Bistability in cell signaling: How to make continuous processes discontinuous, and reversible processes irreversible

James E. Ferrell, Jr.[a] and Wen Xiong
Department of Molecular Pharmacology, Stanford University School of Medicine, Stanford, California 94305-5174

(Received 3 August 2000; accepted for publication 3 January 2001)

Xenopus oocyte maturation is an example of an all-or-none, irreversible cell fate induction process. In response to a submaximal concentration of the steroid hormone progesterone, a given oocyte may either mature or not mature, but it can exist in intermediate states only transiently. Moreover, once an oocyte has matured, it will remain arrested in the mature state even after the progesterone is removed. It has been hypothesized that the all-or-none character of oocyte maturation, and some aspects of the irreversibility of maturation, arise out of the bistability of the signal transduction system that triggers maturation. The bistability, in turn, is hypothesized to arise from the way the signal transducers are organized into a signaling circuit that includes positive feedback (which makes it so that the system cannot rest in intermediate states) and ultrasensitivity (which filters small stimuli out of the feedback loop, allowing the system to have a stable off-state). Here we review two simple graphical methods that are commonly used to analyze bistable systems, discuss the experimental evidence for bistability in oocyte maturation, and suggest that bistability may be a common means of producing all-or-none responses and a type of biochemical memory. © *2001 American Institute of Physics.* [DOI: 10.1063/1.1349894]

One of the key questions of the postgenomic era is how the biological behavior of cells emerges out of the organization of regulatory proteins into cascades and networks. Here we examine one type of signaling circuit that can be used by cells to convert continuous stimuli into discrete responses, and can be used to ''remember'' a stimulus long after the stimulus has been withdrawn; in other words, the circuit exhibits bistability and hysteresis. We have hypothesized that a bistable circuit consisting of the Mos, MEK-1, and p42 MAP kinase proteins is responsible for the all-or-none character of *Xenopus* oocyte maturation, an interesting example of a cell fate induction process that is amenable to a variety of powerful experimental approaches. Here we review what is required to produce a satisfactory bistable signaling circuit, using two simple graphical methods, and review the experimental evidence for bistability in oocyte maturation and other important examples of switch-like biological processes. Our hope is to introduce biologists to the conceptual basis of bistability, and to introduce nonlinear scientists to a biological process where bistability appears to be of critical importance.

I. INTRODUCTION

It has been hypothesized that bistability is at the heart of important cellular processes like differentiation and cell cycle progression.[1–6] The purpose of this article is to examine the basic requirements for a functional bistable cell signaling system. Our laboratory's interest in bistability, and in the properties of signaling networks in general, have been an outgrowth of our experimental work on *Xenopus* oocyte maturation. Maturation is a process of interest to reproductive biologists, because of its importance for understanding fertility and contraception, to developmental biologists because it is an interesting example of cell fate induction, and to cell biologists because of its historical importance in the discovery of cell cycle regulators. However, for the purposes of this review, its main value is that it is a process where it is unusually easy to examine and manipulate the quantitative behavior of signal transduction networks, and to learn lessons about the emergent properties of those networks that might apply to other less tractable systems.

Here we will begin with a brief introduction to *Xenopus* oocyte maturation, and with why it has been hypothesized that the all-or-none character of maturation and, perhaps the irreversibility of maturation arise out of the bistability of the system of signaling proteins that mediates maturation. More detailed information on the biology and biochemistry of oocyte maturation can be found elsewhere.[7–10] Then we will introduce two graphical methods that are commonly used for examining the properties of bistable systems. Finally, we will review the experimental evidence for bistability in *Xenopus* oocyte maturation and several other important all-or-none biological processes.

A. Xenopus oocyte maturation

Like many cell fate decisions, *Xenopus* oocyte maturation is an all-or-none process. This is apparent in experiments where oocytes are incubated with submaximal concentrations of the maturation-inducing stimulus, the steroid hormone progesterone. Some of the oocytes undergo matu-

[a]Author to whom correspondence should be addressed. Telephone: 650 725-0765; Fax: 650 723-2253; electronic mail: james.ferrell@stanford.edu

ration and arrest in metaphase of meiosis II; others fail to undergo maturation and remain arrested in a G2-like state. However, none of the oocytes arrest in an intermediate state. An oocyte either matures or it does not mature, but under normal conditions it does not mature halfway.

In addition, oocyte maturation is essentially irreversible. The irreversible nature of maturation is apparent from experiments where oocytes are incubated transiently with progesterone. Brief exposure to progesterone will not cause oocytes to mature. However, after 1–2 h of incubation with progesterone, most oocytes pass a point-of-no-return. Beyond this point, the oocyte is committed to finishing maturation.

Likewise, if you were to incubate an oocyte with a subthreshold dose of progesterone (say, 1 nM), the oocyte would not mature no matter how long you waited. If you raised the progesterone concentration above the threshold (to, say, 100 nM), the oocyte would mature. But if you then lowered the progesterone concentration to 1 nM, the oocyte would not ''demature'' no matter how long you waited. Thus, you get different stimulus/response curves when you increase and decrease the progesterone concentration. The oocyte exhibits hysteresis in its response to progesterone.

There are a number of special, idiosyncratic aspects of *Xenopus* oocyte maturation that distinguish it from other cell fate determination processes. For example, some of the events of maturation can take place in the absence of gene transcription, whereas in many other cases cell fate determination depends upon the expression of new sets of genes. But in these two basic aspects—the all-or-none character and the irreversibility of the process—oocyte maturation is an absolutely typical example of cell fate induction.

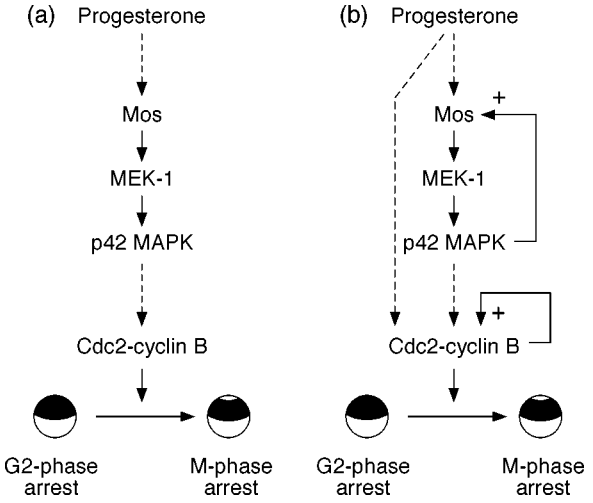

FIG. 1. Schematic view of *Xenopus* oocyte maturation. Oocytes begin in a stable G2-arrest state. The maturation-inducing agent progesterone brings about the activation of the MAP kinase cascade enzymes Mos, MEK-1, and p42 MAPK. The cascade promotes the activation of Cdc2-cyclin B complexes. Active Cdc2-cyclin B causes the oocyte to leave its G2-arrest state, enter meiosis I, and then arrest in metaphase of meiosis II. (a) Simple linear depiction of the signal transduction system that regulates maturation. (b) A more realistic depiction, including the positive feedback loops that are hypothesized to be critical for producing the all-or-none character of oocyte maturation.

