



Regulation of the Eukaryotic Cell Cycle: Molecular Antagonism, Hysteresis, and Irreversible Transitions

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In recent years, molecular biologists have uncovered a wealth of information about the proteins controlling cell growth and division in eukaryotes. The regulatory system is so complex that it defies understanding by verbal arguments alone. Quantitative tools are necessary to probe reliably into the details of cell cycle control. To this end, we convert hypothetical molecular mechanisms into sets of nonlinear ordinary differential equations and use standard analytical and numerical methods to study their solutions. First, we present a simple model of the antagonistic interactions between cyclin-dependent kinases and the anaphase promoting complex, which shows how progress through the cell cycle can be thought of as irreversible transitions (Start and Finish) between two stable states (G1 and S–G2–M) of the regulatory system. Then we add new pieces to the “puzzle” until we obtain reasonable models of the control systems in yeast cells, frog eggs, and cultured mammalian cells.

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Introduction

The cell cycle is the sequence of events by which a growing cell duplicates all its components and divides into two daughter cells, each with sufficient machinery and information to repeat the process. The most important components are the cell's chromosomes, which contain linear DNA molecules in association with many proteins. Each DNA molecule must be accurately replicated and the two copies carefully segregated to daughter cells at division. In eukaryotic cells, these processes occur in temporally distinct

stages (Fig. 1). During S phase, a new copy of each chromosome is synthesized. (The two identical DNA molecules are called sister chromatids.) Some time later, during M phase (mitosis), the sister chromatids are separated so that each daughter cell receives a copy of each chromosome. These two processes, DNA synthesis and sister chromatid separation, make up the chromosome cycle of the cell. In parallel to it runs the growth cycle, whereby the cell's “hardware” (proteins, RNA, phospholipid bilayers, carbohydrates) is also duplicated and partitioned, more-or-less evenly, between daughters. During normal cell proliferation, these two cycles turn at the same rate, so that each round of DNA synthesis and mitosis is balanced by doubling of all other macromolecules in the cell. In this way, the

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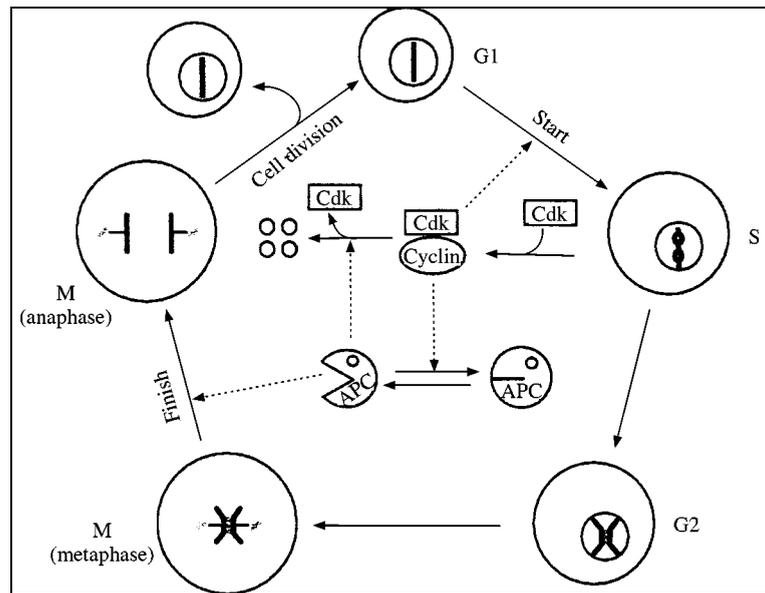


