J. theor. Biol. (2000) **203,** 117–133
doi:10.1006/jtbi.2000.1068, available online at http://www.idealibrary.com on IDEAL®

How to make a Biological Switch

JOSHUA L. CHERRY*† AND FREDERICK R. ADLER*‡

**Department of Biology and ‡Department of Mathematics, University of Utah, Salt Lake City, UT 84112, U.S.A.*

(*Received on* 30 *March* 1999, *Accepted in revised form on* 20 *December* 1999)

Some biological regulatory systems must "remember" a state for long periods of time. A simple type of system that can accomplish this task is one in which two regulatory elements negatively regulate one another. For example, two repressor proteins might control one another's synthesis. Qualitative reasoning suggests that such a system will have two stable states, one in which the first element is "on" and the second "off", and another in which these states are reversed. Quantitative analysis shows that the existence of two stable steady states depends on the details of the system. Among other things, the shapes of functions describing the effect of one regulatory element on the other must meet certain criteria in order for two steady states to exist. Many biologically reasonable functions do not meet these criteria. In particular, repression that is well described by a Michaelis–Menten-type equation cannot lead to a working switch. However, functions describing positive cooperativity of binding, non-additive effects of multiple operator sites, or depletion of free repressor can lead to working switches.

Introduction

During some biological processes, such as differentiation of cells during development, gene regulatory systems must "remember" a state that is set by transient signals. While this memory is achieved in some cases by mechanisms such as heritable DNA methylation (reviewed by Razin & Cedar, 1993), it might also be accomplished by a network of genes that regulate one another through repressor and activator proteins that they encode.

Among the simplest such "genetic switches" is a system in which each of two repressor proteins regulates the synthesis of the other (Monod & Jacob, 1961). In an environment in which both repressors can act (e.g. no inducers are present), the system might have two stable steady states. In one state, the gene for the first repressor is turned on, and the synthesis of the second repressor is therefore turned off. The absence of the second repressor, maintained by the presence of the first, allows the first to be synthesized (in effect, the protein acts as an indirect activator of its own synthesis, since it represses the synthesis of its own repressor). In the other steady state, the second repressor is present and the first is absent.

If represssion can be modulated by some external "inputs", such as inducer molecules, the system can be forced into one or another of the steady states. The presence of an inducer which binds to the first repressor, and thereby inhibits its repressor activity, will lead to the production of the second repressor and repression of the first repressor's synthesis. When inducer is removed,

† Author to whom correspondence should be addressed. E-mail: cherry@biology.utah.edu

0022–5193/00/060117 + 17 $35.00/0

the second repressor will continue to hold the level of the first repressor low. The transient presence of the inducer will have forced the system into a particular steady state which persists after the inducer is removed. Conversely, the temporary presence of an inducer that inhibits the action of the second repressor will force the system into the state where the first repressor is high and the second is low. The system "remembers" which inducer it was last exposed to.

The "inputs" to the switch need not be small molecule inducers that come from the external environment. They might instead be the products of other regulatory genes within the cell, or signals that ultimately come from diffusible morphogens in a developing organism. In this way, a cell and its descendants can "remember" positional information that was present early on but has disappeared, such as morphogen gradients that are present only in early embryogenesis. Thus, the switch may be just a simple component of a complex regulatory network.

The repressor–repressor system described above is analogous to the SR flip-flop of digital electronics. This type of circuit is composed of elements that themselves have no memory, but merely produce an output that is a function of their current inputs. A flip-flop can be made with two NAND or two NOR gates; the version with NOR gates is shown in Fig. 1. Each of the two outputs of the flip-flop is analogous to the presence or absence of repression by one of the repressor proteins. Each NOR gate performs a logical NOR on its two inputs: its output is "true" or "high" when and only when both of its inputs are low, i.e. assertion of either input forces the output to be low. This logical operation corresponds to the fact that repression by a protein may be prevented in two ways. First, the protein's synthesis may itself be repressed by the other protein. Second, inducer, if present, will bind to the protein and inhibit its activity. Repression by the protein will occur when neither the first nor the second of these conditions hold.

In order for a system to function as a switch, it must possess two (or more) stable steady states when inducer is absent. In the qualitative description of a switch given above, a gene was considered to have only two states of transcriptional activation, "on" and "off". In reality, the degree

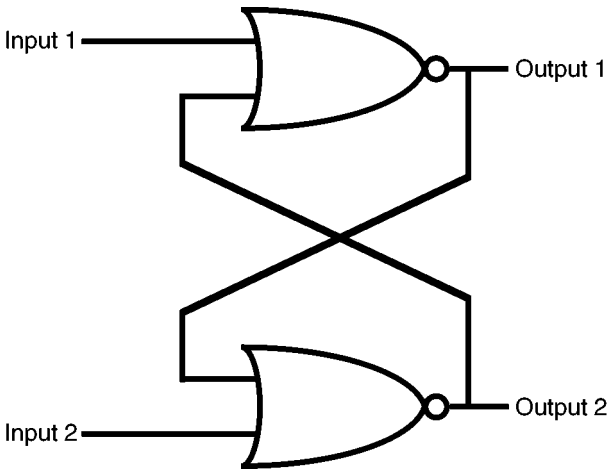

FIG. 1. The SR flip-flop is analogous to a repressor–repressor switch. The circuit, implemented with two NOR gates, has two inputs and two outputs. When neither input is high, the circuit is bistable: output 1 can be high and output 2 low, or output 2 can be high and output 1 low. Assertion of either input forces the system into a particular state and is analogous to addition of an inducer to the biological system. Assertion of input 1, for example, forces output 1 low and allows output 2 to be high. This state will be maintained even after the input is made low.

to which a gene is transcribed can take on a range of values. The question of the existence of multiple steady states must therefore be approached with a quantitative model.

Several previous studies have analysed molecular systems with potential switching behavior. Keller (1995) analysed several types of genetic networks, including some involving mutual repression by two proteins. Thomas (1978), Thomas and D'Ari (1990), and Thomas *et al.* (1995) also discussed several types of regulatory networks, including the two-repressor type. Collier *et al.* (1996) modeled the Delta-Notch system of intercellular signaling, which involves mutual inhibition between adjacent cells, and found conditions that yield bistability. Wolf & Eeckman (1998) modeled both one- and two-gene systems, though their two-gene model lacks bistability. Edelstein-Keshet (1988, pp. 294–295) pointed out the analogy of a bimolecular switch to a pair of species in competition, and applied a competition model to switches. A similar analogy would apply to two predators that prey upon one another (Levin *et al.*, 1977). Several authors have modeled aspects of the regulation of bacteriophage λ (Ackers *et al.*, 1982; Shea & Ackers, 1985;

McAdams & Shapiro, 1995; Thieffry & Thomas, 1995), part of whose regulatory circuitry constitutes a biomolecular switch. Bistability has also been investigated in systems involving only positive regulation (Tyson & Othmer, 1978; Thron, 1995).

We present here criteria for making a working repressor–repressor switch, including conditions on the shapes of the functions that describe repression. Because the shapes of these functions are determined by the underlying mechanisms, some mechanisms can be shown to be insufficient for making a bistable switch. We show that the simplest form of repression is not sufficient for a switch. We also identify several biologically plausible mechanistic features that can make a switch possible.

The Models

A general model for a two-gene network is given by the system

$$\frac{dx}{dt} = f(y) - \mu_1 x,$$

$$\frac{dy}{dt} = g(x) - \mu_2 y, \tag{1}$$

where x and y are the concentrations of the two repressor proteins, f and g are "repression functions" that describe the effect of one protein on the synthesis of the other, and μ_1 and μ_2 are positive constants describing decay. The rate of synthesis of each protein is determined by the level of the other, and each protein decays with first-order kinetics. The first-order "decay" of the proteins might actually be dominated by dilution due to exponential cell growth. Because our main interest is the repressor–repressor case, f and g will be decreasing positive functions, although some of our results apply more generally.

