Considerable insight into genetic switches has been obtained by constructing a synthetic version of a switch in *E. coli*, in which the gene product of the switch is a fluorescent reporter protein. This allows the flipping of the switch to be observed by measuring the fluorescent level of the cells. The underlying gene circuit is based on a mutual repressor model, see Fig. 27. It consists of two repressor proteins *X* and *Y* whose transcription is mutually regulated. That is, the protein product of one gene binds to the promoter of the other gene and represses its output.

For simplicity, the explicit dynamics of transcription and translation are ignored so that we only model the mutual effects of the proteins on protein production. Denoting the concentrations of the proteins by $x_1(t), x_2(t)$, and assuming the two proteins act symmetrically, the resulting kinetic equations are

$$\frac{dx_1}{dt} = -\gamma_p x_1 + \frac{\kappa_p K}{K + x_2^2}, \quad \frac{dx_2}{dt} = -\gamma_p x_2 + \frac{\kappa_p K}{K + x_1^2}. \quad (8.1)$$

Here $\gamma_p$ is the rate of protein degradation, $\kappa_p$ is the rate of protein production in the absence of repression, and $K$ is a binding constant for the repressors. (We have absorbed a factor of $\kappa/\gamma$ into $\kappa_p$, see equation (2.4)).

It is convenient to rewrite the equations in non-dimensional form by measuring $x_1$ and $x_2$ in units of $K^{-1/2}$ and time in units of $\gamma_p^{-1}$:

$$\frac{du}{dt} = -u + \frac{\alpha}{1 + v^2}, \quad \frac{dv}{dt} = -v + \frac{\alpha}{1 + u^2}, \quad (8.2)$$

with $\alpha = \kappa_p \sqrt{K\alpha/\gamma_p}$. Analysis of the fixed point solutions of this pair of equations establishes that the mutual repressor model acts as a bistable switch. The fixed point equation for $u$ is

$$u = \alpha \left[ 1 + \left( \frac{\alpha}{1 + u^2} \right)^2 \right]^{-1},$$

which can be rearranged to yield a product of two polynomials:

$$(u^2 - \alpha u + 1)(u^3 + u - \alpha) = 0.$$
The cubic is a monotonically increasing function of $u$ and thus has a single root given implicitly by

$$u = \frac{\alpha}{1 + u^2} = v.$$  

This solution is guaranteed by the exchange symmetry of the underlying equations. The roots of the quadratic are given by

$$u = U_{\pm} \equiv \frac{1}{2} \left[ \alpha \pm \sqrt{\alpha^2 - 4} \right],$$

with $v = U_{\pm}$. It immediately follows that there is a single fixed point when $\alpha < 2$ and three fixed points when $\alpha > 2$. Moreover, linear stability analysis establishes that the symmetric solution is stable when $\alpha < 2$, and undergoes a pitchfork bifurcation at the critical value $\alpha_c = 2$ where it becomes unstable and a pair of stable fixed points emerge.

### 8.2 Modified mutual repressor model

Consider a simplified mutual repressor model consisting of a single promoter with two operator sites $OR_1$ and $OR_2$ that bind to dimers of protein $Y$ and protein $X$, respectively (see Fig. 28). If the dimer of one protein is bound to its site, then this represses the expression of the other protein. However, both sites cannot be occupied at the same time. Hence, the promoter can be in three states $O_m, m = 0, 1, 2$: no dimer is bound to the promoter ($O_0$); a dimer of protein $Y$ is bound to the promoter ($O_1$); a dimer of protein $X$ is bound to the promoter ($O_2$). Assuming that the number of proteins is sufficiently large, we have the transition scheme

$$O_1 \xrightarrow{\beta K} O_0 \xrightarrow{\beta x_1^2} O_2,$$

where $\beta$ is a transition rate and $K$ is a non-dimensional dissociation constant. Protein $X$ ($Y$) is produced at a rate $\kappa_p$ when the promoter is in the states $O_{0,2}$ ($O_{0,1}$), and both proteins are degraded at a rate $\gamma_p$ in all three states. The kinetic equations become

$$\frac{dx_1}{dt} = -\gamma_p x_1 + \kappa_p \frac{K + x_1^2}{x_1^2 + x_2^2 + K}, \quad \frac{dx_2}{dt} = -\gamma_p x_2 + \kappa_p \frac{K + x_2^2}{x_1^2 + x_2^2 + K}. \quad (8.3)$$

