

# BIOCHEMICAL REGULATION

In this chapter we study the ‘modules’ of biochemical regulation. Our treatment follows that of Tyson, *et al.* [1, 2]. These modules are components of biochemical control systems (“systems biology” is the current buzz word). Assembled into a biochemical circuit, they can represent many cellular processes.

## Signaling via covalent modification

Many enzymes are regulated by attaching (via a kinase) or removing (via a phosphatase) a

phosphate group:  $E \xrightleftharpoons[\text{Phosphatase}]{\text{Kinase}} EP$ . Generally, Kinases are very specific, while phosphatases

are promiscuous. The activity of the kinase can be viewed as an *input*, or *stimulus* to the covalent ‘switch’, and the amount of phosphorylated enzyme the *output*, or *response*. Figure 1a shows the kinetic circuit in the usual informal notation; Figure 1 shows the corresponding Madonna Flowchart.

We can reduce the system to a single reservoir if we assume that *the total amount of enzyme is constant*, so that we can use as the only variable (reservoir) the amount of phosphorylated

enzyme. That is, since  $E + EP = E_T$ , the above reaction becomes  $E_T - EP \xrightleftharpoons[\text{Phosphatase}]{\text{Kinase}} EP$ . Then

the diagram in Figure 1a can be abbreviated as shown in Figure 1b.

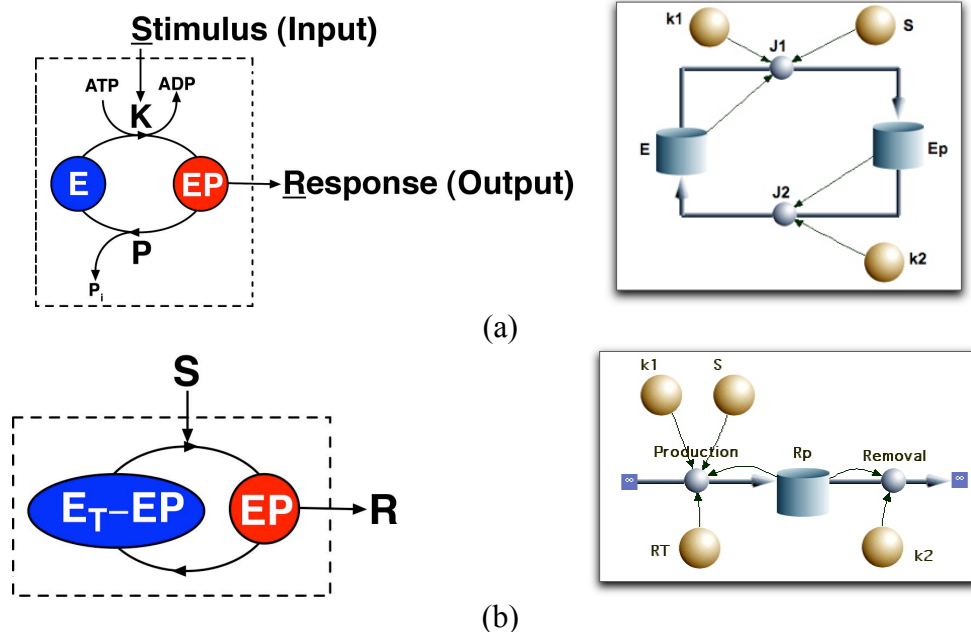
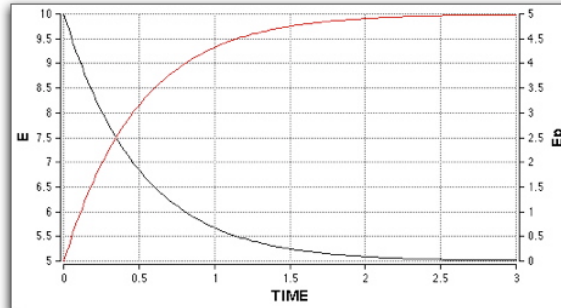


Figure 1. Diagramming covalent modification. (a) Diagram and Madonna Flowchart for the phosphorylation and dephosphorylation of the enzyme E. (b) Reduction of (a) to a single variable using the constraint  $E + E_p = E_T = \text{constant}$ .

In keeping with the notion of stimulus-response behavior, let us switch notation and denote the rate of the kinase by **S** (the *stimulus*), and the amount of phosphorylated (active) enzyme by **R** (the *response*).<sup>1</sup> This can be represented by the Flowchart shown in Figure 1b corresponding to the equation

$$\frac{dR}{dt} = k_f(R_T - R) - k_r R$$



(1)

Exercise 1. Construct the Flowchart in Figure 1b and obtain the corresponding equation (1). Plot the solution using  $k_1 = 1$ ,  $k_2 = 0.5$ ,  $R(0) = 100$ ,  $R_T = 200$ .

Exercise 2. In a separate model, plot the curve  $dR/dt$  vs.  $R$ . (Hint: RENAME TIME =  $R$ ,  $y = (k_1 * S * (R_T - R)) - (k_2 * R)$ , plot  $y$  vs.  $R$  and change the graph labels by clicking on the axes). It should look like Figure 2a.