This model relates the rate of protein production directly to the concentration of repressor protein, and thus ignores the dynamics of messenger RNA (mRNA) levels. The model might be taken as an approximation for the case where the half-life of mRNA is short compared to that of protein. We show in Appendix A that our major conclusions hold in a full four-dimensional system that explicitly considers mRNA concentrations.

Whether the system has multiple stable steady states depends on the functions f and g and on the parameters μ_1 and μ_2. The nullclines for system (1) are

$$x = \frac{f(y)}{\mu_1} \triangleq \bar{f}(y),$$

$$y = \frac{g(x)}{\mu_2} \triangleq \bar{g}(x). \tag{2}$$

The new functions $\bar{f}$ and $\bar{g}$ define the nullclines. These nullclines must have at least one intersection because the x-nullcline starts above and ends below the y-nullcline [Fig. 2(a)]. With only one equilibrium, the system cannot function as a switch because any perturbation that moves the system away from the equilibrium is forgotten as the system returns to equilibrium. With three equilibria [Fig. 2(b)], the central equilibrium becomes unstable. A system resting at one of the stable equilibria will switch to the other only if a perturbation moves the system into the other basin of attraction.

Our goal is to find the conditions under which the nullclines cross more than once. We will first examine two special cases, repressor binding described by Michaelis–Menten and Hill functions, and then derive conditions that identify which repression functions f and g can potentially support a switch for some values of the decay parameters μ_1 and μ_2.

Failure of the Michaelis–Menten Function to Make a Switch

In the simplest model for repression, each repressor binds to its target operator by mass action and the rate of transcription is proportional to the amount of unbound operator. When the molar quantity of repressor is much higher than that of operator, the fraction of bound operator is well approximated by a Michaelis–Menten-type equation. The rate of production of protein x is given by

$$f(y) = k_1 \frac{K_d}{K_d + y},$$

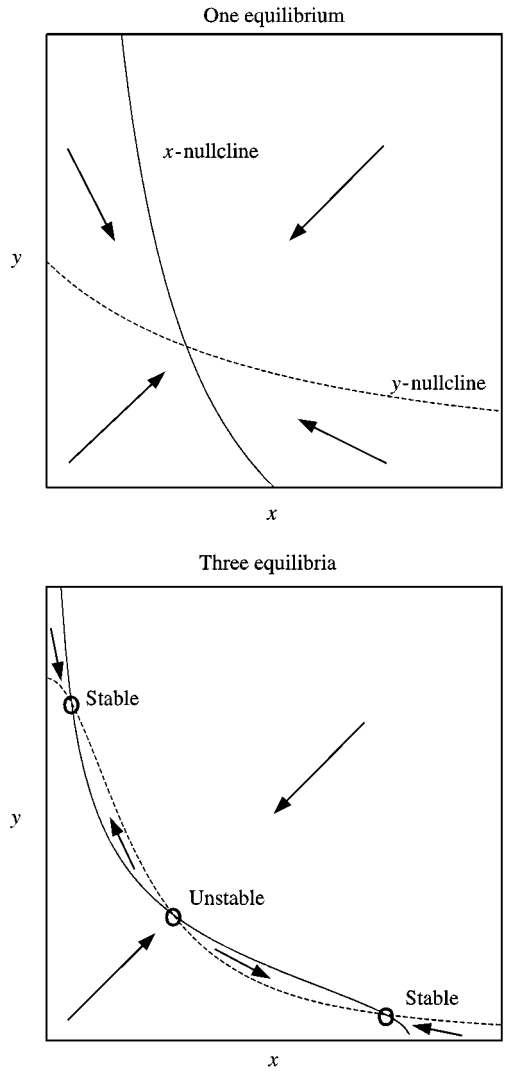

FIG. 2. The phase-plane in cases where (a) the repressor–repressor system does not work as a switch, and (b) where it does work.

where K_d is the equilibrium constant for dissociation of repressor from its operator site and k_1 is the maximum rate of protein production.

By measuring the concentration of repressor y in units of K_d, we can eliminate the parameter and write the rate of production of protein x as

$$f(y) = \frac{k_1}{1+y}. \tag{3}$$

Similarly, the rate of production of protein y is given by

$$g(x) = \frac{k_2}{1+x}, \tag{4}$$

where x has been measured in units of its K_d. With these functional forms, the repressor–represssor system becomes

$$\frac{dx}{dt} = \frac{k_1}{1+y} - \mu_1 x,$$

$$\frac{dy}{dt} = \frac{k_2}{1+x} - \mu_2 y. \tag{5}$$

The nullclines are defined by

$$x = \bar{f}(y) = \frac{k_1}{\mu_1}\left(\frac{1}{1+y}\right),$$

$$y = \bar{g}(x) = \frac{k_2}{\mu_2}\left(\frac{1}{1+x}\right). \tag{6}$$

A requirement for equilibrium can be written in terms of the composition of the nullcline functions:

$$\begin{aligned} x &= \bar{f}(\bar{g}(x)) \\ &= \frac{k_1}{\mu_1}\left(\frac{1}{1+\bar{g}(x)}\right) \\ &= \frac{k_1}{\mu_1}\left(\frac{1}{1+(k_2/\mu_2)\,(1/(1+x))}\right) \\ &= \frac{k_1}{\mu_1}\left(\frac{1+x}{1+(k_2/\mu_2)+x}\right). \end{aligned}$$

In this case, $\bar{f}(\bar{g}(x))$ is a linear fractional transformation (a ratio of linear functions) that has negative second derivative everywhere and cannot have multiple intersections with the diagonal for any value of the parameters. Repression described by a Michaelis–Menten function therefore cannot support a switch.

The Michaelis–Menten form is conventionally based on the approximation that the depletion of free repressor due to binding is insignificant. In the case of a single operator site per cell, the Michaelis–Menten form is exact and no switch can be made through mass action. We show below, however, that inclusion of the depletion

term can make a switch possible when there is more than one operator site per cell.

SUCCESS OF THE HILL FUNCTION

Suppose that the level of repression is described by a Hill function, which models cooperativity of binding. The repression function takes the form

$$f(y) = \frac{k_1}{1 + y^n}. \tag{7}$$

We will consider cooperativity in more detail below, but here show that values of $n > 1$ can support a switch for appropriate values of the parameters.

Substituting the Hill equation into the repressor–repressor system gives

$$\frac{dx}{dt} = \frac{k_1}{1 + y^n} - \mu_1 x,$$

$$\frac{dy}{dt} = \frac{k_2}{1 + x^n} - \mu_2 y. \tag{8}$$

Using the new parameters

$$\tilde{k}_1 = \frac{k_1}{\mu_1},$$

$$\tilde{k}_2 = \frac{k_2}{\mu_2},$$

the nullclines can be written

$$x = \bar{f}(y) = \frac{\tilde{k}_1}{1 + y^n},$$

$$y = \bar{g}(x) = \frac{\tilde{k}_2}{1 + x^n}. \tag{9}$$

The number of equilibria changes when the nullclines are tangent at an equilibrium, or when

$$\bar{f}'(y)\bar{g}'(x) = 1 \tag{10}$$

at an equilibrium. By taking derivatives, simplyfying, and solving eqns (9) and (10) simultaneously for $\tilde{k}_1$, $\tilde{k}_2$, and y in terms of x, we can find parametric curves for the critical values of $\tilde{k}_1$ and $\tilde{k}_2$ as

$$\tilde{k}_1 = \frac{n^2 x^{n+1}}{n^2 x^n - 1 - x^n}, \tag{11}$$

$$\tilde{k}_2 = \left(\frac{1 + x^n}{n^2 x^n - 1 - x^n}\right)^{1/n} (1 + x^n). \tag{12}$$

These equations take positive values for positive x only when the denominator $n^2 x^n - 1 - x^n$ is positive, or when $n > 1$ and $x^n > 1/(n^2 - 1)$. Graphs for various values of n are shown in Fig. 3, with multiple equilibria existing when $\tilde{k}_1$ and $\tilde{k}_2$ lie inside the cusp for that particular value of n. Large values of $\bar{k}_1$ and $\bar{k}_2$ are required for a functional switch when n is near 1, corresponding to large values of the rate constants k_1 and k_2, small values of the decay constants μ_1 and μ_2, or, less obviously, high values of repressor affinities that we have scaled out (low values of the dissociation constant K_d). Any of these conditions lead to high steady-state repressor activity.

