1$. Once inside the cell, TMG is able to inactivate LacI, completing the feedback loop. Combining these equations, we obtain the steady state result

$$y = \alpha \frac{1 + (\beta y)^2}{\rho + (\beta y)^2}. \qquad \textbf{[IV.17]}$$

Here, ρ, α and β are allowed to be arbitrary functions of our external inputs, the extracellular glucose (G) and TMG (T) levels. As these parameters are varied, the system is capable of generating either one or two stable fixed points (Fig. 13).

The boundary between monostability (one stable fixed point) and bistability (two stable fixed points separated by one unstable fixed point) occurs when Eq. IV.17 admits precisely two solutions. Rewriting Eq. IV.17 as a cubic, we obtain

$$y^3 - \alpha y^2 + (\rho/\beta^2)y - (\alpha/\beta^2) = 0. \qquad \textbf{[IV.18]}$$

On the other hand, a general cubic with two identical roots has the form

$$\begin{aligned}(y-a)(y-a)(y-\theta a) \quad &= \\ y^3 - (2+\theta)ay^2 + (1+2\theta)a^2 y - \theta a^3 \quad &\end{aligned} \qquad \textbf{[IV.19]}$$

where θ is the dimensionless ratio of roots. Comparing coefficients, we find

$$\rho = (1+2\theta)(1+2/\theta),$$

$$\alpha\beta = (2+\theta)^{3/2}/\theta^{1/2}. \qquad \textbf{[IV.20]}$$

These are the parametric equations describing the boundary of the bistable region (Fig. 13). The critical point *C* occurs where all three roots coincide, so $\theta = 1$.

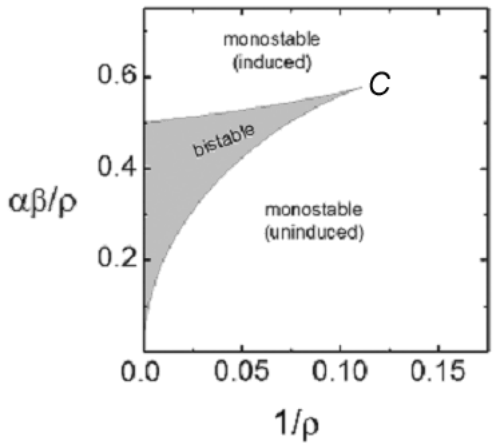

Figure 13. Theoretical phase diagram of the lactose utilization network.