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Bistable biochemical switching and the control of the events of the cell cycle*

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Some of the events of the cell cycle appear to be triggered by a bistable mechanism. A bistable biochemical system can respond to a small, slow signal and is carried by positive feedback from one stable steady state directly to another, in an all-or-none manner. Slow or subthreshold stimuli do not cause accommodation or loss of excitability. Switching is not readily reversible by removing the stimulus, i.e. there is hysteresis: reversal generally requires a stronger, opposite stimulus. Biochemically, bistable biochemical switching requires positive feedback, and mechanisms for stabilizing the system against premature activation and for destabilization in response to a biological signal. Three bistable biochemical models, all suggested by reported experimental observations, are described and analysed. These models suggest that a titratable inhibitor may play an important part in bistable switching, because the end-point of titration can form a natural threshold for enhancement of positive feedback.

Keywords: biochemical switch; bistability; cell cycle; cyclin-dependent kinase inhibitors; positive feedback

Introduction

The onset of mitosis is thought to be caused by maturation promoting factor (MPF), a complex of the protein kinase Cdc2 with cyclin B (Murray and Hunt, 1993). MPF shows the phenomenon of 'amplification': a small added amount of MPF causes activation of a much larger amount from an inactive precursor (Cyert and Kirschner, 1988; Dunphy and Newport, 1988). Amplification is apparently due to the fact that Cdc2/cyclin B activates its own activating kinase Cdc25 (Hoffman *et al*., 1993), giving rise to a positive feedback loop that makes the activation of MPF effectively autocatalytic. Evidently a cell can exist in a stable steady state with low MPF activity; but on displacement of the cell from this steady state, by addition of a small amount of MPF, the autocatalytic effect of MPF causes the cell to shift toward a different stable state, one with high MPF activity. Amplification therefore indicates the existence of two potential stable steady states, i.e. bistability.

Bistable switching is well known in chemical engineering, and a clear and fairly simple explanation is given by Gray and Scott (1990). In biology it has been proposed as a mechanism of memory, of differentiation, or of conversion of continuous chemical gradients into discontinuous patterns of embryonic development (Edelstein, 1971; Seelig and Denzel, 1972; Meinhart, 1982; Murray, 1989). Biochemical switches of the simple types considered here have been described and discussed by Edelstein-Keshet (1988) and Murray (1989). Lewis *et al*. (1977) have particularly stressed that other types of biochemical switch do not show true thresholds of discontinuity, and do not provide either the sharpness or the irrevocability of bistable switches. Bistable switching is typically caused by a change in a rate constant or other parameter which changes the biochemical dynamics and destabilizes the current stable state, and thereby forces the system to go to the alternate stable state. A change of steady state may alternatively be caused by addition of substrate, as in amplification of MPF; but mitosis is not normally initiated by addition of MPF, and we will focus here on switching by destabilization of a steady state.

Some cell cycle events other than mitosis are controlled by cyclin-dependent kinases (Cdks) other than Cdc2, and some of these Cdks appear to be regulated by Cdk inhibitors (CKIs) (Hunter, 1993; Pines, 1994; Elledge and Harper, 1994; Peter and Herskowitz, 1994b). Nasmyth and Hunt (1993) have suggested that Cdks 'build up like water behind a CKI dam,' and that overflow of Cdk from the dam triggers proteolysis or inactivation of the CKI, releasing a flood of Cdks that irrevocably commit the cell to the next phase of the cell cycle. Several CKIs appear to be regulated by proteolysis (McKinney *et al*., 1993; Schwob *et al*., 1994; Pagano *et al*., 1995), and while mechanisms for rapidly activating the proteolysis have not been generally demonstrated, Sherr (1996) cites a report that Cdk2/cyclin E accelerates turnover of $p27^{Kip1}$. If a Cdk activates CKI proteolysis this releases more Cdk, creating positive feedback and possibly leading to complete destruction of CKI and a stable state with CKI obliterated and Cdk freed. To prevent activation, CKI must inhibit Cdk-activated CKI proteolysis enough to protect itself and stabilize a steady state with low Cdk activity. The system is then bistable and has the potential for bistable switching.

The important characteristics of a bistable biochemical switch are (1) it always commits itself to one of two stable steady states, and does not linger in any intermediate or transitional states; (2) it can be switched rapidly from one stable steady state to another, and this may require only a small, slow signal; and (3) it shows hysteresis, in that removing the signal does not undo the response, and restoring the original steady state requires a stronger opposite signal. These characteristics, in a switch controlling the onset of mitosis or other cell cycle events, can prevent an incomplete or vacillating commitment, which might well be catastrophic. This paper attempts to give a qualitative understanding of the dynamics and biochemical kinetics underlying several types of

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bistable switching which may be involved in controlling events of the cell cycle. The models used are based on the experimental literature but are simplified in order to show the dynamical principles.

Autocatalytic activation

We use the terms Cdc2 and Cdc2P to denote the active (tyrosine 15-dephosphorylated) and inactive (tyrosine 15-phosphorylated) forms of the Cdc2/cyclin B heterodimer, respectively.

With any autocatalytic process, a key problem is how to prevent premature activation. In the generally accepted mechanism for Cdc2 activation and inactivation (Figure 1a) the relative strength of autocatalysis must be relatively weak at low Cdc2 concentrations, in order to stabilize the steady state with predominantly the inactive phosphorylated form Cdc2P. Binding of Cdc2 to an inhibitor (X, Figure 1b), preventing autocatalysis, would weaken the autocatalysis preferentially at low Cdc2 concentrations because, by the laws of chemical equilibrium, binding of Cdc2 to a fixed amount of inhibitor would be proportionately greater at lower [Cdc2]. If the binding is very strong, the inhibitor would almost completely inhibit Cdc2 up to a stoichiometric amount, but excess Cdc2 would be uninhibited.