The phase plane diagram shown in Fig. 2(b) describes the case where $n = 2$ and $\tilde{k}_1 = \tilde{k}_2 = 4$.

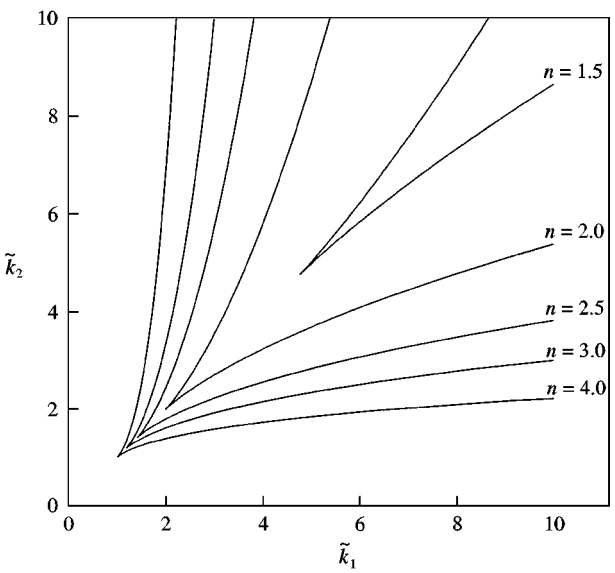

FIG 3. Values of the parameter $\tilde{k}_1 = \kappa_1/\mu_1$ and $\tilde{k}_1 = k_2/\mu_2$ (the ratio of the maximum rate of protein production to the rate of decay) needed for a working switch for particular values of the exponent n in the Hill function. The region inside each cusp works for that particular value of n.

The existence of multiple equilibria might seem to be a consequence of the sigmoidal shape of the nullclines, which generate a sigmoidal composition $\bar{f}(\bar{g}(x))$ (Fig. 4). Perhaps surprisingly, we will show that a sigmoidal repression curve is neither necessary nor sufficient for existence of a switch. In particular, we will construct switches with repression curves that are everywhere concave up, and exhibit whole families of sigmoidal repression curves that cannot support a switch for any choice of parameter values.

THE GENERAL THEORY: COMPUTATION OF $P(f)$

The Michaelis–Menten form of repression [eqn (5)] does not support multiple equilibria, and Hill functions [eqn (7)] with power $n > 1$ can support multiple equilibria for appropriate parameter values. In this subsection, we derive a general condition for whether a pair of repression functions f and g are able to act as a switch for some parameter values.

For each stable equilibrium of the system, the function $\bar{f}(\bar{g}(x))$ crosses the diagonal from above. In order for a continuous function to do this more than once, it must somewhere cross the diagonal from below. Therefore, a crossing from below, which corresponds to an unstable equilibrium is a necessary condition for the existence of multiple stable equilibria (a special case of the analysis in Appendix B). In conjuction with reasonable assumptions about the composition, namely that it is bounded and is non-negative for positive arguments, a crossing from below is also a sufficient condition for multiple stable equilibria. An (x, y) pair can correspond to a crossing from below only if $\bar{f}'(y)\bar{g}'(x) \geqslant 1$.

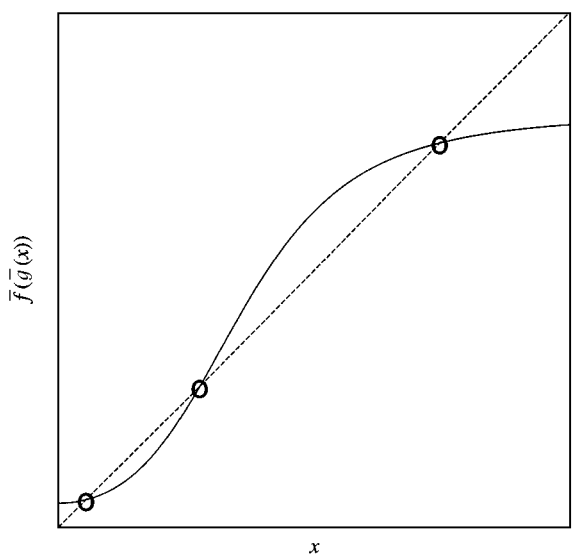

FIG. 4. The composition of two Hill functions is sigmoidal and the system can have multiple equilibria.

An unstable equilibrium (a crossing from below) must satisfy the following three conditions:

1. $\bar{f}(y^*) = x^*$.
2. $\bar{g}(x^*) = y^*$.
3. $\bar{f}'(y^*)\bar{g}'(c^*) \geqslant 1$.

Conditions 1 and 2 readily yield expressions that are equal to one. Multiplication of the left-hand side (l.h.s.) of condition 3 by these expressions gives

$$\left(\frac{y^*\bar{f}'(y^*)}{\bar{f}(y^*)}\right)\left(\frac{x^*\bar{g}'(x^*)}{\bar{g}(x^*)}\right) \geqslant 1. \qquad (13)$$

Note that this is equivalent to

$$\left(\frac{y^* f'(y^*)}{f(y^*)}\right)\left(\frac{x^* g'(x^*)}{g(x^*)}\right) \geqslant 1 \qquad (14)$$

because $\bar{f}$ and $\bar{g}$ are related to f and g by multiplication by a constant. Equation (13) is really a condition on the shapes of the functions, so for some purposes it is unimportant to distinguish between f and $\bar{f}$ or g and $\bar{g}$. We can express this condition more briefly using the following notation.

Definition 1.1. For any decreasing differentiable function, let

$$P(f) = \sup_{y>0}\left[\frac{-yf'(y)}{f(y)}\right].$$

We refer to $F(y) = (-yf'(y)/f(y))$ as the *auxiliary function* of f.

The auxiliary function of f is identical, except for its sign, to the "effective power function" (Clarke, 1980) or "reaction order" (Thron, 1991) of protein production with respect to repressor.

The functional P has several useful properties. First,

$$P(cf) = P(f)$$

for any positive constant c. Furthermore, for the function $f_c(y) = f(cy)$,

$$P(f_c) = \sup_{y>0}\left[\frac{-yf_c'(y)}{f_c(y)}\right]$$

$$= \sup_{y>0}\left[\frac{-cyf'(cy)}{f(cy)}\right]$$

$$= \sup_{y>0}\left[\frac{-yf'(y)}{f(y)}\right] = P(f).$$

The value of $P(f)$ thus depends only on the *shape* of the repression function, not on the magnitudes of the scaling parameters.

Suppose that $P(f)\cdot P(g) > 1$. We could then find values of x^* and y^* which satisfied inequality (14). Furthermore, we can solve conditions 1 and 2 for values of μ_1 and μ_2 for which (x^*, y^*) is indeed an equilibrium. Alternatively, we could adjust f and g by multiplying them by appropriate constants. This corresponds to changing promoter strengths and other determinants of maximal expression levels, or to changing the affinities of repressors for their operator sites. All of these possibilities amount to multiplying the functions $\bar{f}$ and $\bar{g}$ by constants.

We have shown the following proposition.

Proposition 1.1. *For any two repression functions $f(y)$ and $g(x)$, the system defined by differential equations*

$$\frac{dx}{dt} = f(y) - \mu_1 x,$$

$$\frac{dy}{dt} = g(x) - \mu_2 y$$

will have multiple equilibria for some values of μ_1 and μ_2 if $P(f)\cdot P(g) > 1$. Furthermore, if the system has multiple equilibria for some values of μ_1 and μ_2, the $P(f)\cdot P(g) \geqslant 1$.

We do not consider the case $P(f)\cdot P(g) = 1$ in detail. However, the proposition can be strengthened to include this case when the auxiliary functions for f and g have suprema which are not maxima. In this case, even though $P(f)\cdot P(g) = 1$, there are no values of x^* and y^* for which the product of the values of the auxiliary functions is greater than or equal to 1.