![Figure 28: Simplified mutual repressor with a single promoter having two operator sites $OR_1$ and $OR_2$. A dimer of protein X can bind to $OR_2$ and a dimer of protein Y can bind to $OR_1$, but they cannot both be bound at the same time.](image)
Figure 29: (a) Bifurcation diagram for mutual repressor model. (b) Phase-plane dynamics of a modified 3-state mutual repressor model. The black curve shows the $x_2$-nullcline and the grey curve shows the $x_1$-nullcline. The open circles show the stable fixed points, the filled circle shows the unstable saddle. The irregular curve shows a stochastic trajectory leaving the lower basin of attraction to reach the curve (separatrix) that separates the basins of attraction of the two fixed points.

For sufficiently small $K$ one finds that the system exhibits bistability. This is illustrated in Fig. 29.

**Noise-induced switching.** In the absence of molecular or promoter noise, the bistable system will settle into one of the stable fixed points, depending on the initial condition. All initial conditions that approach a given fixed point form the basin of attraction. However, when intrinsic noise is included, it is possible for a stochastic trajectory to leave a fixed point and cross the boundary (separatrix) between the basins of attraction, resulting in a switch to the other fixed point.

### 8.3 The Repressilator

The first reported experimental realization of a synthetic gene oscillator is the *repressilator*. The basic circuit consists of three genes encoding three transcriptional repressors: the lactose repressor LacI, the tetracycline repressor TetR, and the CI repressor from bacteriophage $\lambda$. The synthetic circuit consists of a ring in which the protein of a given gene acts as a repressor of the next gene in the ring, see Fig. 30(a). Representing the action of each repressor as a Hill function, we obtain the following system of equations for the concentrations $x_1, x_2, x_3$ of LacI, TetR and CI, respectively:

\[
\frac{dx_1}{dt} = \kappa_1 + \frac{\alpha_1}{1 + (x_3/K_3)^{n_3}} - \gamma_1 x_1, \\
\frac{dx_2}{dt} = \kappa_2 + \frac{\alpha_2}{1 + (x_1/K_1)^{n_1}} - \gamma_2 x_2, \\
\frac{dx_3}{dt} = \kappa_3 + \frac{\alpha_3}{1 + (x_2/K_2)^{n_2}} - \gamma_3 x_3,
\]

where $\kappa_j, j = 1, 2, 3$ are background production rates. If one also includes the mRNA dynamics and sets $\kappa_j = \kappa$, $\alpha_j = \alpha$, $K_j = K$, $n_j = n$, and $\gamma_i = \gamma$, then one can write down a dimensionless
Figure 30: (a) Schematic diagram of repressilator circuit. (b) Hopf bifurcation diagram. (c) Decaying trajectory for $\beta < \beta_c$. (d) Oscillatory solution for $\beta > \beta_c$. Typically $r = \sim (0.01)\text{min}^{-1}$ whereas $\sigma \sim 10^2\text{min}^{-1}$.

version of the resulting system of equations in the compact form

\[
\frac{da_i}{dt} = -a_i + \frac{\sigma}{1 + x_j^3} + r, \quad (8.5a)
\]

\[
\frac{dx_i}{dt} = -\beta(x_i - a_i), \quad (8.5b)
\]

where $i \in \{1, 2, 3\}$ and $j \in \{3, 1, 2\}$. The parameter $\beta$ denotes the ratio of the protein and mRNA degradation rates, time is rescaled in units of the mRNA lifetime, protein concentrations are in units of $K_i$, and mRNA concentrations are rescaled by their translation efficiency (the average number of proteins produced per mRNA molecule). One finds that the system (8.5) can switch from a stable fixed point to limit cycle oscillations as $\beta$ is increased from zero (Hopf bifurcation), see Fig. 30(b,c).