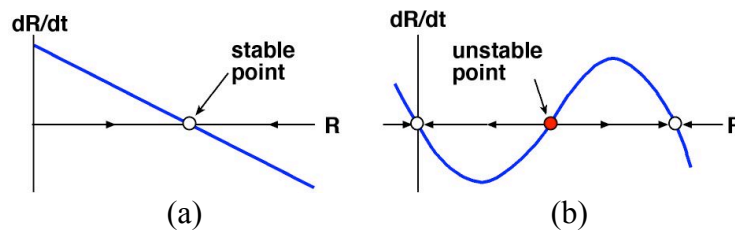


Figure 2. Plotting the rate of a reservoir vs. its content shows whether the equilibrium (Rate = 0) is **stable** or **unstable**. (a) The simple linear system in Figure 1b. (c) A nonlinear system; e.g.  $dR/dt = aR(R-1)(R-2)$ , where  $a$  is a tunable constant. This system shows ‘switch-like’ behavior.

## Signal-response behavior

### Linear

An enzyme (the response,  $R$ ) is synthesized at a basal rate  $k_0$  and degraded with a rate constant  $k_2$ . Its production is enhanced by a rate  $k_1 S$ , where  $S$  is a synthetic enzyme. The system can be diagrammed as shown in Figure 3a.

<sup>1</sup> Of course, many enzymes are ‘normally on’ and are switched off by phosphorylation. However, the modeling proceeds exactly the same.

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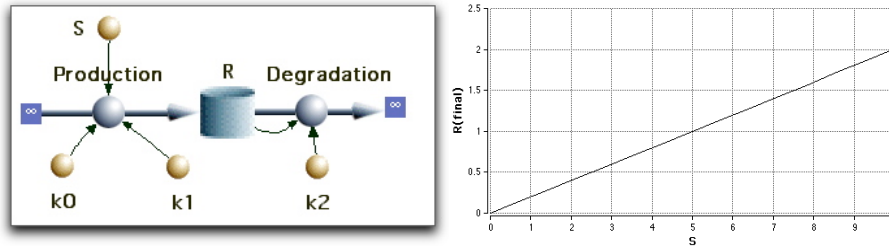


Figure 3. Linear stimulus-response system. (a) Flowchart. (b) Linear response curve:  $R(\text{final})$  vs.  $S$ .

Exercise 3. Write out the equations for the Flowchart in 'normal' mathematical notation:  $dR/dt = f(R, S)$ , where  $R$  is the variable, and  $S$  is a parameter. Find the steady state value of the response,  $R$  by setting reservoir rate  $dR/dt = 0$ . Plot the time behavior and stimulus response behavior (Figure 3b) using the parameter values  $k_0 = 0.01$ ,  $k_1 = 1$ ,  $k_2 = 5$ ,  $S = 10$ ,  $R(0) = 1$ .

### Hyperbolic

The phosphorylation-dephosphorylation system discussed above is re-diagrammed in Figure 4. From the Flowchart, the equation governing the amount of phosphorylated enzyme (i.e. the reservoir,  $R$ ) is:

$$\frac{dR}{dt} = k_1 S (R_T - R) - k_2 R \quad (2)$$

Exercise 4. Plot the time and response behavior of the system using the parameter values  $k_1 = k_2 = 1$ ,  $R_T = 1$ ,  $S = 1$ . Solve equation (2) for the steady state of the response,  $R$ , and show that the 'Michaelis' constant is  $k_2/k_1$ .

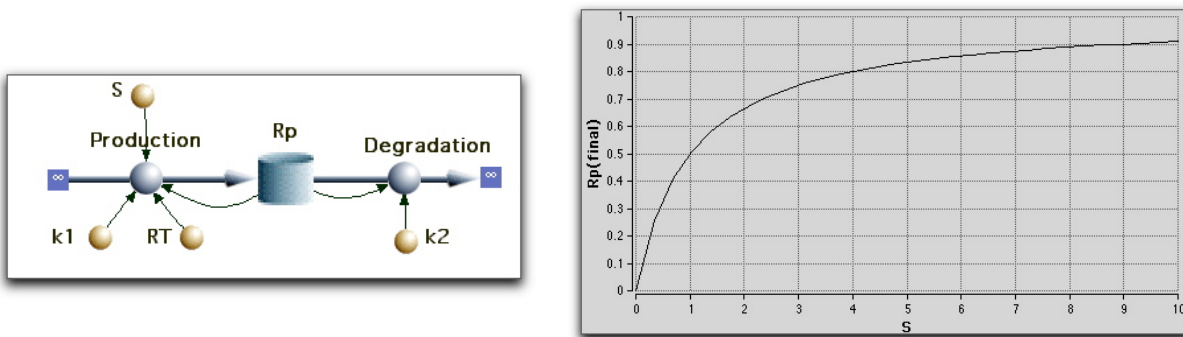


Figure 4. Hyperbolic system. (a) Flowchart for MM. (b). Graph of (a)

### Zero-order ultrasensitivity

In the above two examples the kinetics were governed by mass-action rate laws. *A dramatically different behavior is obtained if the rates are governed by Michaelis-Menten kinetics.* Figure 5 shows the Flowchart for the phosphorylation-dephosphorylation system when the kinase and phosphatase obey MM kinetics. The stimulus-response behavior is sigmoidal, and very sharp (corresponding to a Hill coefficient of  $> 10$ ). This behavior, first discovered by Goldbeter and Koshland [3] has been used to model a variety of biochemical systems [4, 5].

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Exercise 5. Reproduce the Flowchart, write the equations in mathematical notation, and plot the signal-response curve for the system in Figure 5. Use the parameters:  $R_T = 3$ ,  $k_1 = k_2 = 1$ ,  $K_{m1} = 0.05$ ,  $K_{m2} = 0.05$ , where the  $K_m$ 's are the Michaelis constants for phosphorylation and dephosphorylation, respectively.