There is a useful graphical interpretation of $P(f)$. Consider the family of functions $y^p f(y)$ with derivatives equal to

$$\frac{d}{dy}[y^p f(y)] = y^{p-1} pf(y) + y^p f'(y)$$

$$= y^{p-1}(pf(y) + yf'(y)).$$

There is a critical point at some $y > 0$ if

$$p = \frac{-yf'(y)}{f(y)}.$$

Thus, $P(f)$ is the largest value of p for which the function $y^p f(y)$ has a critical point. In simple cases, $P(f)$ can be computed by inspection using this interpretation.

For the Michaelis–Menten form $f(y) = 1/(1+y)$ [eqn (3)] the function $y^p f(y)$ has a maximum for $p < 1$ and is increasing function for $p \geqslant 1$. Therefore, $P(f) = 1$. Alternatively, the auxiliary function is

$$\frac{-yf'(y)}{f(y)} = \frac{y}{1+y}$$

with supremum of 1. If $g(x)$ also has the Michaelis–Menten form, then $P(f)\cdot P(g) = 1$, but neither auxiliary function actually achieves the value 1. A pair of these functions will not work as a switch.

In contrast, with the Hill function

$$f(y) = \frac{1}{1+y^2},$$

the function $y^p f(y)$ has a maximum for $p < 2$ and is increasing for $p > 2$, implying that $P(f) = 2$.

Alternatively, the auxiliary function is

$$\frac{-yf'(y)}{f(y)} = \frac{2y^2}{1+y^2},$$

with a supremum of 2. There are thus parameter values for which a pair of such Hill functions can work as a switch. More generally, if

$$f(y) = \frac{1}{1+y^n},$$

then $P(f) = n$. A pair of Hill functions $f(y)$ and $g(x)$ must have $P(f)\cdot P(g) > 1$ to work as a switch. In the asymmetric case where $f(y) = 1/(1+y)$ and $g(x) = 1/(1+x^n)$ for $n > 1$, $P(f)\cdot P(g) = 1\cdot n > 1$. A combination of the Michaelis–Menten form with any Hill function with exponent greater than one can work as a switch with appropriate parameter values.

In each of these cases, we can compute $P(f)$ with the graphical method by finding the largest value of p for which $y^p f(y)$ approaches 0 as y approaches infinity. Although $y^p f(y)$ must have a critical point for this p (by the Extreme Value Theorem; Adler, 1998), it is possible for $y^p f(y)$ to have a critical point for larger values of p. Comparison of the degrees of the numerator and the denominator provides only a lower bound on the value of $P(f)$.

MULTIPLE OPERATOR SITES

Consider a gene whose upstream region contains multiple sites for repressor binding, each with the same repressor affinity. If the binding of repressor molecules to different sites is independent, and the effects of binding on transcription are additive (diminution of transcription rate is proportional to the number of repressors bound), then the system is indistinguishable (in terms of repression function) from that with a single site. An example of this situation would be a gene with multiple promoters, each with its own independently acting operator site.

Deviations from independence of binding or from additivity of effect will change the repression function and might yield a working switch. If the first bound repressor molecule facilitates the binding of the second, this is called *cooperativity of binding*. Cooperativity of binding is the mechanism underlying the Hill function. This phenomenon can also exist in the negative form, i.e. binding at one site can interfere with binding at another. In addition, the effect of binding of a single repressor molecule need not be precisely half the effect of binding of two repressor molecules. In particular, binding of a single repressor molecule might be sufficient to repress transcription almost completely. This is a likely state of affairs when multiple repressor binding sites are associated with a single promoter.

In this section, we provide a complete analysis of the case where two repressor molecules can bind to the target operator, and some indications of how these results extend to cases where more than two repressor molecules can bind.

Analysis of the Two-site Case

Binding can be described as in Fig. 5. K_1 and K_2 are the equilibrium constants for binding of the first and second repressor molecules and are equal to the ratios of the rate constants for the forward and reverse reactions.

If we ignore depletion due to binding and equate free repressor with total repressor, the relative quantities of states with 0, 1, and 2 bound repressors are given by $1 : K_1 x : K_1 K_2 x^2$, where x is the repressor concentration. To eliminate parameters, we scale x by $\sqrt{K_1 K_2}$ and define

$$b = \sqrt{\frac{K_1}{4K_2}} \tag{15}$$

(the factor of four is a "statistical factor" that accounts for the different numbers of occupied and unoccupied sites in each state; when binding is non-cooperative, $K_1 = 4K_2$). The three states

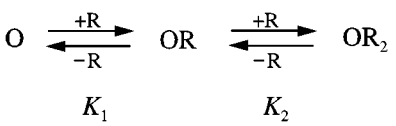

FIG. 5. Binding reactions for an operator site that contains two subsites, each of which can be bound by a repressor molecule. K_1 and K_2 are the equilibrium constants for the binding reactions.

then occur in the ratio $1:2bx:x^2$ and $b < 1$ indicates (positive) cooperativity of binding. With $b = 1$ there is no cooperativity, binding at the two sites is independent, and the three states occur in binomial proportions.

Suppose that genes with one repressor binding site occupied produce protein at rate r_1 and those with both sites occupied produce protein at rate r_2, where $1 \geqslant r_1 \geqslant r_2$. The total protein production in terms of the scaled x is

$$g(x) = \frac{1 + 2br_1x + r_2x^2}{1 + 2bx + x^2}. \tag{16}$$

Our goal is to find the set of values for which $P(g) > 1$.

We know that $P(g) \geqslant 1$ when $xg(x)$ has a critical point. The set of values where $P(g) = 1$ are those where that critical point is also a point of inflection. The equations

$$\frac{\mathrm{d}}{\mathrm{d}x} xg(x) = 0,$$

$$\frac{\mathrm{d}^2}{\mathrm{d}^2x} xg(x) = 0$$

can be expressed as a pair of simultaneous equations and solved for critical values of b and r_1 in terms of r_2 and x as

$$b = \frac{1 - 3r_2x^2}{2r_2x^3},$$

$$r_1 = \frac{x^4r_2^2(x^2 - 3)}{1 - 3r_2x^2}.$$

These are parametric equations for b and r_1 which take on nonnegative values for

$$\sqrt{3} \leqslant x \leqslant \sqrt{\frac{1}{3r_2}}.$$

This inequality immediately requires that $r_2 < 1/9$ to create a switch. With $r_2 = 0$, the critical values fall along the curve

$$4b^2r_1 = 1.$$

Contours for various values of $r_2 < 1/9$ are shown in Fig. 6. The region below each curve can support a switch for that particular value of r_2. Smaller values of each of the parameters, corresponding to more repression or more cooperativity of binding, are more conducive to making a working switch.

With no cooperativity of binding ($b = 1$), there can be a switch only if r_1 and r_2 lie below the critical parametric curve

$$r_1 = \frac{x^2 - 3}{2x(2x + 3)}, \tag{17}$$

$$r_2 = \frac{1}{x^2(2x + 3)} \tag{18}$$

(Fig. 7). Very small values of both r_1 and r_2 are required; repression must be almost complete even when only one repressor molecule is bound.

Several special cases help to illuminate these results. If $r_1 = 0$, indicating that binding at just one site is sufficient for complete repression, $P(g) > 1$ and the system can work as a switch for any b and $r_2 < 1/9$. Similarly, with $b = 0$ there is complete cooperativity of binding, sufficient to make the switch work for any feasible value of r_1 and $r_2 < 1/9$. The Hill function with $n = 2$ is the special case with $b = 0$ and $r_2 = 0$.