**Hopf bifurcation in a planar system.** In general it is difficult to find an explicit oscillatory solution to a nonlinear system of ODEs. A much simpler approach is to investigate how the linear stability of a fixed point varies with some model parameter. If one finds a pair of complex-valued eigenvalues $\lambda = \lambda_R \pm i\lambda_I$ crossing the imaginary axis ($\lambda_R = 0$) as the parameter is varied, then this indicates that a limit cycle oscillation will arise via a so-called Hopf bifurcation. We will illustrate this by considering a planar dynamical system.

Consider a planar dynamical system written as

\[
\frac{dx_1}{dt} = f_1(x_1, x_2), \quad \frac{dx_2}{dt} = f_1(x_1, x_2),
\]
The origin is the only equilibrium point. Then \( r = \sqrt{x^2 + y^2} \) for the entire plane. That it is a spiral can be seen from the polar form of the equations

\[
\begin{align*}
\dot{x} &= \mu x + y - x(x^2 + y^2) \\
\dot{y} &= -x + \mu y - y(x^2 + y^2)
\end{align*}
\]

result.

Thus \( r = c \) for constant coefficients \( c \). Now suppose that \( c = 0 \). By Theorem 10.7 the origin is asymptotically stable and its domain of attraction includes the origin. This proves that the origin of the time-reversed system is stable, so the original system is unstable, and the polar equation confirms that it is a spiral in \( 0 < r < r_1 \).

The corresponding characteristic equation obtained by linearizing about a fixed point \( x^* \) yields a quadratic equation for \( \lambda \), which can be factorized as

\[
(\lambda - \lambda_1)(\lambda - \lambda_2) = 0.
\]

where the roots \( \lambda_{1,2} \) are the eigenvalues. If these eigenvalues are distinct, then the general solution to the linear ODE can be written as

\[
y(t) = \sum_{j=1,2} c_j \mathbf{v}_j e^{\lambda_j t}
\]

for constant coefficients \( c_j \), where \( \mathbf{v}_j \) is the unit eigenvector corresponding to \( \lambda_j \). Expanding the factorized equation and using the fact that \( \text{Tr}[\mathbf{M}] = \lambda_1 + \lambda_2 \) and \( \text{Det}[\mathbf{M}] = \lambda_1 \lambda_2 \), gives

\[
\lambda^2 - \text{Tr}[\mathbf{M}] \lambda + \text{Det}[\mathbf{M}] = 0.
\]

Thus

\[
\lambda_{1,2} = \frac{1}{2} \left[ \text{Tr}[\mathbf{M}] \pm \sqrt{\text{Tr}[\mathbf{M}]^2 - 4\text{Det}[\mathbf{M}]} \right]. \tag{8.6}
\]

It follows from the above analysis that the fixed point \( x^* \) will be stable provided that \( \text{Re}[\lambda_{1,2}] < 0 \), since the perturbations \( y_j(t) = \mathbf{v}_j e^{\lambda_j t} \rightarrow 0 \) as \( t \rightarrow \infty \). Using equation (8.6), the condition for linear stability is \( \text{Tr}[\mathbf{M}] < 0 \).

Now suppose that \( \mathbf{M} \) depends on some model parameter \( \mu \) and that there exists a critical parameter value \( \mu_c \) for which

\[
\text{Tr}[\mathbf{M}(\mu)] < 0 \text{ for } \mu < \mu_c, \quad \text{Tr}[\mathbf{M}(\mu_c)] = 0, \quad \text{Det}[\mathbf{M}(\mu_c)] > 0. \tag{8.7}
\]