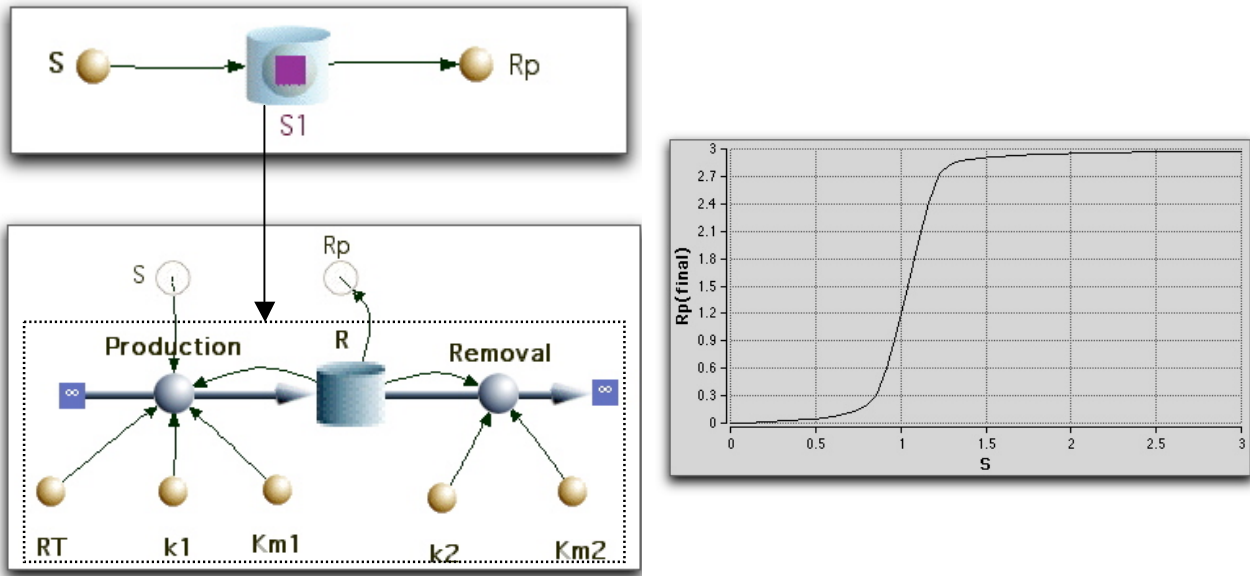


Figure 5. (a) Flowchart for signal response system governed by Michaelis-Menten kinetics. The bottom panel shows the contents of the submodel obtained by grouping the model icons except for the stimulus,  $S$ , and the function  $R_p$  giving the value of the reservoir,  $R$  (dashed line: ---). (b) The sigmoidal signal-response curve that resembles a high order of cooperativity.

## Biochemical feedback systems

There are many kinds of feedback in biochemical systems, some direct, some indirect. Here we give several examples of positive and negative feedback modules. For example, the informal diagram in Figure 6 is a model by B. Goodwin for an oscillating genetic circuit [6]. From the diagram one can write the equations governing the system.

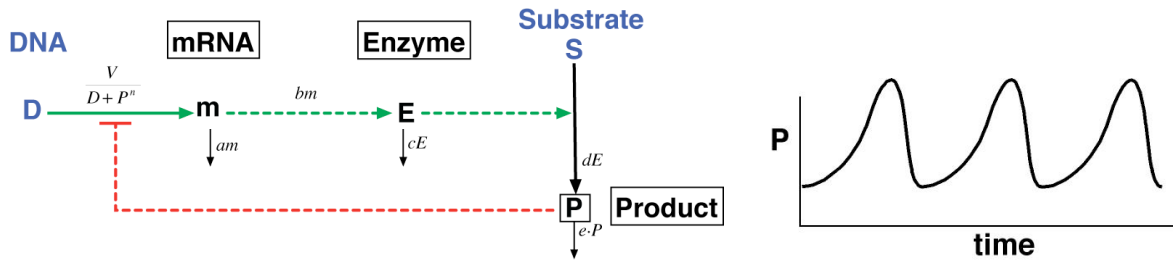


Figure 6. The Goodwin genetic oscillator. Solid lines are flows, dashed lines are signals (green = enhancers, red = inhibitors). The variable quantities (reservoirs) are black, and the parameters are blue.

Exercise 6. See if you can convert the diagram in Figure 9 to equations, and a Berkeley Madonna Flowchart.

### Direct autocatalysis

The simplest positive feedback is pure autocatalysis whereby a protein, A, catalyzes its own production. The Flowchart in Figure 7a shows this feedback. The production flow has the form  $dA/dt = kA^n$ . The graph in Figure 7b shows the explosive growth of A for  $n = 1$ .

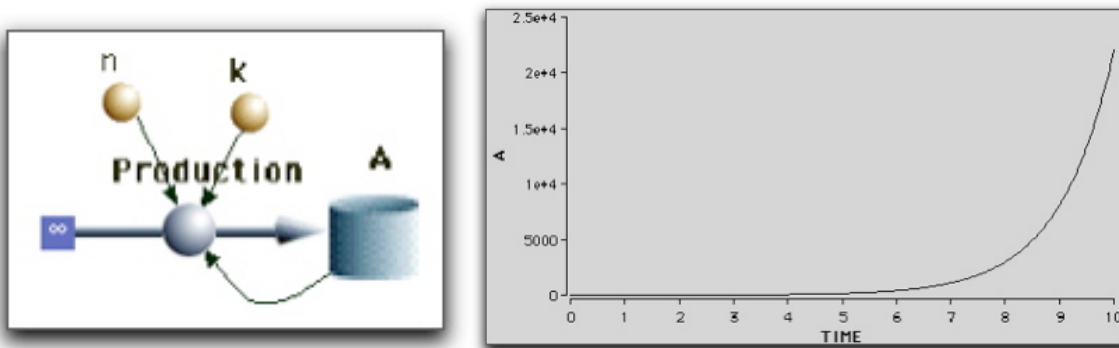


Figure 7. Simple autocatalysis. (a) Flowchart. (b)  $A(t)$  for  $k = 1$ ,  $n = 1$ ,  $A(0) = 1$ ,  $\text{Production} = kA^n$ .