B. The MAP kinase cascade and Cdc2-cyclin B

Xenopus oocyte maturation is mediated by a specific group of protein kinases, as shown schematically in Fig. 1(a). Progesterone brings about the activation of the Mos/MEK-1/p42 MAP kinase (MAPK) cascade, which in turn promotes the activation of the M-phase trigger Cdc2-cyclin B. Activation of both p42 MAPK and Cdc2-cyclin B is required for normal maturation.

At the level of the individual Cdk-cyclin complex, Cdc2 probably acts like a nearly perfect molecular switch. This is known to be the case for the biochemically similar Cdk2-cyclin A complex—the inactive Cdk2 monomer is approximately 3 000 000 000-fold lower in activity than the fully active Cdk2-cyclin A complex in its maximum activity state (Thr 160 phosphorylated; Thr 14 and Tyr 15 dephosphorylated).[11,12] When a Cdc2 molecule is off, it is really off, and when it is on, it is really on. But an oocyte contains $0.5-1\times10^{10}$ Cdc2-cyclin B complexes.[13,14] Thus, in principle, the oocyte could adopt any of an almost unlimited number of nearly continuously variable Cdc2 activity states. The same is true for p42 MAPK—there are approximately 10^{11} MAPK molecules in a single oocyte.[15]

Moreover, at the level of the individual Cdc2 molecule or p42 MAPK molecule, the activation process is reversible. For example, the activity of p42 MAPK is determined by the balance between a MAPK-activating kinase (MEK-1) and one or more MAPK-inactivating phosphatases. Even in M-phase-arrested mature oocytes, where nearly all of an oocyte's p42 MAPK molecules are active, the phosphates are still turning over about once every 5 min,[16] and, consequently, turning off MEK-1 allows the p42 MAPK to rapidly return to its inactive state.

C. Continuous, reversible signal transducers and a discontinuous, irreversible biological response

Somehow the oocyte takes a continuously graded stimulus (the progesterone), transduces it through nearly continuously variable signaling proteins (the MAP kinase cascade and Cdc2-cyclin B), and produces an all-or-none biological response. And somehow it takes a reversible stimulus, transduces it through reversible signaling proteins, and turns it into an irreversible biological response. The all-or-none character of oocyte maturation, the existence of a point-of-no-return, and the ultimate irreversibility of the cell fate choice are all typical of cell fate determination processes. So how does the all-or-none, irreversible character of these processes arise?

The prevailing hypothesis is that both the all-or-none character of oocyte maturation and the irreversibility of it are not properties of the individual signaling proteins, but instead emerge out of the organization of these proteins into a particular type of signal transducing circuit. Both the MAP kinase cascade and Cdc2-cyclin B are embedded in positive feedback loops [Fig. 1(b)]—MAP kinase promotes the accumulation of its upstream activator Mos, and Cdc2-cyclin B promotes both the activation of its upstream activator Cdc25 and the inactivation of its inhibitors Wee1 and Myt1. Signaling circuits that include strong positive feedback can, under

some circumstances, switch between discrete states, with intermediate states possible only transiently. Such systems are termed bistable, because they possess two possible stable steady state outputs for single values of the input stimulus. Bistable systems can exhibit biochemical hysteresis, which, in limiting cases, can be manifested as irreversibility.

Here we will show how all-or-none responses and irreversibility can arise out of signal systems with strong positive feedback. We will present two commonly-used graphical methods for depicting the steady-state responses of these signaling systems, the rate balance plot and the steady state balance plot.[3,17–19] These graphical methods make it easy to see why positive feedback alone does not guarantee a bistable response, and under what circumstances a bistable system might be converted into monostable one experimentally. Finally, we will review the experimental work supporting the hypothesis that the all-or-none character of *Xenopus* oocyte maturation depends upon a bistable signaling transduction system, and speculate about other signal transduction systems where the same sort of signaling circuitry is likely to be important for producing discontinuous, irreversible biological responses. First, though, we will begin with a brief examination of how signaling systems that do not include feedback usually behave.

II. A SIMPLE SIGNAL TRANSDUCTION SYSTEM WITHOUT FEEDBACK

As an example of the simplest type of signaling system, we will consider a monocyclic cascade. A signaling protein A is converted to an activated form A^* by a process catalyzed by a stimulus enzyme S,

$$A \underset{k_{-1}I}{\overset{k_1 S}{\rightleftarrows}} A^*. \tag{1}$$

The stimulus S could be, for example, a protein kinase, and the conversion of A to A^* could be phosphorylation. A^* can be converted back to A through an inactivating enzyme I; if S is a protein kinase, I would be a phosphoprotein phosphatase.

A. Algebraic derivation of the stimulus-response relationship

We can derive a formula for the steady state level of A^* as a function of S. Assume initially that the enzymes S and I are far from saturation, so that the rate of the forward reaction is simply,

$$\frac{dA^*}{dt} = k_1 S[A] = k_1 S[A_{\text{tot}}] - k_1 S[A^*], \tag{2}$$

where $A_{\text{tot}} = A + A^*$. The rate of the back reaction is

$$\frac{dA}{dt} = k_{-1} I[A^*]. \tag{3}$$

At steady state, the rate of the forward reaction equals the rate of the back reaction,

$$k_1 S[A_{\text{tot}}] - k_1 S[A^*] = k_{-1} I[A^*], \tag{4}$$

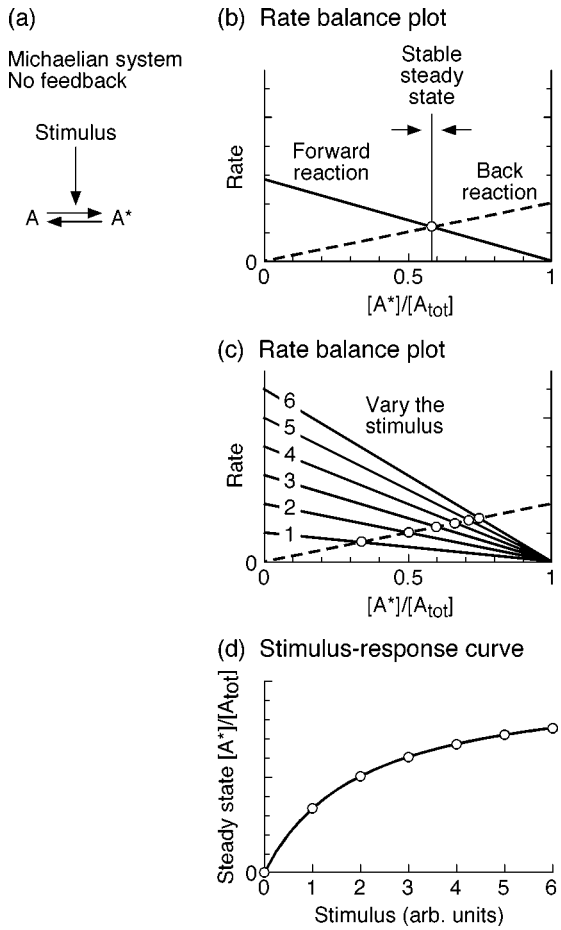

FIG. 2. Steady state responses of a simple Michaelian monocyclic cascade. (a) Schematic depiction of the cascade. (b) Rate balance plot. The forward reaction rate is indicated by the solid line; the back reaction rate by the dashed line. Where the two lines intersect, the system is in a stable steady state. (c) Rate balance plot showing forward reaction rate curves for six levels of stimulus. (d) Stimulus-response curve. The intersection points from panel (c) were plotted as a function of the stimulus. The result is the familiar Michaelian hyperbolic stimulus-response curve.