In the absence of cooperativity of binding ($b = 1$), the value of r_1 must be less than 0.25 even

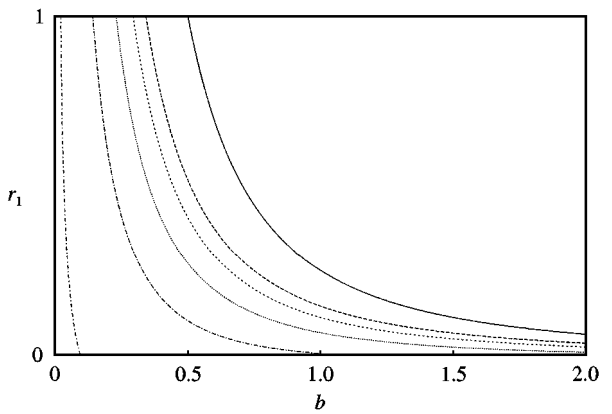

FIG. 6. Contours for six values of r_2 below which the switch operators. The values of r_2, from the highest to the lowest curve, are $r_2 = 0$, $r_2 = 1/160$, $r_2 = 1/80$, $r_2 = 1/40$, $r_2 = 1/20$, and $r_2 = 1/10$. The switch cannot work if $r_2 \geqslant 1/9$.

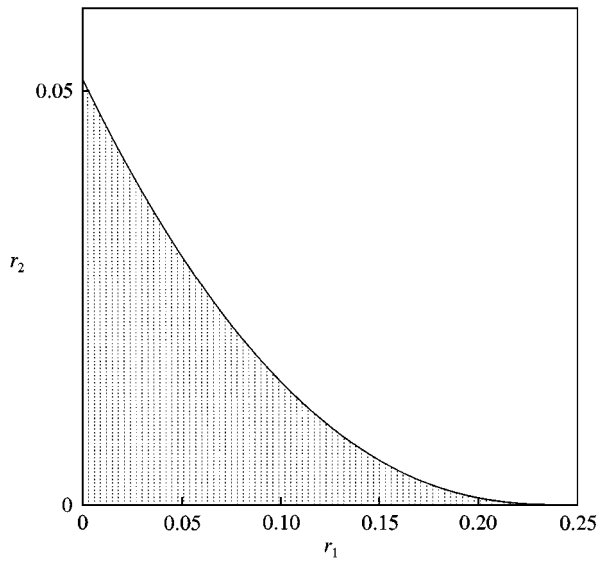

FIG. 7. The shaded region indicates the parameter values for which a switch can work with $b = 1$ (no cooperativity of binding).

in the best case $r_2 = 0$. For example, with $r_1 = 1$ and $r_2 = 0$, i.e. when both sites must be occupied for repression to occur, the switch does not work.

These special cases also provide examples showing that a sigmoidal repression function is neither necessary nor sufficient for making a switch. With $b = 1$, $r_1 = 1$, and $r_2 = 0$ the repression function is

$$g(x) = \frac{1 + 2x}{(1 + x)^2} \tag{19}$$

which has a sigmoidal graph. The auxiliary function is

$$G(x) = \frac{2x^2}{1 + 3x + 2x^2}$$

which never quite takes on its limiting value of 1. Like a pair of Michaelis–Menten functions, a pair of such repression functions could not work as a switch, even though both are sigmoidal.

Conversely, with $b = 1$, $r_1 = 0$ and $r_2 = 0$, the repression function

$$g(x) = \frac{1}{(1 + x)^2} \tag{20}$$

has a graph that is everywhere concave up. The auxiliary function is

$$\frac{-xg'(x)}{g(x)} = \frac{2x}{1 + x}$$

which takes on values greater than 1 for $x > 1$. We can build a switch with a pair of these functions by finding values of μ_1 and μ_2 for which the equations

$$\frac{1}{(1 + y)^2} = \mu_1 x,$$

$$\frac{1}{(1 + x)^2} = \mu_2 y$$

have solutions with $x > 1$ and $y > 1$. If we pick $x = y$, this works for any values $\mu_1 = \mu_2 < 1/4$.

More than Two Sites

With more than two sites, finding general results becomes difficult due to the plethora of parameters. Certain special cases are approachable and yield instructive results.

One analysable case is where binding at n sites is completely cooperative (either zero or all n sites are occupied) and repressor binding inhibits transcription completely. This case is described by the Hill functions analysed above, where it was shown that $P(f) = n$.

Another analysable case is where binding at n sites is non-cooperative and only the completely unbound state is transcribed. The shape of the repression function is given by

$$f(y) = \left(\frac{1}{1 + y}\right)^n.$$

It can be shown that in this case too $P(f) = n$. This is an instance of a general property of the functional P, namely that $P(f^n) = nP(f)$ for any function f. In the limit as the number of sites goes to infinity, the fraction of genes in the unbound state is given by the zero term of the Poisson distribution, and hence has the form $f(y) = e^{-y}$. The auxiliary function is the linear function y, so that $P(f) = \infty$. Any equilibrium with $xy > 1$ is sufficient to make a switch. This provides another

example of a non-sigmoidal (concave up) repression function that can produce a switch.

THE EFFECT OF DEPLETION OF FREE REPRESSOR

The use of the Michaelis–Menten form to describe binding usually rests on an approximation in which depletion of one free component due to binding is ignored. If depletion of free repressor is taken into account, a different family of repression functions results. The ability of these exact functions to form a switch must be investigated.

For simplicity, we assume that the decay rates of free and bound repressor are equal. Decay is therefore linear in total repressor concentration, and the dynamics are described by an instance of system (1). The more general case could be analysed using the results of Appendix B.

The standard treatment of chemical equilibria, which does not apply to isolated cells with a small number of binding sites each, is based on the equilibrium condition

$$\frac{[\mathrm{OR}]}{[\mathrm{O}][\mathrm{R}]} = K_{eq}, \tag{21}$$

where [OR], [O], and [R] are the concentrations of bound operator, free operator, and free repressor, respectively. Suppose total concentrations of repressor (y) and operator (D) are

$$y = [\mathrm{R}] + [\mathrm{OR}],$$

$$D = [\mathrm{O}] + [\mathrm{OR}].$$

The equilibrium condition [eqn (21)] leads to a quadratic equation in the concentration of bound operator sites. If concentrations are measured on a scale such that $K_{eq} = 1$, the relevant solution for $f(y)$, the equilibrium fraction of operator sites that are not bound by repressor, is

$$f(y) = \frac{1}{2D}\left[-y + D - 1 + \sqrt{y^2 - 2yD + 2y + D^2 + 2D + 1}\right]. \tag{22}$$

The rate of transcription is proportional to the fraction $f(y)$. The shape of this function varies with the parameter D; as D becomes small, f approaches the Michaelis–Menten form.

The auxiliary function for $f(y)$, $F(y) = -yf'(y)/f(y)$, has a single critical point at $y = (D+1)^2/D - 1$. A nonnegative critical point exists only for $D > 1$. When $D \leqslant 1$, $F(y)$ is increasing for nonnegative y and the relevant supremum of $F(y)$ is given by $\lim_{y\to\infty} F(y)$, which is always equal to one. Thus for $D \leqslant 1$, $P(f) = 1$, just as for the Michaelis–Menten form. For $D > 1$, $P(f) > 1$ and two such functions can form a switch.

It would seem from the above that mass action is, under some circumstances, a sufficient mechanism to make a working switch. However, eqn (21) applies only when the number of operator sites is large. In reality, each cell contains only a few operator sites. If there is just one operator site in each cell, as there would often be in a haploid cell with one operator site per genome, the Michaelis–Menten form is exact (depletion of repressor, which affects only the rate of binding, occurs only when the single site is already occupied). Mass action therefore cannot suffice to make a switch in such a case. It may be countered, however, that at least one of the repressors will regulate other genes, the ultimate targets of regulation, and that therefore there will be at least two operator sites for one of the repressors. The presence of two operator sites is sufficient to raise P above one (analysis not shown), and a working switch could be constructed with appropriate parameters. As the number of operator sites in each cell becomes large, the repression function approaches eqn (22).