Exercise 7. Simulate the Flowchart using the parameters in the figure caption of Figure 7. For  $n \geq 2$ , use the STIFF solver (Rosenbrock) and a slider for  $n$  and STOPTIME. You will have to reduce the STOPTIME for  $n \geq 2$  considerably because the growth rate of A becomes *much* faster!

### Autocatalysis by inhibition of destruction

The same autocatalytic step can be achieved by inhibiting the destruction or removal of a protein, as shown in Figure 8a.

Exercise 8. Simulate the system in Figure 8a using the parameters in the figure caption.

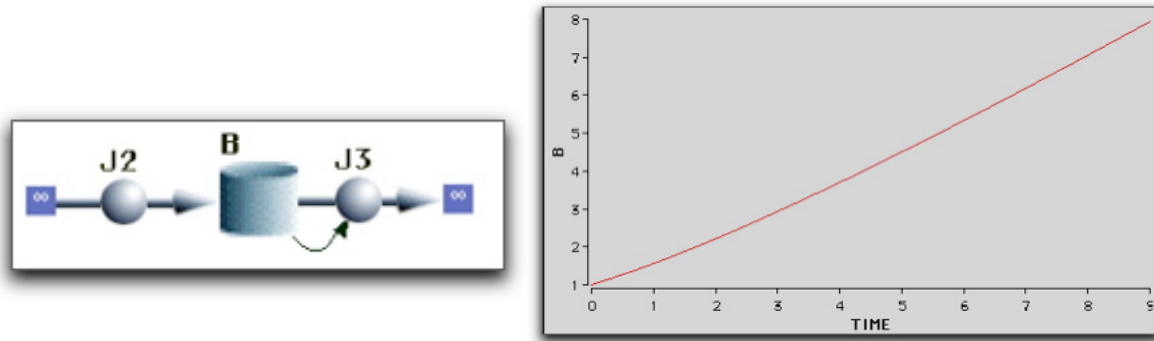


Figure 8. Autocatalysis by removal inhibition. (a) Flowchart. (b) Plot of B vs. time using production ( $J2$ ) = 1, removal ( $J3$ ) =  $1/(1 + B)$ ,  $B(0) = 1$ .

### Indirect autocatalysis

Autocatalysis can arise when each of two chemicals enhance the production of the other (Figure 9a). Autocatalysis can also arise by *negative* feedback via mutual inhibition of production, or by a mixed positive and negative feedback (Figure 9b).

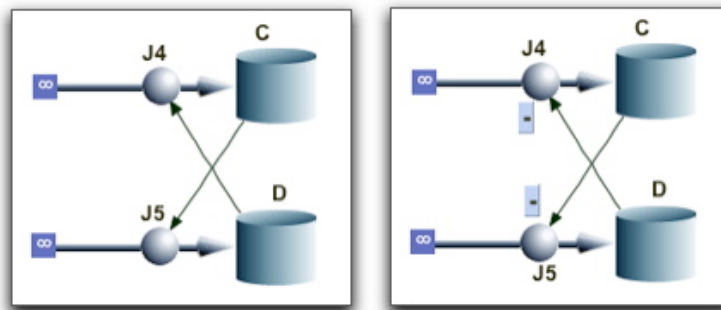


Figure 9. Indirect autocatalysis. (a) Positive feedback via mutual enhancement of production:  $J4 = 2D$ ,  $J5 = C$ . (b) Autocatalysis can also arise by *negative* feedback via mutual inhibition of production:  $J4 = 1/(1 + 2D)$ ,  $J5 = 1/(1 + C)$ . Madonna has no direct way to indicate whether the feedback enhances or inhibits the flow, but minus signs created by the Text tool can be placed adjacent to the parameter arrow to denote inhibition. Finally, autocatalysis can be achieved by a mixed positive ( $J5 = C$ ) and negative ( $J4 = D/(1 + D)$ ) feedback.

Exercise 9. Simulate the unstable (unbounded increase) in C and D for the three types of feedback: positive, negative, and mixed. You can set all constants equal to unity and use the form  $1/(1 + X)$  or  $X/(1 + X)$  as the inhibitory feedback functions. Make a plot showing the difference between those two forms.

### Adaptation

Many—if not most—cellular sensory systems exhibit the property of *adaptation*: The activity of the response returns to its ‘basal’ level despite changes in the stimulus. An example is the run-tumble frequency of *E. coli*. Raising the uniform level of a chemoattractant causes a transient

increase in tumble frequency that soon settles back to its original steady state before the attractant was added.

A circuit for implementing perfect adaptation is shown in Figure 10a. This combines the simple linear response module with a signal pathway through an intermediate, X.