Then \( \lambda_{1,2}(\mu_c) = \pm i \omega_0 \) with \( \omega_0 = \sqrt{\text{Det}[\mathbf{M}(\mu_c)]} \). It can then be shown that a unique curve of periodic solutions bifurcates from the origin as \( \mu \) crosses \( \mu_c \) from below. The amplitude of the limit cycle grows like \( \sqrt{\mu - \mu_c} \) with an approximate period \( 2\pi/\omega_0 \). An example planar system undergoing a Hopf bifurcation is shown in Fig. 31.
8.4 Activator-repressor relaxation oscillator

Another important class of synthetic gene oscillator is based on the combination of both negative and positive feedback loops to create a so-called relaxation oscillator. One of the potential advantages of such oscillators is that they tend to be more robust to noise. For the sake of illustration, consider the network shown in Fig. 32. It consists of two plasmids, both of which contain the same promoter denoted by $P_{RM}^*$. (A plasmid is a genetic structure in a cell that can replicate independently of the chromosomes, typically a small circular DNA strand in the cytoplasm of a bacterium or protozoan. Plasmids are often used for the experimental manipulation of genes.) On the first plasmid, the promoter controls the λ phage cI gene, whereas on the second plasmid, the promoter regulates the lac gene. Each copy of the promoter $P_{RM}^*$ consists of three operator sites $OR_1, OR_2$ and $OR_3^*$. CI dimers can independently bind to $OR_1$ and $OR_2$, whereas a Lac tetramer can bind to $OR_3^*$. (The $P_{RM}^*$ promoter is a mutant of the wild type promoter $P_{RM}$, consisting of sites $OR_1, OR_2, OR_3$, all of which bind CI dimers.) The gene is OFF if $OR_3^*$ is occupied, irrespective of the states of the other two sites, and is ON otherwise. The production rate of protein in the ON state is enhanced by a factor $\alpha$ if both sites $OR_1$ and $OR_2$ are occupied.

Let $X$ and $Y$ denote CI and Lac proteins, respectively, and let $D_i$ denote the promoter region of plasmid $i$. The set of binding reactions are as follows:

$$
2X \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} X_2, \quad 4Y \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} 2Y_2 \overset{k_3}{\underset{k_{-3}}{\rightleftharpoons}} Y_4,
$$

$$
D_i + X_2 \overset{k_4}{\underset{k_{-4}}{\rightleftharpoons}} D_iX_2, \quad D_iX_2 + X_2 \overset{k_5}{\underset{k_{-5}}{\rightleftharpoons}} D_iX_2X_2,
$$

$$
D_i + Y_4 \overset{k_6}{\underset{k_{-6}}{\rightleftharpoons}} D_iY_4, \quad D_iX_2 + Y_4 \overset{k_7}{\underset{k_{-7}}{\rightleftharpoons}} D_iX_2Y_4, \quad D_iX_2X_2 + Y_4 \overset{k_8}{\underset{k_{-8}}{\rightleftharpoons}} D_iX_2X_2Y_4.
$$

For each reversible reaction $a$, $a = 1, \ldots, 8$, we have an equilibrium constant $K_a = k_a/k_{-a}$ (in units of inverse Molars). These are further constrained by taking $K_5 = \sigma K_4$ and $K_6 = K_7 = K_8$.

![Figure 32: Synthetic relaxation oscillator. (a) Circuit diagram consisting of an activator gene cI and a repressor gene lacI. (b) Different states of the mutant promoter $P_{RM}^*$ indicating the production rate in each state. The gene is OFF if a Lac tetramer is bound to site $OR_3^*$ irrespective of the occupancy of sites $OR_1$ and $OR_2.$](image)
Similarly, the production of the proteins are given by the irreversible reactions

\[ \frac{D_1}{D_1 + X}, \quad \frac{D_1 X_2}{D_1 X_2 + X}, \quad \frac{D_1 X_2 X_2}{D_1 X_2 X_2 + X}, \]

\[ \frac{D_2}{D_2 + Y}, \quad \frac{D_2 X_2}{D_2 X_2 + Y}, \quad \frac{D_2 X_2 X_2}{D_2 X_2 X_2 + Y}. \]