Discussion

A two-repressor switch can be made to function, but only with appropriate choice of repression functions and constant parameters. A simple graphical method, similar to that used by Collier *et al.* (1996), allows assessment of the steady states of such a system. This method involves only two functions, each of which describes the steady-state level of one repressor when the level of the other is held constant. While these functions do not completely characterize the dynamics of the system, they are all that we need to know to describe the number, position, and

stability of the system's steady states. This conclusion holds equally well for a four-dimensional system that models mRNA in addition to protein concentrations (Appendix A). It also holds for a more general class of systems than eqn (1) (Appendix B). It is sufficient that $\bar{f}$ and $\bar{g}$ can be defined, i.e. that if one repressor's concentration is held constant, the other's reaches a unique stable equilibrium.

This method leads to a useful characterisation of functions, or rather shapes of functions. A single number, P, characterizes a family of functions with the same shape. In order for a pair of functions to operate together as a switch, it is necessary that the product of the values of P for their families is at least one. If this product is greater than one, a switch can be made using functions from these families if parameter values are chosen appropriately.

MECHANISMS FOR MAKING A SWITCH

A repressor–repressor system with a single repressor-binding site per gene fails as a switch if binding is well described by a Michaelis–Menten equation. However, we have found several features that can enable a regulatory system to function as a switch.

Positive cooperativity of binding is one such feature. It can result from non-independent binding at two adjacent operator sites, but a similar phenomenon might result if repressor functions only as a dimer and the monomer–monomer affinity is sufficiently weak. The model for Delta-Notch signaling presented by Collier *et al.* (1996) depends implicitly on cooperativity of binding for its bistability. Several models of bistable systems involving only positive regulation (Tyson & Othmer, 1978; Wolf & Eeckman, 1998) also invoke cooperativity of binding.

A switch can also work when binding at any one of multiple sites is sufficient for strong repression, even if there is no cooperativity of binding. A simple case of this is where binding at either of two operator sites is sufficient to inhibit transcription completely. This phenomenon is responsible for the bistability of Keller's (1995) model "E" with the parameter values he analysed.

Simple mass action with a single repressor binding site per gene copy can also be sufficient for a switch if depletion of free repressor is significant. For this to work there must be more than one copy of the operator per cell, and the dissociation constant for operator binding must be sufficiently small. The latter requirement might present a problem for the robustness of the switch to stochastic fluctuations, as we discuss next.

ARE YOU A GOOD SWITCH OR A BAD SWITCH?

Our analysis has focused on the *existence* of multiple steady states. While this is a *sine qua non* for a switch, additional considerations are relevant to the quality of the switch.

One important feature is separation of the steady states, as illustrated in Fig. 8(b). Real switches are subject to stochastic fluctuations or noise (McAdams & Arkin, 1997). If the steady states are close to one another, or rather if either is close to the curve that separates the two basins of attraction, then the switch will have poor immunity to noise. Even in the absence of noise it may be desirable to have a large separation between the equilibria. The purpose of the switch is to bring about changes in the expression levels of other genes. The expression levels of the objects of regulation will not be greatly affected by the states of the switch if the steady states are close. While downstream elements of the regulatory circuitry might "sharpen" the distinction between the states, it is simpler and perhaps better if the switch itself provides good separation.

Another robustness criterion applies even to systems with identical steady states. The very existence of the switch may be threatened by real-world fluctuations in parameters. For example, a change of temperature will likely affect the equilibrium constant for repressor binding, and genetic background may affect all of the parameters. With poor transversality of the nullclines [Fig. 8(c)], a slight change to the system can lead to loss of a steady state. Even a transient loss of the switch will lead to loss of the information stored by the switch. With highly transverse nullclines [Fig. 8(a)], the system can tolerate more movement of the nullclines without the disappearance of a steady state.

Stochastic fluctuations in protein levels arise in part because the number of protein and mRNA molecules is finite. Therefore, a switch

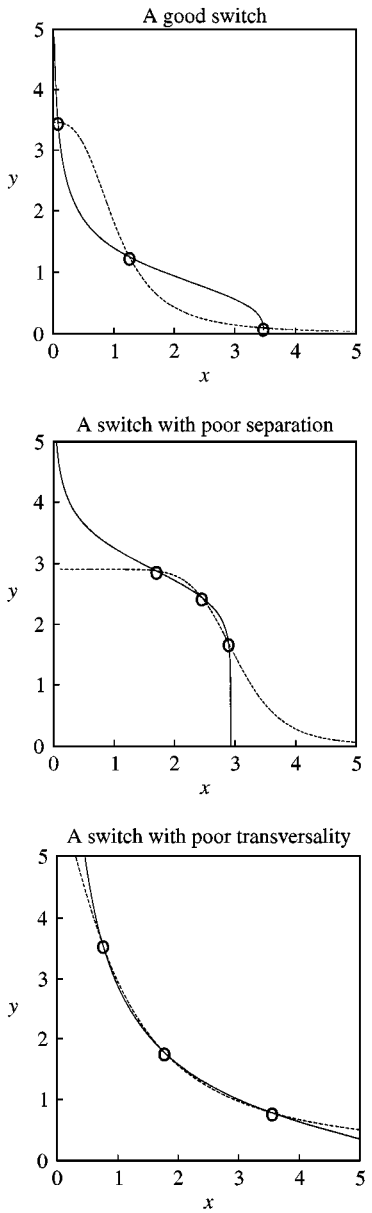

FIG. 8. A good switch, and two types of bad switch.

with high concentrations of low-affinity repressors might be superior to one with low concentrations of high-affinity repressors. The advantage of noise reduction must be balanced against the cost of additional protein production, but this might be quite small. Note that these alternatives are not distinguished by our nondimensionalized equations. If the number of operator sites per cell is specified, our description of mass action with significant depletion of free repressor does distinguish these possibilities. The cases in which simple mass action can form a switch require that the dissociation constant is not large compared to the concentration of repressor sites, which will usually be only a few sites per cell. A consequence is that just a few repressor molecules in a cell will have a significant effect on transcription. This may reduce the robustness of the switch to stochastic events.

REGULATION OF BACTERIOPHAGE λ

Bacteriophage λ is a virus that infects the bacterium *Escherichia coli*. Various aspects of λ gene regulation have been subjects of theoretical and numerical studies (Ackers *et al.*, 1982; Shea & Ackers, 1985; McAdams & Shapiro, 1995). A portion of the λ regulatory circuitry approximates a two-repressor switch (Ptashne, 1992). In reality, this λ system is somewhat more complicated than those we have been discussing because each of the two proteins involved can affect its own rate of synthesis in addition to that of the other protein.

Upon infecting a cell, λ can proceed along two different pathways. In the lytic mode, λ immediately begins directing the host cell's machinery toward making more λ phage. Cell lysis and death follow in a relatively short period of time. In the lysogenic pathway, λ integrates its DNA into the host chromosome. The integrated λ DNA, referred to as a prophage, replicates along with the host DNA indefinitely. In the lysogen, the only phage-encoded protein produced is CI, also known as λ repressor. CI represses the synthesis of all other λ-encoded proteins, including the repressor Cro.

When the "SOS response" of the cell occurs, indicating DNA damage, the prophage is "induced" to excise itself from the chromosome, produce more phage, and eventually lyse the cell. Induction occurs because RecA, altered by the SOS response, cleaves the CI protein. This allows production of Cro, which in turn represses synthesis of CI.

It is not obvious that the CI-Cro network needs to form a switch in the sense of having two stable equilibria. It might be sufficient, from the phage's point of view, for it to react to the current status of the SOS response without any memory. On the other hand, abortive induction by a transient SOS response might be disastrous for the

phage, and a mechanism for irrevocable commitment to excision and lysis might be highly advantageous. Furthermore, part of the phage's repertoire, namely entry into the lytic pathway upon infection, involves repression of CI synthesis by Cro despite the absence of an SOS response.

Experiments with artificial λ-derived constructs suggest that the two-protein system does constitute a switch in our sense (Toman *et al*., 1985). Presumably, this is part of the reason that two regulatory proteins, rather than a single repressor as in the *lac* operon, are involved in the switch from lysogeny to lysis.