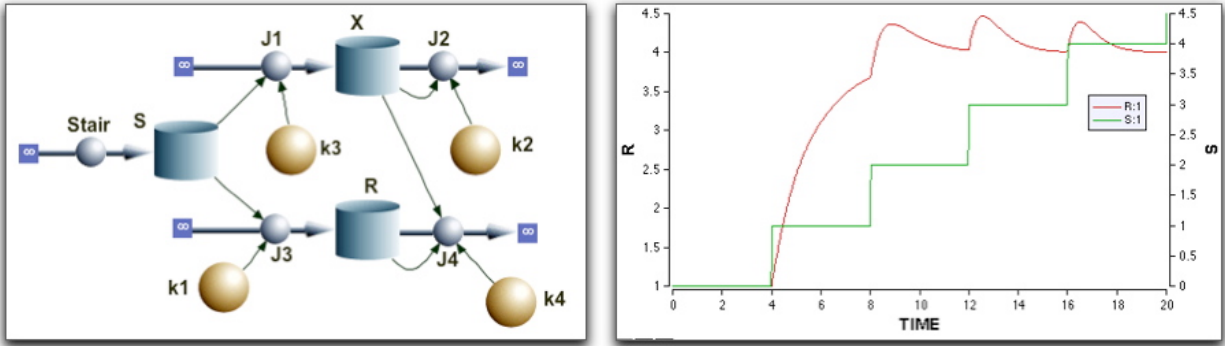


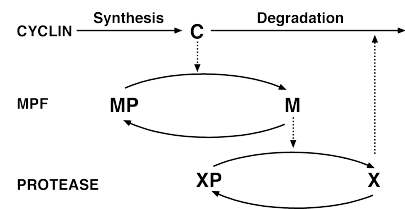
Figure 10. Perfect adaptation. (a) Flowchart combining the linear response module for R with another linear response module for X through which the signal, S, is filtered. (b) Graph of the response, R, when the stimulus is a 'staircase' function. Parameters are:  $k_1 = k_2 = 2$ ,  $k_3 = k_4 = 1$ ,  $X(0) = 0$ ,  $R(0) = 1$ ,  $S(0) = 0$ .

**Note:** The staircase function can be constructed by making the stimulus, S, be a reservoir rather than a function, with an influx consisting of the PULSE function (see the Equation Help sheet under the Help menu). Define STAIR = PULSE(HEIGHT, INITIAL TIME, DURATION).

Exercise 10. Construct the adaptation circuit shown in Figure 10a and reproduce the stimulus-response curve in Figure 10b.

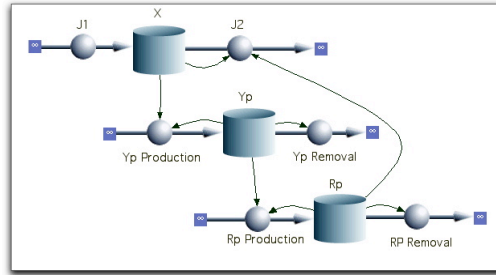
## Oscillations

Combining Michaelis-Menten kinetics with feedback can create a system with stable oscillatory behavior. We will study this phenomenon more thoroughly later; here we simply want to introduce the possibility of more complex dynamics when the modules we have constructed above are connected together.



Exercise 11. Figure 11a shows the Flowchart (with the functions hidden) for negative feedback oscillator using the modules we have shown above and Michaelis-Menten kinetics. Reconstruct the model using the parameter values from the Equation window shown in Figure 11b. Plot a stimulus-response curve for the frequency of oscillations as shown in Figure 11c.

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$\frac{d}{dt}(X) = + J1 - J2$ INIT X = 5 $\frac{d}{dt}(Yp) = + Yp\_Production - Yp\_Removal$ INIT Yp = 0.9 $\frac{d}{dt}(Rp) = - RP\_Removal + Rp\_Production$ INIT Rp = 0.1  $Rp\_Production = k5*YP*(RT-RP)/(Km5+RT-RP)$ $RP\_Removal = k6*RP/(Km6+RP)$ $Yp\_Production = k3*X*(YT-YP)/(Km3+YT-YP)$ $Yp\_Removal = k4*YP/(Km4+YP)$ $J1 = k0 + k1*S$ $J2 = k2*X + k2p*RP*X$	$RT = 1$ $k5 = 0.1$ $k6 = 0.05$ $Km5 = 0.01$ $Km6 = 0.01$ $YT = 1$ $k3 = 0.1$ $Km3 = 0.01$ $k4 = 0.2$ $Km4 = 0.01$ $k0 = 0$ $k1 = 1$ $S = 2$ $k2 = 0.01$ $k2p = 10$
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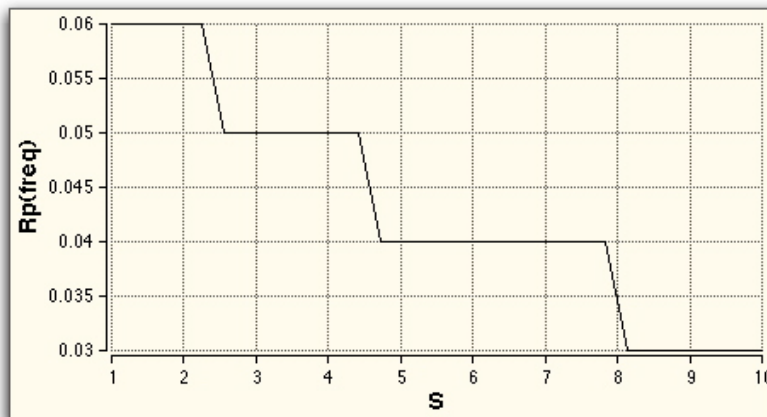


Figure 11. Negative feedback oscillator. (a) Flowchart with the functions hidden. (b) Equation window. (c) Response (Rp) vs. Stimulus (S).