$$\frac{[A^*]}{[A_{\text{tot}}]} = \frac{S}{k_{-1}I/k_1 + S}. \tag{5}$$

This equation is identical in form to the Michaelis–Menten equation, and so this type of response is often termed a Michaelian response. A Michaelian stimulus-response curve is shown in Fig. 2(d). The concentration of active A^* increases linearly with S when S is small. As S increases, each increment of S produces a smaller and smaller increment of A^*. When S is very large, $[A^*] = [A_{\text{tot}}]$; all of the A is active. When $S = k_{-1}I/k_1$, $[A^*]/[A_{\text{tot}}] = 0.5$. Thus $k_{-1}I/k_1$ is the EC50—the concentration or activity of S required to produce a 50% maximal response, and Eq. (5) can be rewritten as

$$\frac{[A^*]}{[A_{\text{tot}}]} = \frac{S}{\text{EC50} + S}. \tag{6}$$

Note that we have *not* assumed that S or I are saturable; enzyme saturation is not the reason that the initially linear response begins to level off. The response levels off because

the larger S gets, the less inactive A there is for the kinase to act on, and the more A^* there is for the phosphatase to act on.

B. The rate balance plot

The algebraic derivation of the stimulus-response relationship for a Michaelian system [Eq. (6)] is not difficult. However, for more complicated systems the algebra is sometimes cumbersome. In these cases, a simple graphical approach, termed the rate balance plot, can often yield the same information much more easily.[17–19] This plot is similar in spirit to the familiar supply/demand plot used by economists—they plot curves for the supply and the demand of a commodity as a function of price, and at the price where the curves cross, supply equals demand and the market is in equilibrium. Here we will plot the forward rate and the back rate for the reactions shown in Fig. 2(a) as a function of $[A^*]$; at the value of $[A^*]$ where the curves cross, the system is in steady-state.

Consider how the rate of the forward reaction varies with $[A^*]$ [Fig. 2(b), solid line]. When $[A^*]=0$, all of the A is nonphosphorylated ($[A]=[A_{tot}]$) and the rate of the forward (kinase) reaction is maximal. When $[A^*]=[A_{tot}]$, $[A]=0$ and the rate of the forward reaction is zero. In between, the rate of the forward reaction increases linearly with $[A]$ and decreases linearly with $[A^*]$. The amount or activity of S determines the steepness of the line.

Now consider how the rate of the back reaction varies with $[A^*]$ [Fig. 2(b), dashed line]. When $[A^*]=0$, the rate of the back reaction is zero (there is no phosphorylated A for the phosphatase to work on); when $[A^*]=[A_{tot}]$ the rate of the back reaction is maximal. In between, the rate of the back reaction increases linearly with $[A^*]$ and decreases linearly with $[A]$. The amount or activity of I determines the steepness of the line.

The system is in steady state when the rate of the forward reaction equals the rate of the back reaction. For the choices of k_1S (the negative slope of the forward reaction line) and $k_{-1}I$ (the slope of the back reaction line) shown in Fig. 2(b), the system is in steady state when about 58% of A_{tot} is phosphorylated.

Now to extract a stimulus-response curve from the rate balance plot, consider how the steady state level of $[A^*]$ would vary as S is varied. As shown in Fig. 2(c), as S increases, the slope of the forward rate line becomes steeper, and the intersection point between the forward and back reaction rate lines occurs at higher values of $[A^*]$. If we plot the steady state level of $[A^*]$ as a function of S, we get the familiar hyperbolic shape of a Michaelian stimulus-response curve [Fig. 2(d)].

C. Stability in a Michaelian system

Suppose that the system starts out in a steady state, and that it is then perturbed by the addition of a little extra A^* (without changing the amounts or activities of S and I). The system has been shifted to the right of the steady state. At this concentration of $[A^*]$, the back reaction rate will be higher than it was at steady state, and the forward reaction rate will be lower [Fig. 2(b)]. Thus there will be an excess of dephosphorylation over phosphorylation, and $[A^*]$ will decrease until the system is returned to the same steady state it was in before it was perturbed. Likewise, if $[A^*]$ is decreased, the forward reaction rate will increase, the back reaction rate will decrease, and again the system will return to the same steady state it was in previously. Thus the steady state in this Michaelian system is stable; perturbations from the steady state set up forces that restore the system to the steady state. Since there is only one steady state for a given choice of k_1S and $k_{-1}I$—the reaction rate lines intersect at only one point—the system is termed *monostable*.

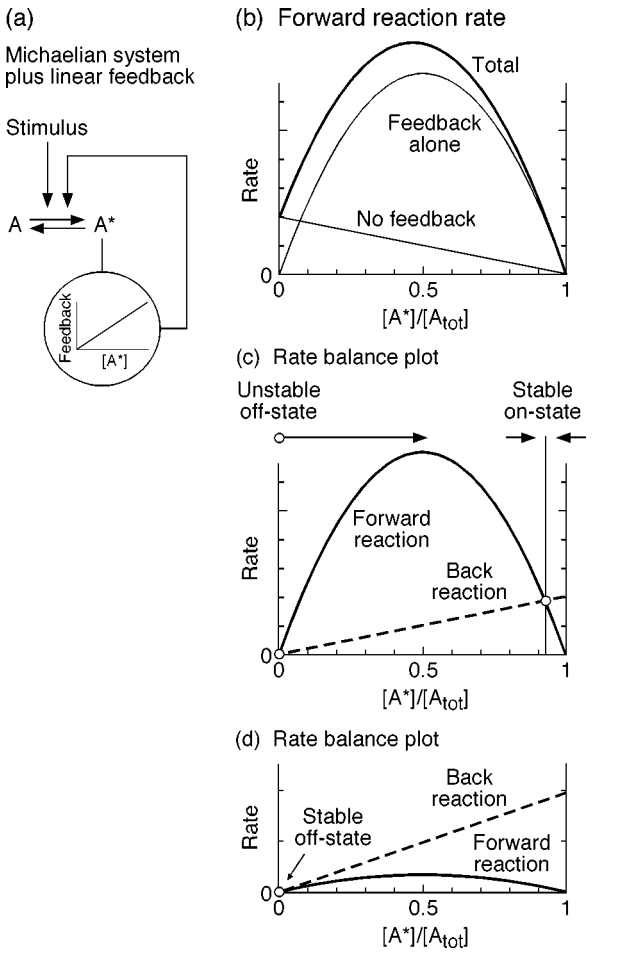

FIG. 3. Steady state responses of a simple system with linear feedback. (a) Schematic depiction of the system. (b) Forward reaction rate. The total forward reaction rate (thick line) has two components: one due directly to the stimulus (no feedback) and the other due to the linear feedback (feedback alone). (c) Rate balance plot. The forward reaction curve assumes that the stimulus level is zero. The forward reaction and back reaction curves intersect at two points, meaning there are two possible steady states. The lower steady state is unstable, because any perturbation of the system from that steady state would drive the system to the (stable) on-state. (d) Rate balance plot. If the feedback is weak, the off-state can be stable, but in this case the on-state collapses down to the off-state.