It has been proposed (Thron, 1994, 1996), that the protein Suc1 acts as the inhibitor X in this model. Suc1 binds to Cdc2 and inhibits activation (Dunphy and Newport, 1989), and this mechanism could explain several of the puzzling effects of Suc1 (Thron, 1994). However this role of Suc1 now seems questionable. Recent findings (Patra and Dunphy, 1996; Pines, 1996) indicate that the action of Suc1 is more complicated. Moreover, the quantity of Suc1 present vastly exceeds the quantity of cyclin B (Kobayashi *et al*., 1991a, b; Patra and Dunphy, 1996). The amount of Cdc2/cyclin B is therefore much too small to titrate Suc1 stoichiometrically. Moreover, if Cdc2/Suc1 binding is partially reversible, the large excess of Suc1 would inhibit all concentrations of Cdc2/cyclin B approximately equally, and there would not be much preferential inhibition of lower concentrations. However other Cdc2-binding substances have been reported (Kumagai and Dunphy, 1995; Lee and Kirschner, 1996) which may act as the inhibitor X in Figure 1b.

In this model, there is continuous metabolic turnover between the active and inactive forms, Cdc2 and Cdc2P respectively. To analyse the mechanism we consider the case where the total of Cdc2 and Cdc2P is constant. Cdc2 is assumed to bind to the inhibitor X, but Cdc2P binding to X is assumed to be relatively much less strong (Thron, 1994, 1996). Unbound Cdc2 effectively (through activation of Cdc25) accelerates its own formation, i.e. the conversion of Cdc2P to Cdc2. Since the total of Cdc2P and Cdc2 is assumed to be constant, the net rate of formation of Cdc2 equals the net rate of decrease of Cdc2P. This rate is equal to the difference between the rate of activation F and the rate of deactivation R:

$$\frac{d[\mathrm{Cdc2}]}{dt} = \frac{d[\mathrm{Cdc2P}]}{dt} = F - R \qquad (1)$$

where [Cdc2] is the total concentration of free and X-bound Cdc2, and F and R are mathematical functions of the concentration of Cdc2. For present purposes these functions need only be described qualitatively, and the mathematical details can be disregarded. (F and R are also functions of the concentrations of free Cdc2 and Cdc2P, but these in turn are functions of [Cdc2]: the proportion of free Cdc2 is determined by the equilibrium of X binding, and [Cdc2P] is equal to the fixed total of [Cdc2] and [Cdc2P] minus [Cdc2]).

The key to the biochemical switching dynamics is in how the rates of activation and inactivation depend on [Cdc2]. In Figure 2a, the rate of Cdc2 deactivation R is shown as directly proportional to [Cdc2] (free and X-bound Cdc2 are assumed to be deactivated at equal rates). The rate of Cdc2 formation F might be expected to be a similar straight line sloping in the opposite direction, because its substrate Cdc2P increases as [Cdc2] decreases (because the total [Cdc2]+[Cdc2P] is constant). However, curve F is more complicated. Over a limited range of Cdc2 concentrations F increases as [Cdc2] increases, because of the autocatalysis. The increase is vanishingly slow at low [Cdc2], however, because most of the Cdc2 is bound to X and is not autocatalytically active. When enough Cdc2 has been added to titrate all the available X, further increase in [Cdc2] produces a relatively large overflow increase in unbound Cdc2, with a corresponding increase in autocatalysis, and F rises more rapidly. However as [Cdc2] increases still further F begins to decrease because of the decreasing concentration of substrate Cdc2P

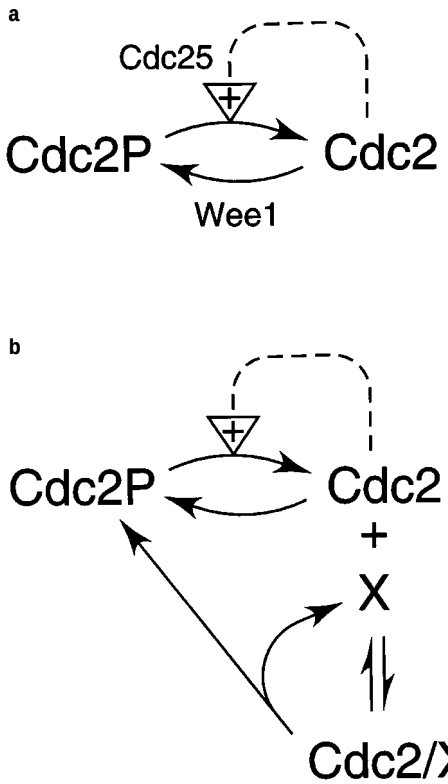

Figure 1 (**a**) The generally accepted mechanism for Cdc2 activation. Cdc2 and Cdc2P denote the active (tyrosine 15-dephosphorylated) and inactive (tyrosine 15-phosphorylated forms of the Cdc2/cyclin B heterodimer, respectively. (**b**) A model with Cdc2 binding to an inhibitor X, weakening the autocatalysis preferentially at low [Cdc2] and thereby stabilizing a steady state with low Cdc2 activity

remaining to be activated; and of course F becomes equal to 0 when all the Cdc2P has been converted to Cdc2.

Note that F has a small positive value when [Cdc2] = 0. The importance of this will appear below.