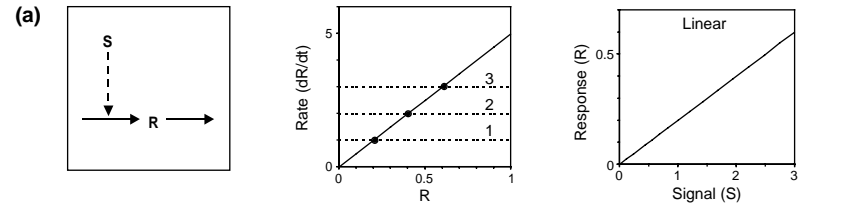


## References

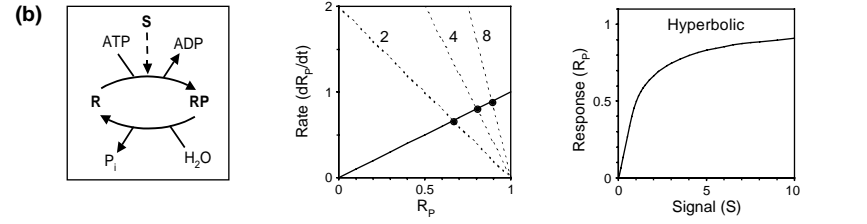
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Figure 1