III. ADDING LINEAR POSITIVE FEEDBACK

Now add to the system a positive feedback loop—for example, imagine that A cannot only be phosphorylated by S, but also be autophosphorylated in trans by A^*,[20] or, alternatively, that A^* can increase the activity of S [Fig. 3(a)].

There are now two components to the forward reaction rate, a basal reaction rate k_1S [Fig. 3(b), thin line] and a feedback component [Fig. 3(b), thin curve]. Initially let us assume that the feedback increases linearly with A^*, so that the rate of the feedback phosphorylation is proportional to $[A^*]$ and $[A]$. The total forward reaction rate is

$$\frac{d[A^*]}{dt} = \text{basal rate} + \text{feedback rate}$$

$$= k_1 S[A] + k_2[A^*][A] \quad (7)$$

$$= (k_1 S + k_2[A^*])([A_{\text{tot}}] - [A^*]). \quad (8)$$

The basal rate of the forward reaction still decreases monotonically as $[A^*]$ increases, but the feedback rate initially increases with $[A^*]$ [Fig. 3(b), thin curves]. The total forward reaction rate curve is a skewed, inverted parabola [Fig. 3(b), thick curve].

The steady state behavior of this system can be inferred from the rate balance plot [Figs. 3(c) and 3(d)]. Let us initially assume that the feedback is strong relative to the phosphatase reaction, $k_2[A_{\text{tot}}] > k_{-1}I$, and that the basal reaction rate is zero [Fig. 3(c)]. The system now has two possible steady states rather than one. It has an off-state where $[A^*]$ is zero, and an on-state where $[A^*]/[A_{\text{tot}}]$ is approximately 0.92. However, if S has any activity at all in the absence of $[A^*]$, the off-state will disappear, and we are back to a system with only one steady state. And even if the basal activity of S is zero, the off-state will be unstable. Adding even one molecule of A^* will push the system out of balance with the forward reaction rate faster than the back reaction rate, which will cause more A^* to accumulate, which makes the forward reaction rate even faster than the back reaction rate, and so on, until ultimately the system arrives in the on-state.

This instability can be avoided by making the positive feedback weaker: $k_2[A_{\text{tot}}] < k_1 I$ [Fig. 3(d)]. However, now the on-state has disappeared, so we are left again with a system with only one steady state. Clearly, feedback alone does not turn a signaling system into a satisfactory biochemical switch.

IV. MAKING THE OFF-STATE STABLE

There are two easy ways to modify the system to allow it to have both a stable off-state and a stable on-state. The first is to have the strength of the feedback increase more than linearly with $[A^*]$. This is equivalent to supposing that the feedback is not mediated by a simple linear (or Michaelian) process, but by a cooperative or ultrasensitive system of signal transducers. The second is to have the back reaction become saturated with $[A^*]$ before the feedback does. Both of these mechanisms essentially allow the system to filter out the first increments of stimulus that impinge upon the positive feedback loop. We will examine these two mechanisms by means of rate balance plots.

A. Nonlinear (ultrasensitive) feedback

There are at least three basic classes of mechanisms that can make the feedback be a sigmoidal function of $[A^*]$. These mechanisms have been discussed in detail elsewhere;[15,21,22] we will review them briefly here.

The first class of mechanism is exemplified by positive cooperativity. Suppose that it takes n molecules of A^* to activate S. Then the feedback will initially be proportional to $[A^*]^n$ rather than to $[A^*]$. There are other ways of making A^* feed into the activation of S more than once that do not, strictly speaking, satisfy the classical definition of cooperativity. For example, A^* could both stimulate an activator of S and inhibit an inactivator of S, a phenomenon termed reciprocal regulation. The term ''multistep ultrasensitivity'' has been proposed to encompass all of these sorts of mechanisms.[15,22]

The second way of producing a sigmoidal response is zero-order ultrasensitivity.[23] The basic idea is that if the phosphatase is operating near saturation, then as $[A^*]$ rises, successive increments of S will become increasingly effective in making $[A^*]$ increase further.

The third way of producing a sigmoidal response is probably the easiest to envision. If there is a low abundance, high affinity stoichiometric (noncatalytic) inhibitor of S, then S will become more effective at causing increases in $[A^*]$ once the stoichiometric inhibitor is saturated. This mechanism has been termed inhibitor ultrasensitivity.[15,24]

All of these mechanisms for generating ultrasensitivity have the potential to endow a feedback system with a stable off-state. We will use rate balance plots to show why this is the case. Suppose that the feedback is a sigmoidal function of $[A^*]$, rather than a linear function of $[A^*]$ [Fig. 4(a)]. For simplicity, we will also suppose that the stimulus is low and the basal activity of the kinase is low, so the ''no feedback'' contribution to the total forward reaction rate [Fig. 4(b)] is negligible [Fig. 4(c)]. The sigmoidal shape of the feedback curve has made it possible for there to be two stable steady states—an on-state and an off-state—with a discrete threshold in between [Fig. 4(c)]. If the system is anywhere to the left of the threshold, it will settle into the off-state; if it is anywhere to the right of the threshold, it will settle into the on-state. The threshold itself is an unstable steady state, since the forward and back rates are equal at this value of $[A^*]/[A_{\text{tot}}]$, but any perturbation will send this system either up to the on-state or down to the off-state.

Note that the bistable system we have constructed is bistable only for a limited range of kinetic parameters. If the basal activity of the kinase is too high, the system will have only an on-state. Likewise if the activity of the phosphatase is too low. If the activity of the phosphatase is too high, the system will have only an off-state. A system is bistable only when the forward and back reactions are properly balanced.

B. Back reaction saturation

A second way to make the off-state stable is to have the back reaction become saturated as more and more $[A^*]$ accumulates.[20] We can show this with rate balance plots, and, again, for simplicity we are supposing that the stimulus level and the basal activity of the kinase are low. If the back reaction is saturable, then we can have the back reaction rate rise more steeply than the feedback as $[A^*]$ is initially in-

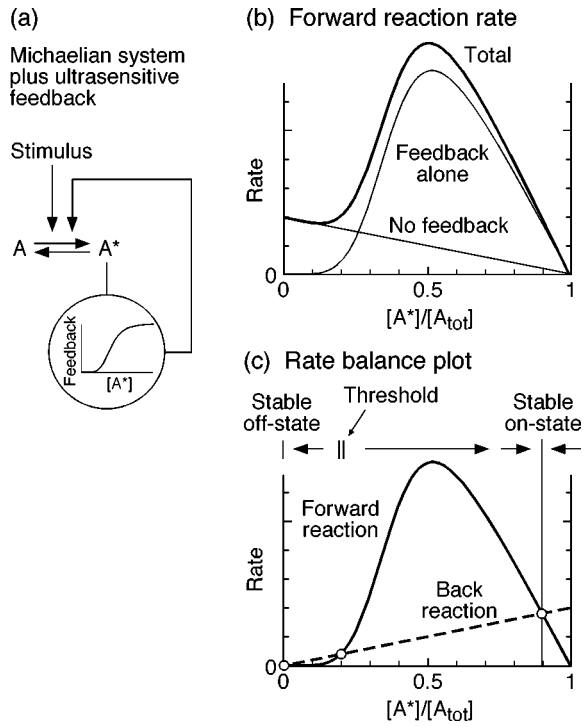

FIG. 4. Steady state responses of a simple system with ultrasensitive (sigmoidal) feedback. (a) Schematic depiction of the system. (b) Forward reaction rate. The total forward reaction rate (thick line) has two components: one due directly to the stimulus (no feedback) and the other due to the linear feedback (feedback alone). (c) Rate balance plot. The shark-fin-shaped forward reaction rate curve can intersect the back reaction rate curve at three points. The lowest point corresponds to a stable off-state. The highest point corresponds to a stable on-state. The middle point is an unstable threshold.

creased, but then level off due to saturation and be overcome by the feedback [Figs. 5(a) and 5(b)]. The back reaction can more than keep up with the first increments of feedback, but then is overwhelmed as the feedback continues to rise.