Given these reactions, the kinetic equations for the monomer CI concentration \( x \) and monomer Lac concentration \( y \) take the form

\[ \frac{dx}{dt} = -2k_1x^2 + 2k_{-1}[X_2] + \kappa_p([D_1] + [D_1 X_2] + \alpha[D_1 X_2 X_2]) - \gamma_x x \]  

(8.8a)

\[ \frac{dy}{dt} = -2k_2y^2 + 2k_{-2}[Y_2] + \kappa_p([D_2] + [D_2 X_2 + \alpha[D_1 X_2 X_2]) - \gamma_y y. \]  

(8.8b)

Since the multimerization and DNA binding processes are much faster than the rates of transcription and degradation, we can carry out an adiabatic approximation. That is, \([X_2], [Y_2] \), etc. are taken to be in quasi-equilibrium. Introduce the dimensionless variables

\[ \tilde{x} = \sqrt{K_1 K_4} x, \quad \tilde{y} = (K_2^2 K_3 K_6)^{1/4} y, \quad \tilde{t} = \sqrt{K_1 K_4 \kappa_p M_1} t, \]  

(8.9)

where \( M_i \) is the copy number concentration of plasmid \( i \):

\[ [D_i] + [D_i X_2] + [D_i X_2 X_2] + [D_i Y_4] + [D_i X_2 Y_4] + [D_i X_2 X_2 Y_4] = M_i. \]

Eliminating the slow variables and dropping the tildes, we obtain the equations

\[ \frac{dx}{dt} = \frac{1 + x^2 + \alpha \sigma x^4}{(1 + x^2 + \sigma x^4)(1 + y^4)} - \gamma_x x \]  

(8.10a)

\[ \frac{dy}{dt} = \frac{1 + x^2 + \alpha \sigma x^4}{(1 + x^2 + \sigma x^4)(1 + y^4)} - \gamma_y x, \]  

(8.10b)

where the degradation rates have also been non-dimensionalized, and

\[ \tau_y = \left( \frac{K_2^2 K_4}{K_2^2 K_3 K_6} \right)^{1/4} \frac{M_1}{M_2}. \]  

(8.11)

Figure 33: Illustration of periodic time variation of activator and repressor concentrations in relaxation oscillator: \( \alpha = 11, \sigma = 2, \gamma_x = 0.2, \gamma_y = 0.3 \) and \( \tau_y = 5 \). (Units are arbitrary.)
Figure 34: Phase-plane analysis of activator-repressor model. The nullcline of the activator is cubic-like, whereas the nullcline of the repressor is monotonic. Point(s) of intersection of the two nullclines are fixed points. (a) If the degradation rate of the activator is around 5 times faster than the repressor, then the repressor nullcline intersects the middle branch of the activator nullcline. The fixed point is unstable and the system exhibits a limit cycle, with most of the time spent in the repressed regime (indicated by red curve). (b) If the degradation rates are comparable, then the repressor nullcline intersects the first branch of the activator nullcline. The fixed point is now stable and the system is excitable. This means that for small perturbations of the fixed point, trajectories make small excursions before relaxing back to the fixed point. On the other hand, large perturbations result in large excursions in the phase plane before returning to a neighborhood of the fixed point, resulting in a single burst of gene expression. This is analogous to the firing of a neuronal action potential.

Since the plasmid copy numbers can be chosen by the designer of the construct, it follows that $\tau_y$ is a design parameter. The degradation rates were taken as experimental control parameters. An illustration of the model’s oscillatory behavior is shown in Fig. 33, based on a high copy plasmid for the CI gene ($M_1 \approx 50$) and a low copy number for the lac gene ($M_2 \approx 1$). Both the activator $X$ and the repressor $Y$ are expressed periodically. Both concentrations grow until there is a sufficient amount of repressor to cut off expression. The concentrations then decrease until repression is released and the next burst of expression begins. The sharp peaks in the concentration profiles, particularly for $X$, is indicative of a relaxation oscillator. A characteristic feature of a relaxation oscillator is that there is a separation of time-scales in which a single cycle can be partitioned into regions of rapid changes in the activator concentration separated by one or more regions of slow variation. (The represilator is a more regular oscillator with sinusoidal like oscillations and no separation of time-scales.) In the activator-repressor model, the slow response occurs in the repressed regime (low $x$). This can be further understood using phase-plane analysis, as detailed in the caption of Fig. 34.