FIG. 1. The cell cycle. Outer ring illustrates the chromosome cycle. The nucleus of a newborn cell contains unreplicated chromosomes (represented by a single bar). At Start, the cell enters S phase and replicates its DNA (signified by replication bubbles on the "chromosome"). At the end of S phase, each chromosome consists of a pair of sister chromatids (X) held together by tethering proteins. After a gap (G2 phase), the cell enters mitosis (M phase), when the replicated chromosomes are aligned on the metaphase spindle, with sister chromatids attached by microtubules to opposite poles of the spindle. At Finish, the tether proteins are removed so that the sister chromatids can be segregated to opposite sides of the cell (anaphase). Shortly thereafter the cell divides to produce two daughter cells in G1 phase. The inner icons represent the fundamental molecular machinery governing these transitions. Start is triggered by a protein kinase, Cdk, whose activity depends on association with a cyclin subunit. Cdk activity drives the cell through S phase, G2 phase, and up to metaphase. Finish is accomplished by proteolytic machinery, APC, which destroys the tethers and cyclin molecules. In G1 phase, APC is active and Cdk inactive, because it lacks a cyclin partner. At Start, the APC must be turned off so that cyclins may accumulate. Cdk and APC are antagonistic proteins: APC destroys Cdk activity by degrading cyclin, and cyclin/Cdk dimers inactivate APC by phosphorylating one of its subunits.

nucleocytoplasmic ratio of the cell is maintained within advantageous limits. Of course, there are exceptions to this rule (Murray & Hunt, 1993), such as oocytes, which grow very large without dividing, and fertilized eggs, which divide rapidly in the absence of growth. Nonetheless, the long-term viability of a cell line depends on balanced growth and division.

START AND FINISH

The chromosome cycle is usually subdivided into four phases (G1, S, G2, M), but it is better to think of it as two alternative "states" (G1 and S-G2-M) separated by two transitions (Start and Finish), as in Fig. 1 (Nasmyth, 1995, 1996; Novak *et al.* 1998a, b). In G1, chromosomes are unreplicated and the cell is uncommitted to the replication-division process. At Start (the transition

from G1 to S phase), a cell confirms that internal and external conditions are favorable for a new round of DNA synthesis and division, and commits itself to the process. The decision is irreversible: once DNA synthesis commences, it goes to completion.

During the process of DNA replication, sister chromatids are tethered together by specific proteins, called cohesins. As the mitotic spindle forms in M phase, microtubules from the spindle poles attach to chromosomes and pull them into alignment at the center of the spindle (metaphase). When DNA replication is complete and all chromosomes are aligned, the second irreversible transition of the cycle (Finish) is triggered. The cohesins are destroyed, allowing sister chromatids to be pulled to opposite poles of the spindle (anaphase). Shortly thereafter, daughter nuclei form around the segregated chromatids

(telophase), and the incipient daughter cells separate.

These major events of the cell cycle must be tightly regulated. For instance, balanced growth and division is achieved in most cells by a size requirement for the Start transition. That is, cells must grow to a critical size before they can commit to chromosome replication and division. If this requirement is compromised by mutation, cells may become morbidly large or small (Moreno & Nurse, 1994). A second crucial regulatory constraint is to hold off the Finish transition if there have been any problems with DNA replication or chromosome alignment. Were anaphase to commence under such conditions, then daughter nuclei would not receive a full complement of chromosomes, which is usually a fatal mistake (Murray, 1995).

MOLECULAR CONTROLS

Cell cycle events are controlled by a network of molecular signals, whose central components are cyclin-dependent protein kinases (Cdks). In the G1 state, Cdk activity is low, because its obligate cyclin partners are missing, because cyclin mRNA synthesis is inhibited and cyclin protein is rapidly degraded. At Start, cyclin synthesis is induced and cyclin degradation is inhibited, causing a dramatic rise in Cdk activity, which persists throughout S, G2 and M (see Fig. 1). High Cdk activity is needed for DNA replication, chromosome condensation, and spindle assembly.