These two ways of endowing the off-state with stability—ultrasensitivity in the feedback loop and back reaction-saturation—are not mutually exclusive. Other things being equal, having both of these mechanisms operating together will produce a more robust, decisive, and complete bistable switch than either mechanism alone would.

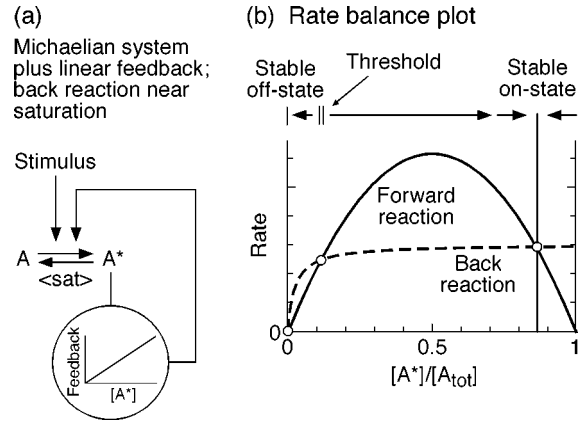

FIG. 5. Steady state responses of a simple system with a back reaction operating near saturation. (a) Schematic depiction of the system. (b) Rate balance plot. The forward and back reaction rate curves can intersect at three points, corresponding to a stable off-state, an unstable threshold, and a stable on-state.

V. BISTABILITY WITHOUT POSITIVE FEEDBACK

Bistability can also arise in systems that do not possess positive feedback. For example, suppose that A^* negatively regulates the phosphatase that dephosphorylates it. The resulting circuit is a ''double-negative'' feedback loop. Like the positive feedback system described above, this double-negative system may be able to switch between two discrete states: one with A phosphorylated and the A^* phosphatase inhibited, and one with A dephosphorylated and the A^* phosphatase disinhibited. One example of a biologically important double-negative system is the Notch/Delta system, where Notch and Delta mutually antagonize each other's expression in pairs of neighboring cells.[25]

The common feature of the double-negative circuit and the positive feedback circuit is that both possess a ''vicious cycle'' type of logic—the more A^* there is, the faster A gets phosphorylated (the positive feedback case) or the slower A^* gets dephosphorylated (the double-negative feedback case). Other variations on positive and negative feedback that can give rise to bistability are discussed elsewhere.[17,26,27]

VI. SWITCHING FROM THE OFF-STATE TO THE ON-STATE

A. Getting over the threshold

So far we have constructed a bistable system two ways [Figs. 4(a) and 5(b)], and in both cases we have ignored any contribution from the basal activity of the kinase, or from any feedback-independent stimulus that could promote the forward reaction. We have produced systems that can reside in either a stable off-state or a stable on-state, but we have not provided a mechanism for the systems to make a transition from one state to the other. One way of flipping the switch to send the system from the off-state to the on-state is to continuously increase the feedback-independent stimulus. The bistable system then converts this continuous change in the activity of an enzyme (the stimulus) into a discontinuous change in the steady state output of the system (the concentration of A^*).

This is shown in a rate balance plot in Fig. 6(a), which starts with the bistable system described in Fig. 4 (ultrasensitive feedback, no saturation of the back reaction). However, instead of showing a single curve for the forward reaction rate as a function of $[A^*]/[A_{tot}]$, we now show a family of seven such curves, each corresponding to a different level of the continuously variable stimulus. The lowest of the seven curves corresponds to the case of no stimulus; the other curves correspond to six successive increments of stimulus.

Suppose that the stimulus is initially zero, and the system is starting out in the off-state, with the steady state concentration of $[A^*]$ being zero. There is an on-state too, where $[A^*]/[A_{tot}]$ would be approximately 0.72, but it would take a mighty perturbation to drive the system out of the off-state, past the threshold (at $[A^*]/[A_{tot}] \approx 0.28$), and into the territory of the on-state.

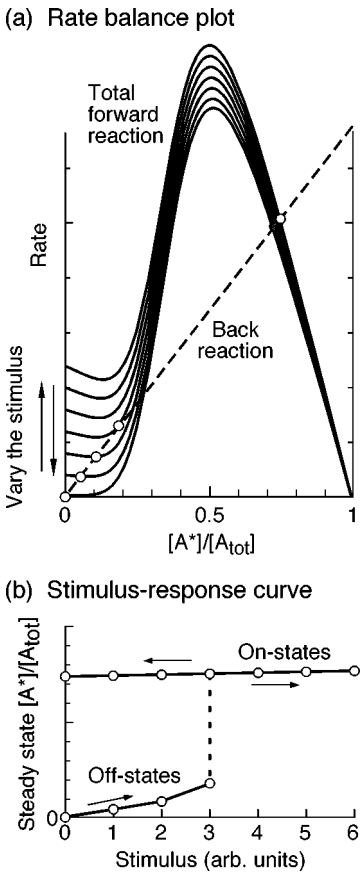

FIG. 6. Graphical derivation of the stimulus-response curve for the system depicted in Fig. 4(a). (a) Rate balance plot. The total forward reaction rate curves represent seven levels of stimulus. For low levels of stimulus, there is an off-state, an on-state, and a threshold. As the stimulus increases, the off-state shifts up toward the threshold and the threshold shifts down towards the off-state. Eventually the off-state and threshold disappear and the system has only an on-state. (b) Stimulus-response curve. The intersection points from panel (a) are plotted as a function of stimulus. When the stimulus is increased, the system can switch from the off-state to the on-state. However, when the stimulus is decreased back to zero, the system remains stuck in the on-state.

Now add one increment of stimulus to the system. The stimulus provides an additional component to the total forward reaction rate, skewing the curve upward [Fig. 6(a)]. Now the off-state has shifted upward slightly, to $[A^*]/[A_{tot}] \approx 0.06$, and the threshold has shifted down slightly, to $[A^*]/[A_{tot}] \approx 0.26$. The off-state and threshold are beginning to approach each other.

Add one more increment of stimulus to the system. The total forward reaction rate curve skews upward a little farther [Fig. 6(a)]. The off-state shifts to $[A^*]/[A_{tot}] \approx 0.12$, and the threshold shifts down a little further to $[A^*]/[A_{tot}] \approx 0.23$. With one more increment of stimulus, the off-state and the threshold have become about equal at $[A^*]/[A_{tot}] \approx 0.19$. The total forward reaction curve and the back reaction line are barely touching at this value of $[A^*]/[A_{tot}]$. If the stimulus is increased any further, the off-state and the threshold no longer exist. There is now only a single possible steady state for the system, the on-state. The system leaves the off-state and aims for the on-state. Initially the driving force for this transition will be very small; the difference between the forward reaction rate and back reaction rate will be small. As the stimulus increases further, the driving force for the transition to the on-state becomes higher.