The CI protein represses the production of Cro, and Cro represses the production of CI. In addition to this mutual repression, CI activates its own synthesis, and each protein can inhibit its own synthesis, although this inhibition only becomes important at relatively high concentrations. The genes that encode the CI and Cro proteins are adjacent and are transcribed, in opposite directions, from the region between the two genes. A compound operator site (O_R), consisting of three subsites (O_R1, O_R2, and O_R3), is located between the genes and is involved in their regulation. Both CI and Cro have affinities for all three operator subsites, but they have different preferences among the sites. Several features of the system can be identified that promote the existence of two stable equilibria and hence a working switch:

- Binding of CI to either O_R1 or O_R2 is sufficient to turn off production of Cro. As discussed above, this effect alone can lead to the existence of two stable equilibria.
- Binding of CI to O_R1 and O_R2 exhibits a positive cooperativity. This effect could be sufficient for the existence of a switch even if only one of the sites were involved in turning off transcription.
- Like most bacterial repressors, functional CI is a dimer. However, CI is unlike many other repressors in that the affinity of monomers for each other is sufficiently low that monomers are common over part of the physiological range of protein concentrations. The need for dimerization can be considered to be a form of cooperativity of binding. This effect alone could yield a switch, even if there were only one binding site for repressor dimer.
- CI activates its own synthesis. This effect, when combined with simple mutual repression described by Michaelis–Menten functions, would be sufficient to yield a switch (analysis not shown).

It is gratifying that this λ regulatory system has some features that we have predicted to support a switch. It is striking that the system possesses at least four switch-promoting features when any one would suffice for the existence of a switch. As discussed above, though the existence of two stable equilibria is sufficient for a switch, other considerations are important for the quality of the switch.

We thank an anonymous reviewer for comments that greatly improved this paper.

REFERENCES

ACKERS, G. K., JOHNSON, A. D. & SHEA, M. A. (1982). Quantitative model for gene regulation by λ phage repressor. *Proc. Nat. Acad. Sci.* **79,** 1129–1133.

ADLER, F. R. (1998). *Modeling the Dynamics of Life: Calculus and Probability for Life Scientists*. Pacific Grove: Brooks/Cole.

CLARKE, B. L. (1980). Stability of complex reaction networks. In: *Advances in Chemical Physics* (Prigogin, I. & Rice, S. A., eds), Vol. XLIII, pp. 1–215. New York: John Wiley & Sons.

COLLIER, J. R., MONK, N. A. M., MAINI, P. K. & LEWIS, J. H. (1996). Pattern formation by lateral inhibition with feedback: a mathematical model of delta-notch intercellular signalling. *J. theor. Biol.* **183,** 429–446.

EDELSTEIN-KESHET, L. (1988). *Mathematical Models in Biology*. New York: Random House.

KELLER, A. D. (1995). Model genetic circuits encoding autoregulatory transcription factors. *J. theor. Biol.* **172,** 169–185.

LEVIN, S. A., LEVIN, J. E. & PAINE, R. T. (1997). Snowy owl predation on short-eared owls. *Condor* **79,** 395.

MCADAMS, H. H. & ARKIN, A. (1997). Stochastic mechanism in gene expression. *Proc. Nat. Acad. Sci.* **94,** 814–819.

MCADAMS, H. H. & SHAPIRO, L. (1995). Circuit simulation of genetic networks. *Science* **269,** 650–656.

MONOD, J. & JACOB, F. (1961). General conclusions: Teleonomic mechanisms in cellular metabolism, growth, and differentiation. In: *Cold Spring Harbor Symp. Quant. Biol*., Vol. 26, pp. 389–401. Long Island Biological Association.

MURRAY, J. D. (1993). *Mathematical Biology*. New York: Springer-Verlag.

PTASHNE, M. (1992). *A Genetic Switch*. Cambridge: Cell Press and Blackwell Scientific Publications.

RAZIN, A. & CEDAR, H. (1993). DNA methylation and embryogenesis. In: *DNA Methylation: Molecular Biology and Biological Significance* (Jost, J. P. & Saluz, H. P., eds), pp. 343–357. Basel, Switzerland: Birkhauser-Verlag.
SHEA, M. A. & ACKERS, G. K. (1985). The O_r control system of bacteriophage λ: a physical–chemical model for gene regulation. *J. Mol. Biol.* **181,** 211–230.
THIEFFRY, D. & THOMAS, R. (1995). Dynamical behaviour of biological regulatory networks—II. Immunity control in bacteriophage lambda. *Bull. Math. Biol.* **57,** 277–297.
THOMAS, R. (1978). Logical analysis of systems comprising feedback loops. *J. theor. Biol.* **73,** 631–656.
THOMAS, D. & D'ARI, R. (1990). *Biol. Feedback*. Boca Raton, FL: CRC Press.
THOMAS, R., THIEFFRY, D. & KAUFMAN, M. (1995). Dynamical behaviour of biological regulatory networks—I. Biological role of feedback loops and practical use of the concept of the loop-characteristic state. *Bull. Math. Biol.* **57,** 247–276.
THRON, C. D. (1991). A model for a bistable biochemical trigger of mitosis. *Science* **254,** 122–123.
THRON, C. D. (1995). A model for a bistable biochemical trigger of mitosis. *Biophys. Chem.* **57,** 239–251.
TOMAN, Z., DAMBLY-CHAUDIERE, C., TENENBAUM, L. & RADMAN, M. (1985). A system for detection of genetic and epigenetic alterations in *Escherichia coli* induced by DNA-damaging agents. *J. Mol. Biol.* **186,** 97–105.
TYSON, J. J. & OTHMER, H. G. (1978). The dynamics of feedback control circuits in biochemical pathways. In: *Progress in Theoretical Biology* (Rosen, R. & Snell, F. M., eds), Vol. 11, pp. 1–62. New York: Academic Press.
WOLF, D. M. & EECKMAN, F. H. (1998). On the relationship between genomic regulatory element organization and gene regulatory dynamics. *J. theor. Biol.* **195,** 167–186.

APPENDIX A

Much gene regulation, including that mediated by repressor proteins, occurs at the level of transcription. A proper model of such a system must take account of the dynamics of mRNA as well as protein concentrations. Suppose that the rate of protein production is proportional to the concentration of the mRNA that encodes it, that the rate of mRNA production is a function of the concentration of the repressor protein that regulates it, and that all components decay with first-order kinetics. If N and M are the concentrations of the mRNAs measured on an appropriate scale, then the two-repressor system is described by the system

$$\frac{dx}{dt} = N - \mu_1 x,$$

$$\frac{dN}{dt} = f(y) - \delta_1 N,$$

$$\frac{dy}{dt} = M - \mu_2 y,$$

$$\frac{dM}{dt} = g(x) - \delta_2 M. \tag{A.1}$$

The behavior of this system is similar in many ways to that of system 1. If y is held constant, then N reaches a stable equilibrium at $f(y)/\delta_1$, and x will then settle at $N/\mu_1 = f(y)/\mu_1\delta_1$. Thus, if the concentration of one repressor is held constant, that of the other will settle at some value. It is therefore possible to define $\bar{f}(y)$ as the equilibrium value of x when y is held constant, and analogously define $\bar{g}$. For the four-dimensional model,

$$\bar{f}(y) = \frac{f(y)}{\mu_1\delta_1},$$

$$\bar{g}(x) = \frac{g(x)}{\mu_2\delta_2}. \tag{A.2}$$

In the four-dimensional case, as in the two-dimensional simplification, $\bar{f} = c_1 f$ and $\bar{g} = c_2 g$. Furthermore, it is again a necessary and sufficient condition for an equilibrium of the system that $\bar{f}(\bar{g}(x)) = x$. Crossings of the diagonal by the function $\bar{f}(\bar{g}(x))$ therefore correspond exactly to the equilibria of the system. Recall that for the two-dimensional system, crossings from below correspond to unstable equilibria whereas crossings from above represent stable ones. The stability of the four-dimensional system can be evaluated using the Routh–Hurwitz criteria (Murray, 1993). The Jacobian matrix for system (A.1) is

$$\begin{bmatrix} -\mu_1 & 1 & 0 & 0 \\ 0 & -\delta_1 & f'(y) & 0 \\ 0 & 0 & -\mu_2 & 1 \\ g'(x) & 0 & 0 & -\delta_2 \end{bmatrix}.$$