At Finish, a group of proteins, making up the anaphase-promoting complex (APC), is activated (Zachariae & Nasmyth, 1999). The APC attaches a "destruction label" to specific target proteins, which are subsequently degraded by the cell's proteolysis machinery. The APC consists of a core complex of about a dozen polypeptides plus two auxiliary proteins, Cdc20 and Cdh1, whose apparent roles (when active) are to recognize specific target proteins and present them to the core complex for labeling (Visintin *et al.*, 1997; Zachariae & Nasmyth, 1999). Activation of Cdc20 at Finish is necessary for degradation of cohesins at anaphase, and for activation of Cdh1. Together, Cdc20 and Cdh1 label cyclins for degradation at telophase, allowing the control system to return to G1. We must distinguish

between these two different auxiliary proteins, because Cdc20 and Cdh1 are controlled differently by cyclin/Cdk, which activates Cdc20 and inhibits Cdh1.

There are additional complexities in the Cdk network (Mendenhall & Hodge, 1998). The control systems found in budding and fission yeasts consist of two different families of cyclins, a stoichiometric inhibitor of cyclin/Cdk complexes, kinases and phosphatases that modify the Cdk subunit, transcription factors that control the expression of cell-cycle genes, and a carefully regulated phosphatase that opposes cyclin/Cdk activity at crucial control points in the network (Chen *et al.*, 2000; Novak *et al.*, 1999). The control system in mammalian cells is more complicated still, with seven different Cdks, seven families of cyclins, a dozen stoichiometric inhibitors, and hundreds of relevant interactions among these components (Bartek *et al.*, 1996; Kohn, 1999). In addition, signal transduction pathways ("surveillance mechanisms") convey information from the interior and exterior milieus to control progress through the cell cycle (Elledge, 1996; Hanahan & Weinberg, 2000).

A major challenge for theoretical molecular biologists is to explain the physiology of cell proliferation in a variety of unicellular and multicellular eukaryotes in terms of their underlying molecular control systems. Of necessity, such connections will be made by ambitious computational models that reflect some of the inescapable complexity of real cell cycle controls (Chen *et al.*, 2000; Novak & Tyson, 1993). In order to design such models and understand how they work, we first need a solid grasp of the basic control principles of the cell cycle.

The purpose of this paper is to draw attention to a simple theme that runs through the morass of molecular details (Tyson *et al.*, 1995; Aguda, 1999; Thron, 1999): the irreversible transitions of the cell cycle (Start and Finish) are consequences of the creation and destruction of stable steady states of the molecular regulatory mechanism by dynamic bifurcations. At the core of the cell cycle is a hysteresis loop deriving from the fundamental antagonism between Cdk and APC (Novak *et al.*, 1998): the APC extinguishes Cdk activity by destroying its cyclin partners, whereas cyclin/Cdk dimers inhibit APC activity by

phosphorylating Cdh 1 (Fig. 1). This antagonism creates two, alternative, stable steady states of the control system: a G1 state, with high Cdh1/APC activity and low cyclin/Cdk activity, and an S-G2-M state, with high cyclin/Cdk activity and low Cdh1/APC activity. Transitions between these two states are facilitated by “helper” molecules that are insensitive to the antagonists.

In the following sections, we first construct and analyse a simple model of this antagonism. Next, we add a cyclin-dependent kinase inhibitor (CKI) to the model, to create a simple yet accurate model of yeast cell cycle controls. Finally, we speculate on how to extend the yeast model to a useful picture of cell cycle regulation in multicellular eukaryotes.

Our approach is a tribute to the spirit of Joel Keizer, who recognized that many interesting and important problems in molecular cell biology can be formulated as physicochemical processes in space and time and studied successfully by modern tools of nonlinear dynamical systems.

Hysteresis in the Interaction of Cyclin B/Cdk and Cdh1/APC

The biochemical reactions in the center of Fig. 1 can be described by a pair of nonlinear ordinary differential equations (ODEs):

$$\frac{d[\text{CycB}]}{dt} = k_1 - (k'_2 + k''_2 [\text{Cdh1}])[\text{CycB}], \quad (1)$$

$$\frac{d[\text{Cdh1}]}{dt} = \frac{(k'_3 + k''_3 A)(1 - [\text{Cdh1}])}{J_3 + 1 - [\text{Cdh1}]} - \frac{k_4 m [\text{CycB}] [\text{Cdh1}]}{J_4 + [\text{Cdh1}]}. \quad (2)$$