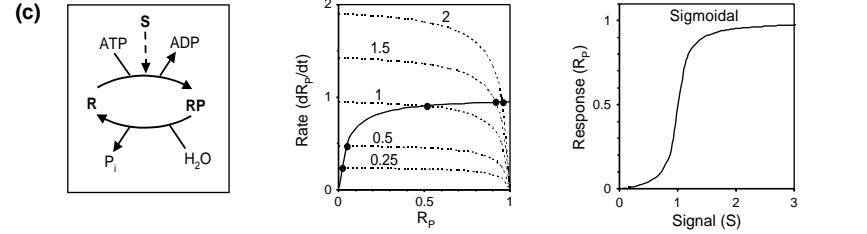
(a) *Linear Response*



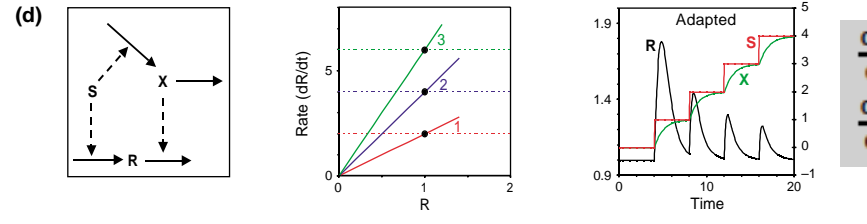
(b) *Hyperbolic Response*



(c) *Sigmoidal Response*



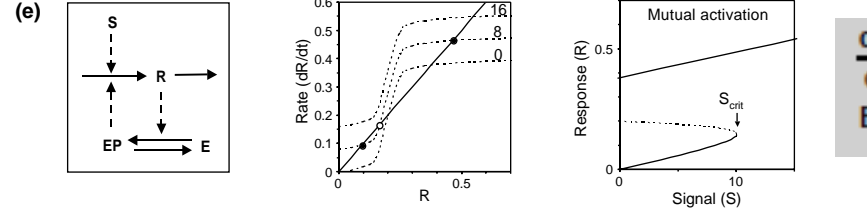
(d) *Perfect Adaptation*



$$\frac{dR}{dt} = k_1 S - k_2 X \cdot R \quad R_{ss} = \frac{k_1 k_4}{k_2 k_3}$$

$$\frac{dX}{dt} = k_3 S - k_4 X \quad X_{ss} = \frac{k_3 S}{k_4}$$

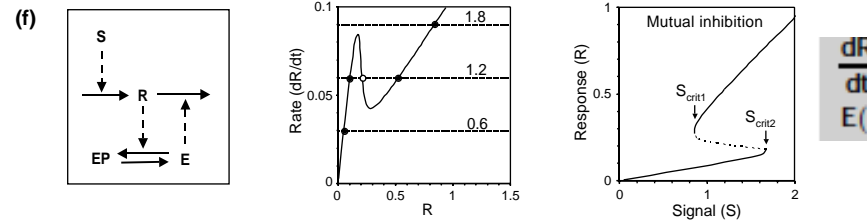
(e) *Mutual Activation*



$$\frac{dR}{dt} = k_0 E_p(R) + k_1 S - k_2 X \cdot R$$

$$E_p(R) = G(k_3 R, k_4, J_3, J_4)$$

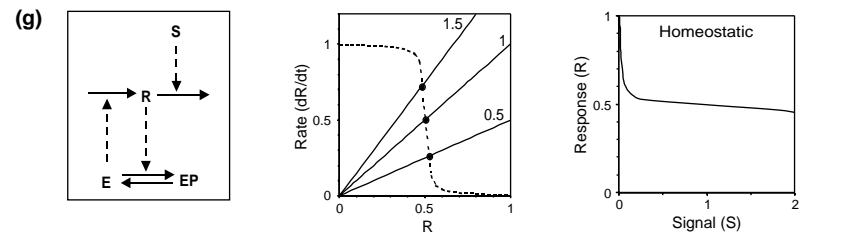
(f) *Mutual Inhibition*



$$\frac{dR}{dt} = k_0 + k_1 S - k_2 R - k_2' E(R) \cdot R$$

$$E(R) = G(k_3, k_4 R, J_3, J_4)$$

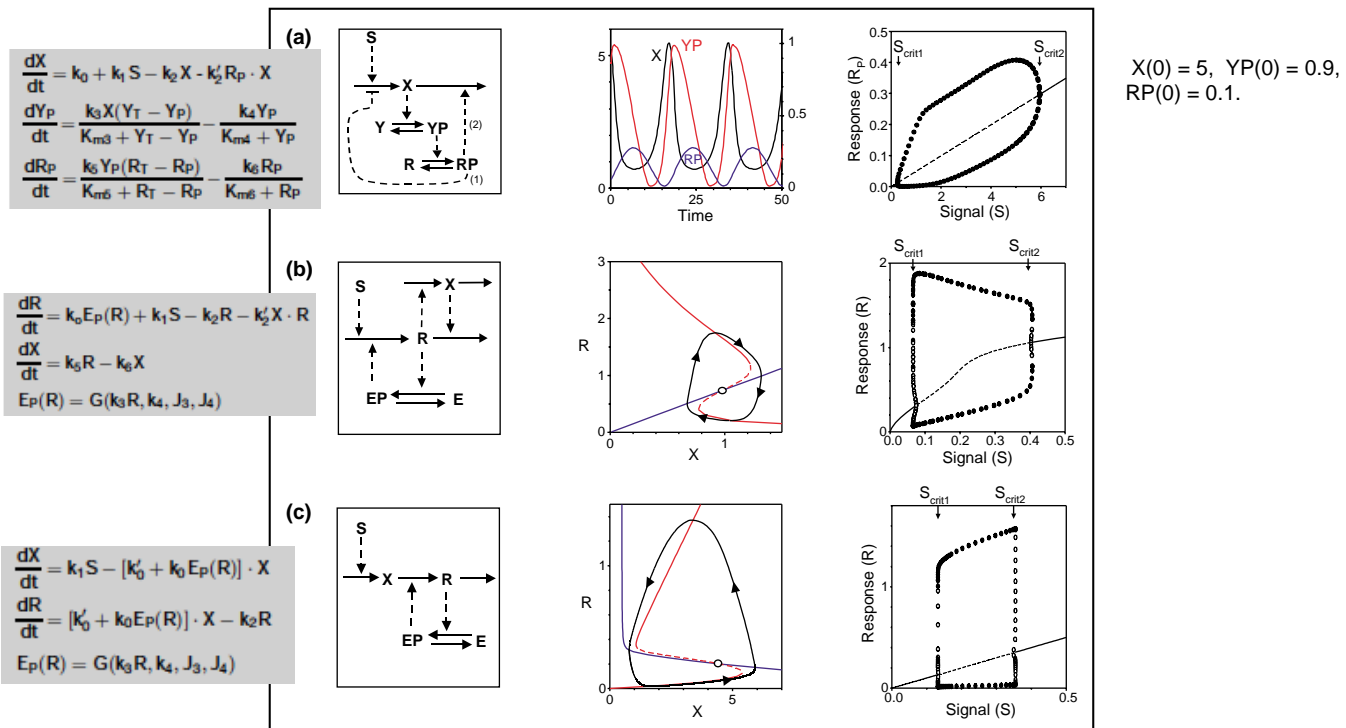
(g) *Negative Feedback Homeostasis*



$$\frac{dR}{dt} = k_0 E(R) - k_2 S \cdot R$$

$$E(R) = G(k_3, k_4 R, J_3, J_4)$$

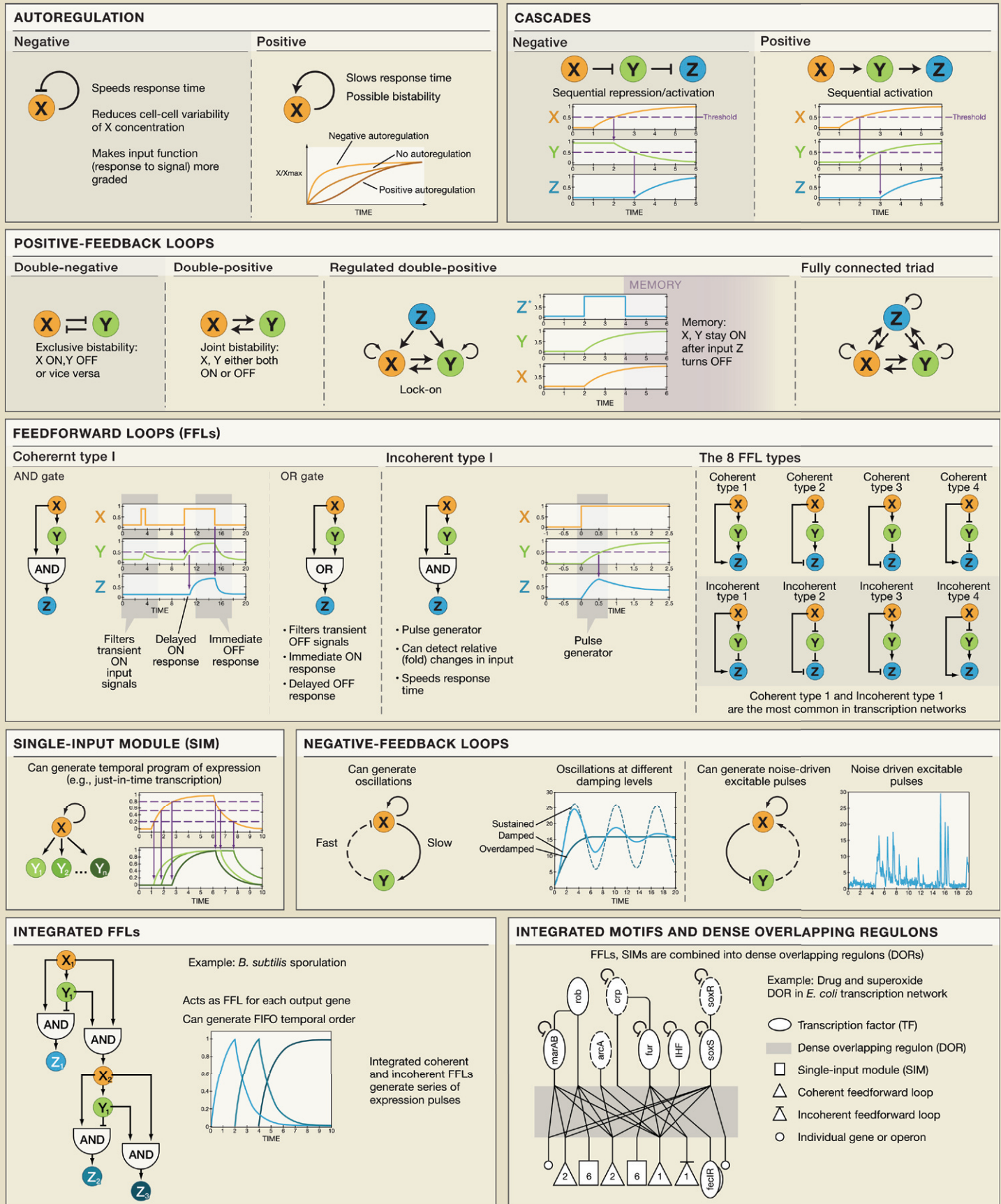
Figure 2



# SnapShot: Network Motifs

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# SnapShot: Network Motifs

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Cell

Transcription regulation and signaling networks are composed of recurring patterns called network motifs. Network motifs are much more prevalent in biological networks than would be expected by comparison to random networks and comprise almost the entire network structure. The same small set of network motifs has been found in diverse organisms ranging from bacteria to plants to humans. Experiments show that each network motif can carry out specific dynamic functions in the computation done by the cell. Here we review the main classes of network motifs and their biological functions.

## Autoregulation

Negative autoregulation (NAR) occurs when a transcription factor represses the transcription of its own gene. (We use transcription to help make examples concrete; all circuits described here could operate also in other regulatory modes, e.g., a protein inhibiting its own activity by autophosphorylation.) This occurs in about half of the repressors in *E. coli* and can speed up the response time of gene circuits and reduce cell–cell variation in protein levels that are due to fluctuations in production rate. Positive autoregulation occurs when a transcription factor enhances its own rate of production. Response times are slowed and variation is usually enhanced. This motif, given sufficient cooperativity, can lead to bimodal (all-or-none) distributions, where the concentration of X is low in some cells but high in others.

## Cascades

Cascades of gene expression create sequential activation of genes. The downstream gene is activated when its regulator reaches the relevant threshold. Using negative regulation, the genes can be sequentially activated and repressed.

## Positive-Feedback Loops

Developmental transcription networks often use positive-feedback loops that are made of two transcription factors that regulate each other. The double-negative loop, in which two repressors repress each other, has two steady states: X is ON and Y is OFF, or the opposite. In the double-positive loop, either both X and Y are OFF, or both are ON. In either case, a transient signal can cause the loop to lock irreversibly into a steady state, providing memory of an input signal. Often, X and Y also positively regulate themselves. In a regulated feedback loop, an upstream regulator Z regulates X and Y, which locks the feedback loop into one of its steady states. Triads of mutually activating transcription factors are also common network motifs.