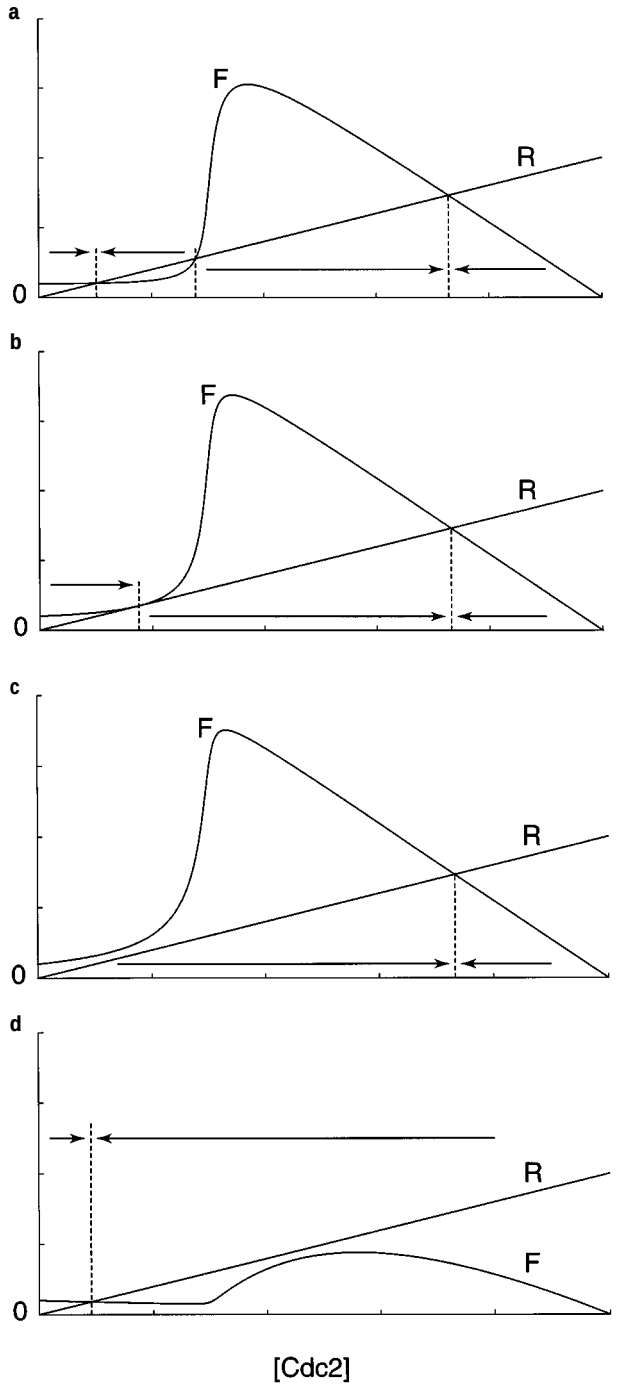

Figure 2 Rates of activation F and deactivation R of Cdc2 in the autocatalytic model of Figure 1b. The intersections of curves F and R (where $F = R$) mark steady state concentrations of Cdc2, shown by dashed lines. Between steady states, arrows show the direction of spontaneous change: [Cdc2] increases if $F > R$, decreases if $R > F$. (**a**) Three steady states, two stable and one unstable. (**b**) With increasing Cdc25 activity, curve F is shifted upward until the left-hand steady state merges with the middle (unstable) steady state in a point of tangency between curves F and R. (**c**) Further increase in Cdc25 activity causes F to rise further, breaking contact with R, and abolishing the left-hand steady state; the system must shift to the right-hand steady state. (**d**) A very marked decrease in Cdc25 activity is needed to extinguish the right-hand steady state and cause the system to return to the left-hand steady state. See text for further explanation

The model attributes it to the hypophosphorylated low-activity form of Cdc25 (Zheng and Ruderman, 1993; Thron, 1996).

From Figure 2a can be extracted the essential information on how the system behaves. Where curves R and F intersect, R equals F and the spontaneous rate of change of [Cdc2] is zero, by equation (1). The intersections of R and F therefore mark concentrations of Cdc2 at which the system has no tendency to change, i.e. the system is in a steady state. These steady state concentrations are indicated by dashed lines in Figure 2a. As can be seen in Figure 2a, the double curvature of curve F, caused by the autocatalysis and the X binding, results in three intersections with R, and hence three (potential) steady states. Between the steady states, [Cdc2] tends to change, by equation (1), increasing where $F > R$ and decreasing where $R > F$, as shown by the arrows in Figure 2a. From these directions of spontaneous change it can be seen that the left-hand steady state, at low [Cdc2], is a *stable* steady state, because if [Cdc2] is slightly increased or decreased it returns spontaneously to this steady state. Likewise the right-hand steady state is a stable steady state. In contrast, the middle steady state is *unstable*, because if [Cdc2] is increased or decreased it does not return spontaneously to this steady state, but rather moves away toward the left-hand or right-hand steady state.

In the stable steady state with low Cdc2 activity this sytem can be triggered by adding enough Cdc2 to bring [Cdc2] above the level of the unstable steady state. Once [Cdc2] is above that level, it goes spontaneously to the high-activity steady state. This is apparently what happens experimentally in 'amplification' of MPF.

A different mode of activation is produced by increasing Cdc25 activity in the model (Figure 2b, c). This increases the rate of Cdc2 activation and causes a portion of the curve F to shift upward until it breaks contact with curve R. In Figure 2b, curve F has shifted high enough to be just tangent to curve R at a point where the low-activity and unstable steady states have merged. In Figure 2c, a further increase in Cdc25 activity has broken this contact between F and R, and the low-activity and unstable steady states no longer exist. The direction of spontaneous change is now everywhere toward the high-activity steady state and [Cdc2] is compelled to change completely to the high-activity stable steady state.