B. Switching implies hysteresis

So far, by increasing the stimulus we have gotten the system out of the off-state and into the on-state. What happens if we now lower the stimulus back down? Once the stimulus is lowered to three units, the off-state will reappear. But there will be no driving force for the system to leave the on-state and make the transition back to the off-state. The on-state is stable, and the system is stuck in it. Thus, the stimulus-response curve you get when the stimulus is rising is not the same as the one you get when the stimulus is falling [Fig. 6(b)]; the system exhibits hysteresis.[26–28] For the example shown here, the hysteresis is so substantial that the transition from the off-state to the on-state is irreversible.

The potential significance of hysteresis in biological switching is twofold. First, it decreases the likelihood that a system will repeatedly switch back and forth between two states (a possibility termed ''chattering'' by Thron[17]) when the stimulus that drives the switching is hovering near its threshold value. Second, it is a potential mechanism for a type of biochemical memory. Unless something happens to fundamentally change (e.g., break) the positive feedback loop, a system like those shown in Figs. 4 and 6 can remain on indefinitely. A bistable signaling system could be the mechanism through which cells ''remember'' that they are differentiated long after the differentiation stimulus has been withdrawn, and even long after all of the protein molecules that make up the feedback loop have been replaced by new protein molecules.

VII. THE STEADY STATE BALANCE PLOT

Another commonly used way of representing bistable systems is the steady state balance plot. This type of plot is particularly useful when one can identify two key enzymes in a feedback system that mutually activate each other (A^* promotes the activation of B, and B^* in turn promotes the activation of A; a positive feedback system) or mutually inactivate each other (A^* promotes the inhibition of B^*, and B^* in turn promotes the inhibition of A^*; a double-negative feedback system). Here we will consider the positive feedback system [Fig. 7(a)].

For the moment we will ignore the feedback reactions and consider only the direct activation of B by A^* [designated ''1'' in Fig. 7(a)]. The steady state response of B^* to A^* will be described by a stimulus-response curve of some shape; here we have assumed it is a Michaelian curve [Fig. 7(b)]. For the whole system to be in steady state, $[B^*]$ must be unchanging with respect to time. The curve shown in Fig. 7(b) constitutes the only pairings of $[A^*]/[A_{tot}]$ and $[B^*]/[B_{tot}]$ where this can be true.

Now we will consider only the feedback reactions [designated ''2'' in Fig. 7(a)]. In these reactions, the steady state level of $[A^*]/[A_{tot}]$ is a function of $[B^*]$. Again, some sort of stimulus-response curve will describe the dependence of $[A^*]/[A_{tot}]$ upon $[B^*]/[B_{tot}]$; here we have assumed that

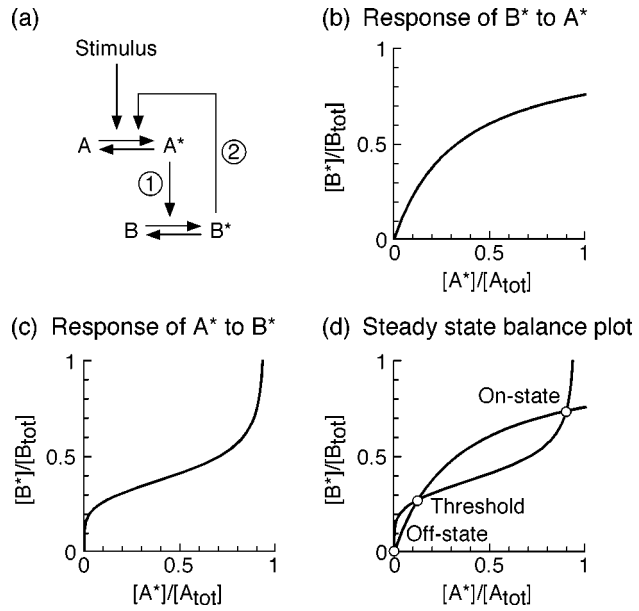

FIG. 7. Steady state balance plots for a system with ultrasensitive positive feedback. (a) Schematic depiction of the system. (b) Assumed Michaelian steady state response of B^* to A^*. (c) Assumed ultrasensitive steady state response of A^* to B^*. Note that we have flipped the x- and y-axes from what would be customary for depicting A^* as a function of B^*. (d) Steady state balance plot. The curves from (b) and (c) are plotted together. Where the two curves intersect, the system is in steady state. There are three intersection points, corresponding to an off-state, a threshold, and an on-state.

this stimulus-response cure is sigmoidal [Fig. 7(c)]. The points on this curve represent the only pairings of $[A^*]/[A_{\mathrm{tot}}]$ and $[B^*]/[B_{\mathrm{tot}}]$, where $[A^*]$ can be unchanging with respect to time.

Finally, we can plot A^* as a function of B^* and B^* as a function of A^* on the same set of axes [Fig. 7(d)]. Only points that lie on both curves—the three points of intersection—can represent a system where neither $[A^*]$ nor $[B^*]$ is changing with respect to time. These three intersection points correspond to the off-state, threshold, and on-state.

VIII. BISTABILITY IN OOCYTE MATURATION

The all-or-none character of oocyte maturation suggests that a bistable signaling system may mediate the process. In support of this idea, both of the key protein kinases involved in oocyte maturation—MAPK and Cdc2—are embedded in positive feedback loops. Not only does Mos bring about activation of p42 MAPK, but activation of p42 MAPK brings about accumulation of Mos, even in the absence of progesterone.[29–32] Two factors appear to contribute to p42 MAPK-dependent Mos accumulation. First, Mos mRNA polyadenylation and protein synthesis is stimulated by p42 MAPK;[32] second, Mos stability is increased by p42 MAPK, possibly through the phosphorylation of Ser 3.[29,31] Likewise, multiple factors contribute to positive feedback in the activation of Cdc2. Cdc2 activation stimulates the polyadenylation of the cyclin B1 mRNA and translation of the cyclin B1 message.[32] Moreover, Cdc2 can bring about the inactivation of Myt1,[33,34] the main Cdc2-inhibitory kinase present in maturing oocytes [Wee1 is not present until late in meiosis 2 (Ref. 35)], and activation of Cdc25C,[36,37] the dual-specificity phosphatase that reverses the effects of Myt1 and activates Cdc2.

In addition, the response of p42 MAPK to Mos in oocyte extracts is known to be highly ultrasensitive—equivalent to the response of a cooperative enzyme with a Hill coefficient of about 5.[38] Thus the MAPK cascade has a mechanism for endowing a positive feedback loop with a stable off-state. It is not known whether the feedback loop in the Cdc2 system is ultrasensitive as well, but many of the factors that can generate ultrasensitivity, such as multistep phosphorylation reactions and stoichiometric inhibitors, are present in Cdc2 regulation.