## Feedforward Loops (FFLs)

The feedforward loop (FFL) appears in hundreds of gene systems in *E. coli* and yeast as well as in other organisms. This motif consists of a regulator, X, which regulates Y and Z, where Y also regulates Z. Because each of the three interactions in the FFL can be either activation or repression, there are eight possible structural types of FFLs. X and Y combine to regulate Z, often approximately as AND or OR gates. The two most common FFLs are the coherent type 1 FFL (C1-FFL) and the incoherent type 1 FFL (I1-FFL). The C1-FFL with an “AND” gate is a “sign-sensitive delay” element and a persistence detector. The I1-FFL is a pulse generator and response accelerator. For a range of parameters, the I1-FFL can also act as a fold-change detector, where the response dynamics depend only on the fold-change (rather than absolute change) of the input signal.

## Single-Input Module (SIM)

In single-input modules, a regulator X regulates a group of target genes (typically X also regulates itself). This motif allows coordinated expression of genes with a shared function and can generate a temporal expression program, with a defined order of activation or repression of each of the target promoters. Stochastic pulses of X can provide proportional control according to the pulse frequency (as in CRZ1 in yeast).

## Negative-Feedback Loops

Negative-feedback loops between two genes or proteins are often made up of interactions that take place on different timescales. For example, X can slowly activate Y, which in turn quickly inhibits X (for instance, slow transcriptional activation and rapid inhibition by degradation). This circuit can create oscillations. A symmetrically opposed motif, with fast activation and slow negative feedback, can generate noise-driven excitable pulses.

## Integrated FFLs

FFLs may be combined into larger integrated structures and more complex transcription circuits. For example, integrated coherent and incoherent FFLs generate temporal waves of gene expression during the sporulation process of *B. subtilis*.

## Integrated Motifs and Dense Overlapping Regulons (DORs)

Dense overlapping regulons are sets of regulators that combinatorially control a set of output genes. The DOR can be thought of as a gate-array, carrying out a computation by which multiple inputs are translated into multiple outputs.

Network motifs combine to form the global structure of the network. In the example shown, viewing an image of the network using symbols to denote the different motifs helps to portray the network in a compact way. Note that FFLs and SIMs are integrated into DORs. Usually the DORs occur in a single layer, thus most computations are carried out in a single “cortex.” Developmental networks can have deeper layers of cascades.

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