This extinction or disappearance of the low-activity steady state is the mechanism for this mode of triggering activation of Cdc2, according to this model. This is a *qualitative change in the dynamics*: a bistable system becomes monostable. The general mathematical term for such a qualitative change is *bifurcation* (Hale and Kocak, 1991). This particular type of bifurcation, in which a pair of steady states, one stable and one unstable, appears or disappears, is a *saddle-node bifurcation* (the name comes from multi-variable systems, where there is what is called a 'saddle point' instead of an unstable steady state).

The small positive value of F at [Cdc2] = 0 is important for the reliability of triggering. If F and R were both 0 when [Cdc2] = 0 (i.e. if the low-activity steady state were at the origin) then increasing the rate of Cdc2 activation could destabilize this point, but

could not obliterate it. Curves *F* and *R* would never break contact at this point and the low-activity steady state would always remain as an unstable steady state, where the system might remain indefinitely unless there happened to be a trace of active Cdc2 to start the autocatalysis.

Reversing the increase in Cdc25 activity in Figure 2b and c would restore the three intersections in Figure 2a, but it would not destabilize the high-activity steady state, and would not cause the system to shift back to the low-activity steady state. In this sense the activation of Cdc2 has been irrevocable. To force a return to the low-activity steady state would require a much more marked reduction in Cdc25 activity, to the point where the maximum in curve *F* no longer contacts curve *R* (Figure 2d). Again there is a change in the number of steady states, this time by a different saddle-node bifurcation; and there remains only the low-activity steady state, to which the system must return. The Cdc25 activity at which inactivation occurs is therefore lower than the Cdc25 activity required for activation. This behavior, with a difference between the thresholds for activation and inactivation, is *hysteresis*. Because of hysteresis, small random fluctuations in the rate of Cdc2 activation will never switch the system repeatedly between high- and low-activity states. Hysteresis, together with the fact that there are never any stable intermediate-activity states, ensures that commitment to the next phase of the cell cycle is complete and effectively irrevocable. (The restoration of the switch, in the course of the cell cycle, actually proceeds by an entirely different pathway involving cyclin degradation).

This system is also *non-accommodating*: a slow or subthreshold stimulus does not desensitize the autocatalytic mechanism in the way that a subthreshold current desensitizes a nerve membrane and renders it inexcitable. Because of this, the system does not require to be stimulated by a sharp pulse, but can be triggered by a slight change in the level of a slowly-changing cellular signal. In effect, it can transduce a continuous signal into a discrete event.

The high-activity state in Figure 2c has less than 75% Cdc2 activated. More complete activation is possible with different parameter values (Figure 3), but the illustration is less satisfactory because low-activity and unstable steady states cannot be seen well without magnification. Other factors may favor complete activation. For example the decrease in Wee1 activity that occurs in M phase (Smythe and Newport, 1992; Tang *et al*., 1993; Atherton-Fessler *et al*., 1994) would effectively lower the right-hand portion of curve *R*, so that it intersects curve *F* at a higher [Cdc2]. The shapes of these curves for real systems are presently unknown.

Note that triggering depends on the balance of Cdc2 turnover (activation and deactivation) in relation to the effective threshold for autocatalysis, so there are several parameter changes that might bring about triggering. These would include (in addition to Cdc25 activation) reducing the rate of Cdc2 deactivation (reducing *R*, inhibiting Weel) and increasing the total of Cdc2 and Cdc2P (cyclin B biosynthesis). These other ways also cause saddle-node bifurcation.

The sigmoid shape of curve *F* in Figure 2 is due to inhibitor binding; but a sigmoid shape can also arise without an inhibitor if the autocatalysis is proportional to the second or higher power of [Cdc2], as in the models of Novak and Tyson (1993) and Obeyesekere *et al*. (1995). Also, if curve *F* is not sigmoid, curvature in curve *R* may produce three intersections and bistability if *R* shows saturable (e.g. Michaelis-Menten) kinetics or if, as mentioned above, Wee1 activity decreases in M phase (Thron, 1996, 1997a, Figure 4).

The model of Figure 1b is essentially the same mechanism previously used to drive an oscillatory cell

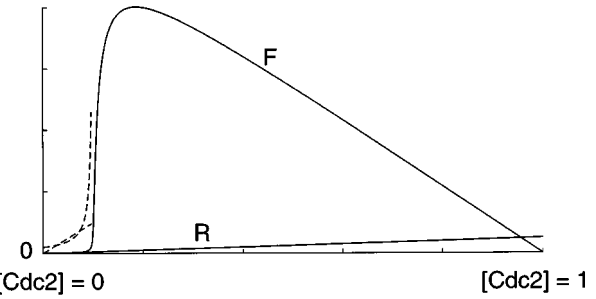

Figure 3 Rates of activation and deactivation of Cdc2 in the autocatalytic model with parameters adjusted to give >95% activation of Cdc2 in the high-activity steady state. The dashed curves show a 20-fold magnification of the ordinate scale