In these equations, $[\text{CycB}]$ and $[\text{Cdh1}]$ are the average concentrations (grams of protein per gram of total cell mass) of cyclin B/Cdk dimers and active Cdh1/APC complexes, respectively. The k 's are rate constants, the J 's are Michaelis constants, m represents cell “mass” (not to be confused with M for “mitosis”). The terms in eqn (1) represent synthesis and degradation of CycB, with degradation rate dependent on Cdh1

activity (assuming that APC cores are in excess). We assume that cyclin B molecules combine rapidly with an excess of Cdk subunits, so that we do not have to keep track of CycB and Cdk monomers. The terms in eqn (2) represent activation and inactivation of Cdh1, formulated as Michaelis–Menten rate laws. We assume that the total Cdh1 concentration is constant (scaled to 1), and that J_3 and J_4 are both $\ll 1$, so that Cdh1 behaves like a “zero-order ultra-sensitive switch” (Goldbeter & Koshland, 1981). This switch-like behavior of Cdh1 is crucial to hysteresis in our model. Cdh1 is activated by a generic “activator”: for now A is simply a parameter, but, in the next section, we will show how A relates to Cdc20. We assume that the inactivation of Cdh1 by CycB/Cdk1 takes place in the nucleus, where CycB accumulates. Under this assumption, the effective concentration of CycB in the nucleus will increase as the cell grows, so $[\text{CycB}]$ is multiplied by m in the second term in eqn (2). This mass-dependence plays a crucial role in our model by connecting the Start transition to cell growth.

The phase plane portrait for system (1)–(2) is illustrated in Fig. 2. The nullclines are described

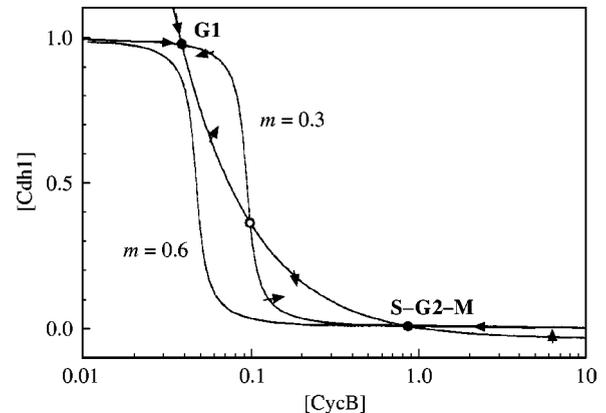


FIG. 2. Phase plane portrait for the pair of nonlinear ODEs (1) and (2). Parameter values are given in Table 1. Curves are nullclines (see text) for $A = 0$ and $m = 0.3$ or 0.6 . Arrows indicate direction field for $m = 0.3$ only. For $m = 0.3$, the control system has three steady states: a stable node (G1) at $([\text{CycB}], [\text{Cdh1}]) \approx (0.039, 0.97)$, a saddle point near $(0.10, 0.36)$, and another stable node (S-G2-M) near $(0.90, 0.0045)$. Suppose a newborn cell resides at G1 (Cdh1 active and CycB missing). As the cell grows (m increases), the G1 steady state is lost by a saddle-node bifurcation (at $m \approx 0.53$), and the control system is forced to the S-G2-M steady state.

by simple algebraic equations:

$$[\text{CycB}] = \frac{\beta}{J_2 + [\text{Cdh1}]} \quad (\text{CycB nullcline}),$$

$$[\text{CycB}] = p \frac{(1 - [\text{Cdh1}])(J_4 + [\text{Cdh1}])}{[\text{Cdh1}](J_3 + 1 - [\text{Cdh1}])}$$

(Cdh1 nullcline),

where $\beta = k_1/k_2$, $J_2 = k'_2/k''_2$ and $p = (k'_3 + k''_3 A)/(k_4 m)$. The CycB nullcline is a simple hyperbola. For $J_3 = J_4 \ll 1$, the Cdh1 nullcline is a sigmoidal curve passing through $[\text{CycB}] = p$ at $[\text{Cdh1}] = \frac{1}{2}$.