The characteristic polynomial of this matrix is

$$\lambda^4 + (\delta_1 + \delta_2 + \mu_2 + \mu_1)\lambda^3$$

$$+ (\mu_1\delta_1 + \delta_1\delta_2 + \delta_1\mu_2 + \mu_2\delta_2 + \mu_1\delta_2 + \mu_1\mu_2)\lambda^2$$

$$+ (\mu_1\delta_1\delta_2 + \mu_1\delta_1\mu_2 + \delta_1\mu_2\delta_2 + \mu_1\mu_2\delta_2)\lambda$$

$$+ \mu_1\delta_1\mu_2\delta_2 - f'(y)g'(x).$$

The Routh–Hurwitz criteria for stability involve the non-leading coefficients of the characteristic polynomial, designated a_1–a_4. For system (A.1),

$$a_1 = \delta_1 + \delta_2 + \mu_2 + \mu_1,$$

$$a_2 = \mu_1\delta_1 + \delta_1\delta_2 + \delta_1\mu_2 + \mu_2\delta_2 + \mu_1\delta_2 + \mu_1\mu_2,$$

$$a_3 = \mu_1\delta_1\delta_2 + \mu_1\delta_1\mu_2 + \delta_1\mu_2\delta_2 + \mu_1\mu_2\delta_2,$$

$$a_4 = \mu_1\delta_1\mu_2\delta_2 - g'(x)f'(y).$$

The criteria for stability are

$$a_1 > 0,$$

$$a_1 a_2 > a_3,$$

$$a_1 a_2 a_3 > a_3^2 + a_1^2 a_4,$$

$$a_4 > 0.$$

The first two of these criteria are met by virtue of the fact that δ_1, δ_2, μ_1, and μ_2 are all positive. The third criterion is equivalent to

$$(\mu_2+\mu_1)(\delta_2+\mu_2)(\delta_2+\mu_1)(\mu_2+\delta_1)(\delta_1+\mu_1)(\delta_1+\delta_2)$$

$$+ (\delta_1 + \delta_2 + \mu_2 + \mu_1)^2 f'(y)g'(x) > 0.$$

This condition is guaranteed to be satisfied when the signs of f' and g' are the same, as must always be the case for a repressor–repressor or activator–activator system. The fourth condition, $a_4 > 0$, is equivalent to

$$\mu_1\delta_1\mu_2\delta_2 - f'(y)g'(x) > 0.$$

We can rewrite the inequality as

$$\frac{f'(y)}{\mu_1\delta_1}\frac{g'(x)}{\mu_2\delta_2} < 1$$

or

$$f'(y)\bar{g}'(x) < 1.$$

At an equilibrium point, $y = \bar{g}(x)$, and the condition is equivalent to

$$\bar{f}'(\bar{g}(x))\bar{g}'(x) < 1.$$

The l.h.s. of this inequality is the derivative of the function $\bar{f}(\bar{g}(x))$. Thus when $\bar{f}(\bar{g}(x))$ crosses the diagonal with derivative less than one, the equilibrium is stable, whereas when it crosses with derivative greater than one it is unstable (we leave unanalysed the case where an intersection occurs with derivative equal to one). Thus, our conclusions regarding the ability of different shapes of repression functions to make a switch continue to hold when the dynamics of mRNA are taken into account.

APPENDIX B

System 1 does not apply when decay is other than first order or when proteins affect the rates of their own synthesis. Nonetheless, many biologically relevant instances of the more general form

$$\frac{dx}{dt} = f(x, y),$$

$$\frac{dy}{dt} = g(x, y) \qquad \text{(B.1)}$$

will have the property that if the concentration of one of the proteins is held constant, that of the other will settle at a unique value. This occurs when the one-dimensional system $dx/dt = f(x, y)$, with constant y, always has exactly one equilibrium in the range of interest, and this equilibrium is stable, and the symmetrical criteria hold for $dy/dt = g(x, y)$. Let the one-dimensional equilibrium points be given by $\bar{f}$ and $\bar{g}$, respectively [$\bar{f}(y)$ and $\bar{g}(x)$ are the x and y nullclines of the two-dimensional system]. The equilibria of the two-dimensional system are exactly those points where both $x = \bar{f}(y)$ and $y = \bar{g}(x)$. At such points $x = \bar{f}(\bar{g}(x))$, just as in the special case discussed in the main text [system (1)], and equilibria correspond to crossings of the diagonal by $\bar{f}(\bar{g}(x))$. The stability of an equilibrium can be evaluated based

on the Jacobian matrix of the system, namely

$$\begin{bmatrix} f_1(x, y) & f_2(x, y) \\ g_1(x, y) & g_2(x, y) \end{bmatrix}$$

where the f_i and g_i are the partial derivatives of f and g. From the supposition that the one-dimensional equilibria are stable, we know that f_1 and g_2 are non-positive. We will ignore the cases where they are equal to zero. The trace of the matrix must be negative. The stability of an equilibrium then depends on the value of the determinant. If the determinant is positive, the equilibrium is stable. If it is negative, the equilibrium is unstable (in particular it is a saddle point). The condition for stability is therefore

$$f_1(x, y)g_2(x, y) - f_2(x, y)g_1(x, y) > 0.$$

Because the one-dimensional equilibria are assumed to be stable, $f_1(x, y)$ and $g_2(x, y)$ must both be negative at any equilibrium of the full system. Rearrangement gives

$$\left(\frac{f_2(x, y)}{f_1(x, y)}\right)\left(\frac{g_1(x, y)}{g_2(x, y)}\right) < 1.$$

At equilibrium points, $y = \bar{g}(x)$ and $x = \bar{f}(y)$, and the condition can be written

$$\left(\frac{f_2(\bar{f}(y), y)}{f_1(\bar{f}(y), y)}\right)\left(\frac{g_1(x, \bar{g}(x))}{g_2(x, \bar{g}(x))}\right) < 1. \qquad \text{(B.2)}$$

Note that

$$f(\bar{f}(y), y) = 0$$

and therefore

$$\frac{\mathrm{d}(f(\bar{f}(y), y))}{\mathrm{d}y} = f_1(\bar{f}(y), y)\bar{f}'(y) + f_2(\bar{f}(y), y) = 0.$$

It follows that

$$\frac{f_2(\bar{f}(y), y)}{f_1(\bar{f}(y), y)} = -\bar{f}'(y)$$

at an equilibrium point, and similarly that

$$\frac{g_1(x, \bar{g}(x))}{g_2(x, \bar{g}(x))} = -\bar{g}'(x).$$

Substitution into eqn (B.2) yields

$$\bar{f}'(y)\bar{g}'(x) < 1$$

or, because this is an equilibrium,

$$\bar{f}'(\bar{g}(x))\bar{g}'(x) < 1.$$

The l.h.s. of this inequality is the derivative of the function $\bar{f}(\bar{g}(x))$. Therefore when this composition crosses the diagonal from above, except with derivative equal to one, the corresponding equilibrium is stable. Where it crosses from below with derivative not equal to one, the equilibrium is unstable. Our analysis of the shapes of functions, including our condition on the products of the values of P for two functions, therefore can be extended to all systems of the form of system (B.1) where $\bar{f}$ and $\bar{g}$ exist. In the special case given by system (1), $\bar{f}$ was proportional to f, $P(f)$ was equal to $P(\bar{f})$, and we freely interchanged f and $\bar{f}$ in our analysis of functional shapes. In the more general case, such interchange is not possible, and only the shapes of the nullclines can be analysed. The condition for a working switch is $P(\bar{f}) \cdot P(\bar{g}) > 1$. When this condition holds, bistability is possible with nullclines of the forms $k_1\bar{f}(y)$ and $k_2\bar{g}(x)$ for some values of k_1 and k_2. These are the nullclines of $f(x/k_1, y)$ and $g(x, y/k_2)$.