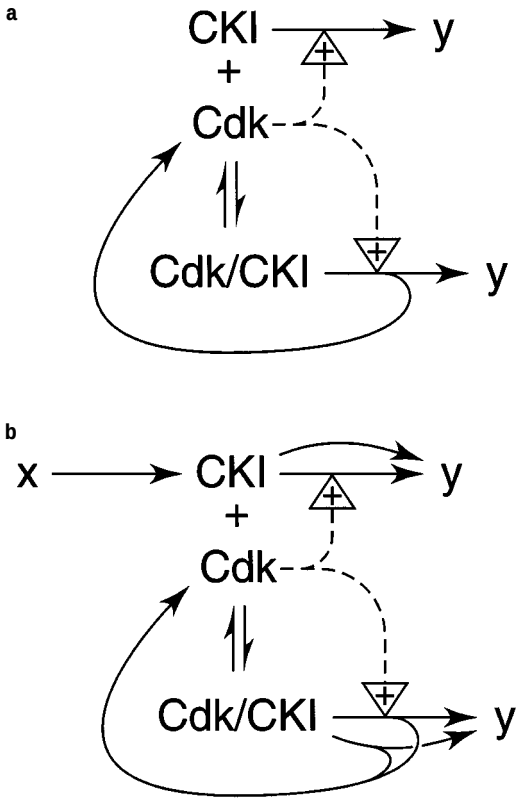

Figure 4 A model in which Cdk activates the proteolysis of a Cdk inhibitor (CKI). Precursors and proteolysis products of CKI are represented by *x* and *y*, respectively. (**a**) Without a replacement process for the CKI destroyed there is no stable steady state until all CKI is destroyed and Cdk liberated. (**b**) A process of continual CKI biosynthesis allows for steady states, but to make a *stable* steady state with low Cdk activity there must be a non-Cdk-dependent process of CKI destruction, in addition to the Cdk-activated CKI destruction (see Figure 5)

cycle model (Thron, 1994). Oscillatory cell cycle models have received considerable attention as models for repetitive cell division (Thron, 1994; Obeyesekere *et al*., 1995; Novak and Tyson, 1993, 1995), but oscillations and repetitive cell division are outside the scope of this paper.

Activation by inhibitor proteolysis

This second model assumes that Cdk is regulated by a titratable inhibitor (CKI) and is released by sudden, rapid, and complete proteolysis of CKI. As shown in Figure 4a, CKI is assumed to bind Cdk and thereby inhibit it, and free Cdk is assumed to accelerate the proteolysis of CKI whether or not the latter is bound to Cdk. As with the previous model, a key problem is how to prevent premature activation. Any trace of free Cdk will cause slight CKI proteolysis and will thereby amplify itself by positive feedback; so the model as it stands in Figure 4a has no stable steady state until all the CKI is destroyed, i.e. it does not have a stable steady state with Cdk inhibited. In order to have a steady state with low free Cdk activity, there must be a process for continually restoring CKI, as in Figure 4b. In addition, in order to provide a *stable* steady state with Cdk inhibited, it is necessary that part of the CKI proteolysis not be activated by Cdk. The need for this non-Cdk-dependent proteolysis is explained below in the discussion of Figure 5.

In this second model, total [Cdk] (free plus CKI-bound) is constant, and none is in an inactive form, except through binding to CKI. The net rate of formation of CKI is the difference between the rate of formation F and the rate of destruction R:

$$\frac{d[\mathrm{CKI}]}{dt} = F - R \qquad (2)$$

Here [CKI] is the total concentration of CKI, whether or not bound to Cdk. Again the key to the biochemical switching dynamics is in how the rates of formation and destruction depend on the state variable, here [CKI]. The rate of CKI formation F is assumed constant (Figure 5a), independent of [CKI]. The rate of CKI destruction R is more complicated. At low [CKI] R increases with [CKI] and soon intersects curve F, creating a stable steady state with little Cdk inhibited. With increasing [CKI] R may become quite large because of Cdk-activated CKI proteolysis; but as [CKI] increases further it binds more Cdk, decreasing the rate of Cdk-activated CKI proteolysis. Eventually R intersects F at another steady state, this time an unstable steady state. At still higher [CKI], Cdk-activated proteolysis becomes negligible, R begins to increase again slowly because of non-Cdk-dependent proteolysis, and R intersects F a third time, at a stable steady state with a low concentration of free Cdk. Without non-Cdk-dependent proteolysis, concentrations of CKI above the unstable steady state would tend to increase indefinitely, and switching would be impossible.

Increase in [Cdk] accelerates proteolysis, raising curve R until it breaks contact with curve F on the right (Figure 5b). The right-hand stable steady state, which has low free Cdk concentration, is extinguished by merging with the unstable steady state in a saddle-node bifurcation, and the system must shift to the remaining stable steady state, by proteolysis of most of the CKI, with liberation of most of the Cdk. Note that triggering does not require a sharp pulse of Cdk. Like the autocatalytic model of Figure 2, this system can be triggered by a small slow signal without showing accommodation. This system also shows hysteresis and irrevocability: to return the system in Figure 5b from the high-activity to the low-activity state would require drastic reduction of Cdk, enough to make the 'hump' of curve R lie entirely below curve F.

As can be understood from Figure 5, proteolysis and Cdk release could also be triggered by an increase in the rate constants for Cdk-activated or non-Cdk-dependent proteolysis or a decrease in F, and the triggering would be by saddle-node bifurcation.