So the potential is there for bistability. The question is, does this bistability really occur? Eric Machleder and I addressed this question by incubating oocytes with submaximal concentrations of progesterone, letting the oocytes come to a new steady state, and then measuring the level of p42 MAPK phosphorylation (a surrogate for p42 MAPK activation) in each oocyte.[39] We found that the oocytes either activated essentially all of their p42 MAPK (p42 MAPK phosphorylation $>90\%$ maximal) or essentially none of it ($<10\%$ maximal phosphorylation). None of the oocytes (out of 209 examined) showed an intermediate level of p42 MAPK phosphorylation. These findings established that p42 MAPK responds to progesterone in an all-or-none manner.

The all-or-none character of maturation could arise within the MAPK cascade, or it could arise upstream of the cascade and merely be propagated by the cascade. To test these possibilities we microinjected oocytes with different concentrations of Mos protein, which activates the MAPK cascade directly without activating the most upstream elements of the progesterone signaling pathway, and measured their p42 MAPK responses. Once again, the oocytes responded in an all-or-none fashion, indicating that the MAPK cascade can generate, not simply propagate, an all-or-none response.[39]

Finally, we examined whether manipulations that should block the positive feedback loop would convert the all-or-none MAPK response into a more graded one. We blocked protein synthesis with cycloheximide, which prevents MAPK from inducing Mos accumulation, and then examined the response of p42 MAPK to microinjected Mos protein. We found that many oocytes now had intermediate steady state levels of p42 MAPK activation. Abolishing positive feedback abolished the all-or-none character of the p42 MAPK response.[39]

All of these observations support the idea that p42 MAPK exhibits bistable responses, and it seems plausible that the all-or-none character of p42 MAPK biochemistry contributes to the all-or-none character of oocyte maturation.

IX. OTHER EXAMPLES OF BISTABILITY IN BIOLOGY

The lysis-lysogeny transition in phage-infected *E. coli* is another bistable switch, where feedback is provided by the lambda repressor and filtering is (probably) provided by a dimerization requirement and cooperativity.[40] Muscle differentiation constitutes another important example; transcrip-

tional regulators that can induce muscle differentiation have been shown to induce their own transcription.[41] The natures of the proteins involved and the time scales of the responses are quite different, but in all cases the all-or-none switches are thought to be built out of the same basic ingredients—positive feedback plus ultrasensitivity.

In addition, bistable switches can be coupled to slower negative feedback loops to produce biological oscillators. One example is the action potential in electrically excitable cells. Positive feedback is provided by the voltage-dependent sodium channel and filtering is provided by the nonlinearity of the channel's initial response, producing an all-or-none firing.[42] However, in this case, the on-state is transitory, not permanent; the sodium channel auto-inactivates, breaking the feedback, and resetting the system to its initial off-state. A second example is the cyclin-dependent protein kinases (Cdks), which drive cell cycle transitions. As described by Tyson elsewhere in this issue,[43] this system appears to be built out of bistable switches, which allow for sharp transitions between one phase of the cell cycle and the next, coupled to slower negative feedback mechanisms, which keep the cell cycle from getting stuck in an on state. The positive feedback is provided by the action of the Cdks on their upstream activators, filtering is probably provided by a number of different mechanisms, and negative feedback is provided by the Cdk-triggered destruction of their activating cyclin subunits.

In a very satisfying test of these principles, several groups have now constructed artificial bistable[44,45] or oscillatory[46] systems by expressing circuits of regulatory proteins in *E. coli* and *S. cerevisiae*. These studies are important both from a biotechnology perspective and from a more basic one as well—studies of this sort have the potential to inform our understanding of why nature has or has not made certain choices in the design of signaling circuits.

X. SUMMARY

We have shown that signaling systems that include strong positive feedback have the potential to exhibit bistability; to convert continuous stimuli into discontinuous steady state responses; and to exhibit hysteresis or irreversibility. There are two essential ingredients for a successful bistable switch. The first is positive feedback; the second is some mechanism for filtering small stimuli out of the positive feedback loop, so that the system can have a stable off-state. One way of producing a stable off-state is to have the positive feedback be relayed by a signaling protein or cascade that exhibits a sigmoidal (ultrasensitive) stimulus-response curve. Another way is to have the enzyme that opposes the positive feedback operating close to saturation.

In addition, the kinetic properties of the forward and back reactions must be in proper balance for a system to exhibit bistability. Increasing or decreasing the amounts or activities of various components has the potential to turn a bistable system into a monostable one, which acts more like a rheostat than a switch.

Bistable cell signaling systems appear to be responsible for the digital, all-or-none character of the action potential, various cell fate induction processes, and progression from one discrete phase of the cell cycle to another. Bistable cell signaling systems also offer the possibility of providing a sort of biochemical memory, which may contribute to the irreversibility of some cell differentiation processes.

ACKNOWLEDGMENTS

We thank members of our lab group for helpful suggestions on this manuscript. Our work in this area is supported by National Institute of Health Grant No. GM61276.

[1] J. Monod and F. Jacob, ''General conclusions: Teleonomic mechanisms in cellular metabolism, growth, and differentiation,'' Cold Spring Harbor Symp. Quant. Biol. **26**, 389–401 (1961).
[2] J. Lewis, J. M. Slack, and L. Wolpert, ''Thresholds in development,'' J. Theor. Biol. **65**, 579–590 (1977).
[3] C. D. Thron, ''Bistable biochemical switching and the control of the events of the cell cycle,'' Oncogene **15**, 317–325 (1997).
[4] L. A. Segel, ''Multiple attractors in immunology: Theory and experiment,'' Biophys. Chem. **72**, 223–230 (1998).
[5] P. Smolen, D. A. Baxter, and J. H. Byrne, ''Frequency selectivity, multistability, and oscillations emerge from models of genetic regulatory systems,'' Am. J. Physiol. **274**, C531–542 (1998).
[6] M. Laurent and N. Kellershohn, ''Multistability: A major means of differentiation and evolution in biological systems,'' Trends Biochem. Sci. **24**, 418–422 (1999).
[7] Y. Gotoh and E. Nishida, ''The MAP kinase cascade: Its role in Xenopus oocytes, eggs, and embryos,'' Prog. Cell Cycle Res. **1**, 287–297 (1995).
[8] J. E. Ferrell, Jr., ''Building a cellular switch: More lessons from a good egg,'' BioEssays **21**, 866–870 (1999).
[9] J. E. Ferrell, Jr., ''Xenopus oocyte maturation: New lessons from a good egg,'' BioEssays **21**, 833–842 (1999).
[10] N. Sagata, ''What does Mos do in oocytes and somatic cells?'' BioEssays **19**, 13–21 (1997).
[11] L. Connell-Crowley, M. J. Solomon, N. Wei, and J. W. Harper, ''Phosphorylation independent activation of human cyclin-dependent kinase 2 by cyclin A *in vitro*,'' Mol. Biol. (Moscow) **4**, 79–92 (1993).
[12] J. C. Hagopian, M. P.Kirtley, L. M. Stevenson, R. M. Gergis, A. A. Russo, N. P. Pavletich, S. M. Parsons, and J. Lew, ''Kinetic basis for activation of Cdk2/CyclinA by phosphorylation,'' J. Biol. Chem. (in press).
[13] H. Kobayashi, J. Minshull, C. Ford, R. Golsteyn, R. Poon, and T. Hunt, ''On the synthesis and destruction of *A*- and *B*-type cyclins during oogenesis and meiotic maturation in *Xenopus laevis*,'' J. Cell Biol. **114**, 755–765 (1991).
[14] H. Kobayashi, R. Golsteyn, R. Poon, E. Stewart, J. Gannon, J. Minshull, R. Smith, and T. Hunt, ''Cyclins and their partners during Xenopus oocyte maturation,'' Cold Spring Harbor Symp. Quant. Biol. **56**, 437–447 (1991).
[15] J. E. Ferrell, Jr., ''Tripping the switch fantastic: How a protein kinase cascade can convert graded inputs into switch-like outputs,'' Trends Biochem. Sci. **21**, 460–466 (1996).
[16] M. L. Sohaskey and J. E. Ferrell, Jr., ''Distinct, constitutively active MAPK phosphatases function in Xenopus oocytes: Implications for p42 MAPK regulation in vivo,'' Mol. Biol. (Moscow) **10**, 3729–3743 (1999).
[17] C. D. Thron, ''A model for a bistable biochemical trigger of mitosis,'' Biophys. Chem. **57**, 239–251 (1996).
[18] J. J. Tyson, B. Novak, G. M. Odell, K. Chen, and C. D. Thron, ''Chemical kinetic theory: Understanding cell-cycle regulation,'' Trends Biochem. Sci. **21**, 89–96 (1996).
[19] D. C. LaPorte and D. E. Koshland, Jr., ''Phosphorylation of isocitrate dehydrogenase as a demonstration of enhanced sensitivity in covalent regulation,'' Nature (London) **305**, 286–290 (1983).
[20] J. E. Lisman, ''A mechanism for memory storage insensitive to molecular turnover: A bistable autophosphorylating kinase,'' Proc. Natl. Acad. Sci. U.S.A. **82**, 3055–3057 (1985).
[21] A. Goldbeter and D. E. Koshland, Jr., ''Sensitivity amplification in biochemical systems,'' Q. Rev. Biophys. **15**, 555–591 (1982).
[22] D. E. Koshland, Jr., A. Goldbeter, and J. B. Stock, ''Amplification and adaptation in regulatory and sensory systems,'' Science **217**, 220–225 (1982).
[23] A. Goldbeter and D. E. Koshland, Jr., ''Am amplified sensitivity arising