8.5 The circadian clock gene

The circadian rhythm plays a key physiological role in the adaptation of living organisms to the alternation of night and day. Experimental studies of a wide range of plants and animals have established that in almost all cases, autoregulatory feedback on gene expression plays a central role in the molecular mechanisms underlying circadian rhythms. The resulting circadian oscillator has a natural period of approximately 24 hours, which can be entrained to the external light-dark cycle. Here we consider a minimal model of the circadian clock in the fungus *Neurospora*. A related model
Figure 35: Minimal model for a negative auto regulation network underlying circadian rhythms. Transcription of a clock gene (X) produces mRNA (M), which is transported outside the nucleus and then translated into cytosolic clock protein (X_C). The protein is either degraded or transported into the nucleus (X_N) where it exerts negative feedback on the gene expression.

For circadian rhythms in *Drosophila* was previously introduced by Golbeter. A schematic diagram of the basic model is shown in Fig. 35. A clock gene X (frq in Neurospora, per in Drosophila) is transcribed to form mRNA (M), which exits the nucleus and is subsequently translated into cytoplasmic clock protein (X_C). The resulting protein either degrades or enters the nucleus (X_N) where it inhibits its own gene expression.

The governing equations for the concentrations \( m, x_C, x_N \) of mRNA, cytosolic protein and nuclear protein, respectively, are

\[
\frac{dm}{dt} = \kappa - \frac{K^m_m}{K^m_m + x_N^n} - \gamma \frac{m}{K^m_m + m} \quad (8.12a)
\]

\[
\frac{dx_C}{dt} = \kappa_p m - \frac{x_C}{K_p + x_C} - k_1 x_C + k_2 x_N \quad (8.12b)
\]

\[
\frac{dx_N}{dt} = k_1 x_C - k_2 x_N. \quad (8.12c)
\]

Here \( \kappa \) is the unregulated rate of transcription, \( \kappa_p \) is the rate of translation, and \( \gamma, \gamma_p \) are the rates of mRNA and protein degradation; degradation is assumed to obey Michaelis-Menten kinetics. The negative regulation of transcription is taken to be cooperative, with a Hill coefficient of \( n \). Finally, the rate constants \( k_1, k_2 \) characterize the transport of protein into and out of the nucleus. It can be shown that the above model exhibits oscillations in physiologically reasonable parameter regimes, and thus provides a molecular basis for the sustained oscillations of the circadian clock under constant darkness.

**Effects of noise on genetic oscillators.** There are at least two different issues that arise when noise is included. First, to what extent is a genetic oscillator robust to noise arising from low copy numbers, for example? This has been explored in some detail within the context of circadian rhythms. In the absence of noise, one can represent the dynamics in terms of motion around a closed curve known as a limit cycle, see Fig. 36. Each point on the limit cycle can be assigned a
phase $\theta \in [0, 2\pi]$ such that the dynamics is given by

$$\frac{d\theta}{dt} = \omega$$  \hspace{1cm} (8.13)

where $\omega = \frac{2\pi}{\Delta}$ and $\Delta$ is the period of the oscillation. When noise is included, one tends to observe an irregular trajectory around the limit cycle. If the trajectory wanders too far from the limit cycle then it may escape completely, and the notion of phase breaks down. However, even if the trajectory remains close to the limit cycle, the resulting stochastic phase of the oscillator undergoes diffusion around the limit cycle, so all phase information is eventually lost. The effective diffusion coefficient of this process is one measure of robustness.

A second effect of noise is that it can extend the parameter regime over which oscillations can occur. That is, even though the deterministic system converges to a fixed point, the system exhibits oscillatory behavior when noise is included. This can be established by looking at the power spectrum of the protein concentration.