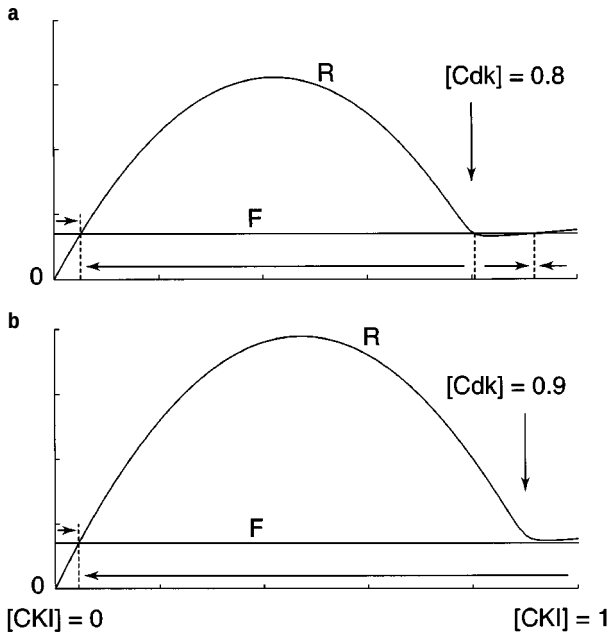

Figure 5 Rates of formation F and destruction R of CKI in the proteolysis model of Figure 4b. The intersections of curves F and R mark steady state concentrations of CKI; elsewhere horizontal arrows show the direction of spontaneous change. Vertical arrows show where [CKI] = [Cdk]. At lower CKI concentrations there is not enough CKI to bind all the Cdk, so there is free Cdk which activates CKI proteolysis (curve R). (**a**) three steady states, two stable and one unstable. (**b**) With increased concentration of Cdk, curve R rises until the right-hand steady state merges with the middle (unstable) steady state, then breaks contact with F, abolishing the right-hand steady state (high [CKI], Cdk inhibited), and forcing a shift to the left-hand steady state, where [CKI] is low and most Cdk is uninhibited. See text for further explanation

Biphasic autoregulation

The cell cycle-regulatory transcription factor E2F-1 can apparently activate its own promoter (Johnson *et al*., 1994; Hsiao *et al*., 1994; Neuman *et al*., 1994). On the other hand, complexes of the retinoblastoma gene product (RB) or related proteins with E2F-1, may be active transcriptional repressors (Neuman *et al*., 1994; Qin *et al*., 1995; Johnson, 1995). Therefore E2F-1 can apparently have a biphasic effect on its own transcription: low concentrations inhibit transcription by binding RB to the promoter, while concentrations exceeding [RB] competitively displace the RB-E2F complexes from the promoter and exert a positive

effect on transcription, i.e. positive autoregulation (Figure 6). At least in some cells, RB is normally present in considerable excess over E2F-1 (WH Lee, personal communication); but RB loses its affinity for E2F-1 when it becomes phosphorylated, and E2F-1 may then exceed the concentration of hypophosphorylated RB. This sytem may be bistable, as shown in the following model.

E2F-1 probably acts as a heterodimer with DP-1 (LaThangue, 1994). In the model, 'E2F' denotes the active transcription factor, and it is assumed that translation and heterodimerization closely follow transcription, so that in effect E2F directly regulates its own rate of formation. 'RB' denotes just the active, hypophosphorylated form of the retinoblastoma protein. There is metabolic turnover of E2F (Hofmann *et al*., 1996; Hateboer *et al*., 1996), and the net rate of formation of E2F is the difference between the rate of formation F and the rate of destruction R:

$$\frac{d[\mathrm{E2F}]}{dt} = F - R \qquad (3)$$

Here [E2F] is the total concentration of E2F, whether or not bound to RB (the amounts bound to promoter are assumed to be negligible). It is known that binding to RB inhibits the destruction of E2F (Hofmann *et al*., 1996; Hateboer *et al*., 1996), but for simplicity the rate of E2F destruction R is here assumed proportional to the total [E2F]. The effect of RB inhibition will be discussed below. The rate of formation F (Figure 7a) is slow at [E2F] = 0, and decreases with increasing E2F because of binding of RB-E2F complexes to the promoter. When the concentration of E2F exceeds that of RB, F begins to increase because of displacement of RB-E2F by E2F, with activation of the promoter. The equation for F assumes that the promoter exists in three forms (unoccupied, occupied by two RB-E2F complexes, and occupied by two E2Fs), and that the proportions of these forms depend on the free concentrations of E2F and RB-E2F, raised to the second power, according to the usual dissociation equilibrium laws. A decrease in [RB], due for example to inactivation of RB by phosphorylation, can then obliterate the low-activity stable state by a saddle-node bifurcation (Figure 7b), and cause an all-or-none shift to the high-activity stable state. As with the two previous models, this system does not show accommodation, but does show hysteresis and irrevocability.

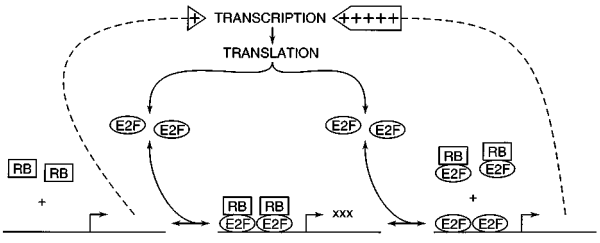

Figure 6 Proposed model of biphasic autoregulation. The E2F-1 promoter has two sets of overlapping E2F-binding sites (Johnson *et al*., 1994; Hsiao *et al*., 1994; Neuman *et al*., 1994). E2F here indicates a transcription factor containing the gene product, e.g. a heterodimeric complex with DP-1. The E2F-1 gene is transcribed to some degree when the E2F sites on the promoter are unoccupied. In the presence of retinoblastoma factor RB, E2F binds RB to the promoter, inhibiting transcription. Excess E2F competitively displaces the E2F-RB complexes from the promoter and strongly activates transcription. The resulting further increase in E2F creates a positive feedback loop