from covalent modification in biological systems,'' Proc. Natl. Acad. Sci. U.S.A. **78**, 6840–6844 (1981).
[24] C. D. Thron, ''Theoretical dynamics of the cyclin B-MPF system: A possible role for p13suc1,'' BioSystems **32**, 97–109 (1994).
[25] S. Artavanis-Tsakonas, M. D. Rand, and R. J. Lake, ''Notch signaling: Cell fate control and signal integration in development,'' Science **284**, 770–776 (1999).
[26] J. F. Hervagault and S. Canu, ''Bistability and irreversible transitions in a simple substrate cycle,'' J. Theor. Biol. **127**, 439–449 (1987).
[27] G. M. Guidi and A. Goldbeter, ''Bistability without hysteresis in chemical reaction systems: A theoretical analysis of irreversible transitions between multiple steady states,'' J. Phys. Chem. A **101**, 9367–9376 (1997).
[28] H. S. Hahn, P. J. Ortoleva, and J. Ross, ''Chemical oscillations and multiple steady states due to variable boundary permeability,'' J. Theor. Biol. **41**, 503–521 (1973).
[29] L. M. Roy, O. Haccard, T. Izumi, B. G. Lattes, A. L. Lewellyn, and J. L. Maller, ''Mos proto-oncogene function during oocyte maturation in Xenopus,'' Oncogene **12**, 2203–2211 (1996).
[30] Y. Gotoh, N. Masuyama, K. Dell, K. Shirakabe, and E. Nishida, ''Initiation of Xenopus oocyte maturation by activation of the mitogen-activated protein kinase cascade,'' J. Biol. Chem. **270**, 25898–25904 (1995).
[31] W. T. Matten, T. D. Copeland, N. G. Ahn, and G. F. Vande Woude, ''Positive feedback between MAP kinase and Mos during Xenopus oocyte maturation,'' Dev. Biol. **179**, 485–492 (1996).
[32] E. L. Howard, A. Charlesworth, J. Welk, and A. M. MacNicol, ''The MAP kinase signaling pathway stimulates Mos mRNA cytoplasmic polyadenylation during *Xenopus* oocyte maturation,'' Mol. Cell. Biol. **19**, 1990–1999 (1999).
[33] P. R. Mueller, T. R. Coleman, A. Kumagai, and W. G. Dunphy, ''Myt1: A membrane-associated inhibitory kinase that phosphorylates Cdc2 on both threonine-14 and tyrosine-15,'' Science **270**, 86–90 (1995).
[34] A. Palmer, A. C. Gavin, and A. R. Nebreda, ''A link between MAP kinase and $p34^{cdc2}$/cyclin B during oocyte maturation: $p90^{rsk}$ phosphorylates and inactivates the $p34^{cdc2}$ inhibitory kinase Myt1,'' EMBO J. **17**, 5037–5047 (1998).
[35] M. S. Murakami and G. F. Vande Woude, ''Analysis of the early embryonic cell cycles of Xenopus; regulation of cell cycle length by Xe-wee1 and Mos,'' Development (Cambridge, U.K.) **125**, 237–248 (1998).
[36] M. J. Solomon, M. Glotzer, T. H. Lee, M. Philippe, and M. W. Kirschner, ''Cyclin activation of $p34^{cdc2}$,'' Cell **63**, 1013–1024 (1990).
[37] A. Kumagai and W. G. Dunphy, ''Regulation of the cdc25 protein during the cell cycle in Xenopus extracts,'' Cell **70**, 139–151 (1992).
[38] C.-Y. F. Huang and J. E. Ferrell, Jr., ''Ultrasensitivity in the mitogen-activated protein kinase cascade,'' Proc. Natl. Acad. Sci. U.S.A. **93**, 10078–10083 (1996).
[39] J. E. Ferrell, Jr. and E. M. Machleder, ''The biochemical basis of an all-or-none cell fate switch in Xenopus oocytes,'' Science **280**, 895–898 (1998).
[40] M. Ptashne, A. D. Johnson, and C. O. Pabo, ''A genetic switch in a bacterial virus,'' Sci. Am. **247**, 128–130 (1982); **247**, 132 (1982); **247**, 134–140 (1982).
[41] M. J. Thayer, S.J. Tapscott, R. L. Davis, W. E. Wright, A. B. Lassar, and H. Weintraub, ''Positive autoregulation of the myogenic determination gene MyoD1,'' Cell **58**, 241–248 (1989).
[42] E. Kandel, J. Schwartz, and T. Jessell, in *Principles of Neural Science* (Appleton & Lange, Norwalk, CT, 1991), pp. 104–118.
[43] J. J. Tyson and M. C. Mackey, Chaos **11**, 81 (2001).
[44] T. S. Gardner, C. R. Cantor, and J. J. Collins, ''Construction of a genetic toggle switch in Escherichia coli,'' Nature (London) **403**, 339–342 (2000).
[45] A. Becskei and L. Serrano (personal communication).
[46] M. B. Elowitz and S. Leibler, ''A synthetic oscillatory network of transcriptional regulators,'' Nature (London) **403**, 335–338 (2000).