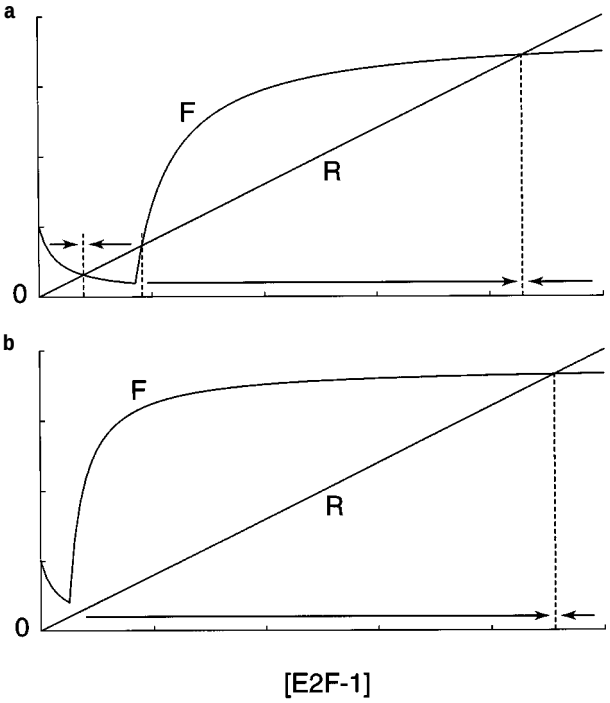

Figure 7 Rates of formation F and destruction R of E2F in the model with biphasic autoregulation (Figure 6). The intersections of curves F and R mark steady state concentrations of E2F; elsewhere arrows show the direction of spontaneous change. (**a**) Three steady states, two stable and one unstable. (**b**) With decreased concentration of RB, curve F breaks contact with R, abolishing the steady state at low [E2F], and forcing a shift to the steady state with high [E2F]. See text for further explanation

As noted above, binding of E2F to RB inhibits the destruction of E2F. This would decrease the slope of R where [E2F] < [RB]. This model can be modified to include substantial RB inhibition, as long as the inhibition is not complete. If E2F removal is inhibited completely then there is no steady state unless E2F production is stopped by some other switching mechanism.

The curves in Figure 7 are somewhat similar to those in Figure 2, but in Figure 7 curve F passes through minimum, rather than just rising sigmoidally as in Figure 2. This is because of the biphasic autoregulation. Also the possible depletion of substrate for E2F biosynthesis has been ignored in the model of Figure 7, so curve F does not decrease at higher E2F concentrations, in contrast to Figure 2.

More complicated models

In the model of Figure 1 the total of Cdc2 and Cdc2P is assumed constant. In life this total changes as a result of cyclin biosynthesis. This requires a second differential equation and a somewhat different graphical mode of analysis, namely 'phase-plane analysis' (Edelstein-Keshet, 1988; Novak and Tyson, 1995; Tyson *et al*., 1995, 1996). However these more complicated models switch in much the same way as the simpler models discussed here, by coalescence of a

stable steady state with an unstable steady state (actually a saddle point), with resulting disappearance of the stable steady state; and they show hysteresis and not accommodation.

Discussion

These models are presented as illustrations of a dynamical principle, and not as established mechanisms. A number of assumptions and inferences have been made which have not been demonstrated experimentally. It has not been established whether a titratable inhibitor plays the role proposed here in MPF activation, or whether there is steady-state turnover of CKIs, or whether transcription of E2F-1 operates as a bistable switch. The application of any of these models to real data would undoubtedly require some re-evaluation, reinterpretation, and modification of the model, and would probably also require some additional experiments. However, the object here has not been quantitative simulation of real systems, but rather insight into qualitative system behavior, principally the disappearance of a stable steady state by saddle-node bifurcation. The analysis in terms of formation and removal rate curves can be done for any system whose formation and removal rate curves are known or can be estimated.

If bistable switching is to occur, the system must have an unstable steady state, because the saddle-node bifurcation is produced by the merger of the low-activity stable steady state with an unstable steady state, with the extinction of both. In order to have an unstable steady state the biochemical kinetics must be somewhat unusual. As can be seen in the figures, an unstable steady state is one where curve F crosses curve R from below upwards, as substrate concentration increases. This means that either F must increase with increasing concentration of the substance formed by F (effective autocatalysis, as in Figures 2, 3 and 7), or R must decrease with increasing concentration of the substance removed by R (effective substrate inhibition, as in Figure 5). In short, the substrate must tend to increase its own net rate of production, i.e. it must be 'self-activating' (Higgins, 1967), or positively autoregulated. The term 'autoregulation' is often used as if it were synonymous with negative autoregulation, because of the preoccupation with homeostasis in biology. However these reactions are not homeostatic. Their function is to produce change; and abrupt, complete, irrevocable change requires to be effected by positive feedback.

In each of these examples there is a titratable inhibitor. The role of the inhibitor is not merely to slow a rate process quantitatively, but more importantly to alter the concentration-dependence of the rate process in a way that affects the dynamics, e.g. to put a 'kink' in curve F or R that allows multiple intersections between F and R. The end-point of titration of a titratable inhibitor forms a more or less sharp threshold for the enhancement of positive feedback. This threshold effect is a distinctive property of a titratable or stoichiometric inhibitor, and is not produced by competitive or non-competitive inhibitors. It has already been recognized (Kato *et al.*, 1994; Hall *et al.*, 1995) that the stoichiometric CKIs $p27^{Kip1}$ and $p21^{Cip1}$ may set a threshold for Cdk activation. On the other hand $p15^{INK4b}$ and $p16^{INK4a}$, which are essentially competitive (Hall *et al.*, 1995), would not by themselves create a threshold, though by displacing $p27^{Kip1}$ from Cdk4 and Cdk6 (Reynisdóttir *et al.*, 1995) they presumably alter the threshold created by $p27^{Kip1}$ for Cdk2/cyclin E.

Though the titration threshold is important in these examples, there are other biochemical mechanisms, without titratable inhibitors, for which the F and R curves may make the three intersections required for bistable switching, as already noted, and as shown elsewhere (Thron, 1996; 1997a).

The stabilization of the low-activity steady state is presumably not delay for its own sake, but is important for cellular function. In the case of MPF, it may be important to prevent activation during the period of cyclin B accumulation, and thereby allow accumulation of enough pre-MPF to produce a sudden high level of MPF when activated (Thron, 1994). The CKIs may likewise function to allow the accumulation of a reservoir of Cdk for sudden release (Nasmyth and Hunt, 1993). Since too small a rise in MPF or Cdk might fail to initiate the next phase of the cell cycle, these inhibitors may be necessary for progress through the cell cycle, though in a sense they inhibit progress through the cell cycle.

With a stable low-activity state, and a saddle-node bifurcation, these models are potential 'checkpoints' (Hartwell and Weinert, 1989; Elledge, 1996) where progress through the cell cycle can be held up indefinitely until some required condition is met. As noted above, the models of Figures 2 and 5 can be triggered in any of several ways, which might be controlled by specific signals of cell size or completeness of DNA replication or repair. Novak and Tyson (1995), in their model of the *S. pombe* cell cycle, have modeled checkpoints as 'stable steady state[s] inserted into and removed from the cell cycle by saddle-node bifurcations' (JJ Tyson, personal communication). A number of other theoretical checkpoint models have been suggested (Tyson *et al.*, 1995, 1996; Thron, 1997b). What is proposed here is that checkpoint arrest is a *steady state*, with continual turnover of an effectively 'self-activating' substance. Turnover is necessary because the unbalancing of the turnover is what terminates the arrest. A titratable inhibitor may be the most obvious mechanism of checkpoint arrest, but there are other possible mechanisms of bistability, as noted above. Ultimately checkpoint arrest depends on whether the continuing turnover process maintains the self-activating substance below the critical level for triggering. On this view, turnover reactions are crucial for checkpoint control. Not all of these turnover reactions have been well studied, and some, for example those proposed for the inhibitor proteolysis model (Figure 4b), are essentially conjectural.

Experimental evidence for bistability would include (1) amplification, as with MPF, (2) a sudden, discontinuous change of stable state during a gradual change of a rate constant or other parameter (saddle-node bifurcation), and (3) hysteresis or lack of reversibility. Any system showing a rapid change after a period of apparent stability could in principle be tested for amplification as was done by Cyert and Kirschner (1988), by adding a small amount of the

product of a previously activated sample; and if this precipitates or accelerates the activation it would be evidence of autocatalysis or positive feedback. Novak and Tyson (1993; Tyson *et al*., 1996) have suggested that differing thresholds for activation and inactivation (hysteresis) might be experimentally demonstrable in the activation of $p34^{cdc2}$ by cyclin (Solomon *et al*., 1990); and the suggestion still seems valid in principle, although more recent findings (Lee and Kirschner, 1996) would have to be taken into account. Titration curves of Cdks with $p21^{Cip1}$ or $p27^{Kip1}$ are sometimes quite steep (Xiong *et al*., 1993; Polyak *et al*., 1994; Jackson *et al*., 1995; Fotedar *et al*., 1996), and this suggests the possibility of positive feedback, if not bistability. Some considerations in evaluating experimental observations are (1) on destabilization of a steady state, the change to a new steady state may not occur instantaneously, but may rather be gradual, though inevitable; (2) in multicellular systems, abrupt changes in individual cells tend to be disguised by cellular asynchrony; and (3) in cell extracts the molecular architecture may be disrupted in a way that affects the biochemical kinetics.

The picture of the cell cycle is becoming increasingly complicated (Sherr, 1996), and there are now a number of proposed positive-feedback loops, in addition to those already mentioned, that might produce bistable switching: (a) E2F-1 activates the cyclin E promoter (Ohtani *et al*., 1995; Geng *et al*., 1996) and Cdk2/cyclin E probably phosphorylates RB (Weinberg, 1995), liberating E2F-1; (b) Cdk2/cyclin E activates Cdc25A, which may also activate cyclin E/Cdk2 (Hoffmann *et al*., 1995); (c) expression of cyclin D1 leads to an apparently E2F-1-dependent activation of the *MYC* promoter (Oswald *et al*., 1994) and Myc induces cyclin D1 expression (Daksis *et al*., 1994); (d) Mos activates MAP kinase, and MAP kinase induces synthesis of Mos (Roy *et al*., 1996); and (e) *CLN2* in *Saccharomyces cerevisiae* activates its own transcription (Nasmyth, 1993; Stuart and Wittenberg, 1995), and Far1 is apparently a titratable inhibitor (Peter and Herskowitz, 1994a). Myc also activates cyclin E/Cdk2 (Steiner *et al*., 1995) and activates transcription of Cdc25A (Galaktionov *et al*., 1996). Finally, proteolysis of Cdk activators, as well as inhibitors, plays an important part in cell cycle progression (King *et al*., 1996), and this may well be switched by a bistable mechanism.

Whether these positive-feedback loops actually produce bistable switching is a question that can only be answered experimentally. It is suggested here that this question can be approached by analysis of formation and removal rates, with identification of the nature of the positive feedback, the mechanism preventing premature activation, the turnover reactions stabilizing the low-activity steady state, and the mechanism of destabilization.

Note added in proof

It has been reported that Cdk2/cyclin E causes phosphorylation of $p27^{Kip1}$ which results in elimination of $p27^{Kip1}$ from the cell (Sheaff RJ, Groudine M, Gordon M, Roberts JM and Clurman BE, *Genes Devel*., in press). This system may therefore be similar to the inhibitor proteolysis model of Figure 4.

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