The Cdh1 nullcline can be rewritten for $[\text{Cdh1}]$ as a function of $[\text{CycB}]$, $[\text{Cdh1}] = G(p, [\text{CycB}], J_3, J_4)$, where G is the Goldbeter-Koshland function (Goldbeter & Koshland, 1981):

$$G(V_a, V_i, J_a, J_i) = \frac{2\gamma}{\beta + \sqrt{\beta^2 - 4\alpha\gamma}}, \quad \alpha = V_i - V_a,$$

$$\beta = V_i - V_a + V_a J_i + V_i J_a, \quad \gamma = V_a J_i.$$

This function will come in handy later.

The control system has steady-state solutions wherever the nullclines intersect. The number of intersections depend on the value of p (Fig. 3). For $p_1 < p < p_2$, the ODE system (1)–(2) has three steady states: two stable nodes separated by a saddle point. The stable nodes we refer to as **G1** (Cdh1 active, CycB low) and **S-G2-M** (Cdh1 inactive, CycB high); in bold face to distinguish the theoretician's stable steady state from the experimentalist's cell cycle phase. It can be shown that, when $J_2 = J_3 = J_4 \equiv \varepsilon \ll 1$, the saddle-node bifurcations occur at

$$p_1 \approx \beta \{1 + 2\sqrt{\varepsilon} + O(\varepsilon^{3/2})\}$$

$$[\text{Cdh1}] \approx 1 - \sqrt{\varepsilon} + \varepsilon$$

$$[\text{CycB}] \approx \beta(1 + \sqrt{\varepsilon} - \varepsilon), \quad \text{and}$$

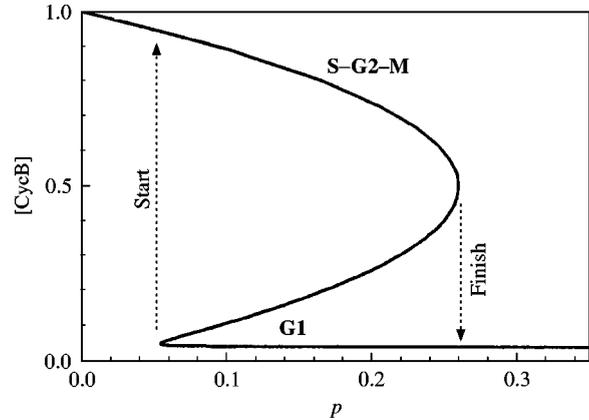


FIG. 3. Bifurcation diagram for eqns (1) and (2). The steady-state concentration of CycB is plotted as a function of the bifurcation parameter, $p = (k'_3 + k''_3 A)/k_4 m$. Other parameters: $\beta = \varepsilon = 0.04$. Saddle-node bifurcations occur at $p_1 \approx 0.0545$ and $p_2 \approx 0.261$.

$$p_2 \approx \beta/(4\varepsilon)$$

$$[\text{Cdh1}] \approx \varepsilon \quad [\text{CycB}] \approx \beta/(2\varepsilon).$$

Progress through the cell cycle can be thought of as a tour around the hysteresis loop in Fig. 3. For a small, newborn cell in G1 phase (with $A \approx 0$ and $p \approx k'_3/k_4 m > p_1$), the CycB–Cdh1 control system is attracted to the stable **G1** steady state. As the cell grows, m increases and p decreases. Eventually, p drops below p_1 , and the **G1** steady state disappears, forcing the control system to jump irreversibly to the **S-G2-M** steady state. High CycB/Cdk activity initiates the processes of DNA synthesis and mitosis, as the cell continues to grow. We assume that, when DNA replication is complete and the chromosomes are properly aligned on the mitotic spindle, the parameter A increases abruptly, forcing p to increase above p_2 . Consequently, the **S-G2-M** steady state is lost by a saddle-node bifurcation, and the control system jumps irreversibly back to the **G1** state. The cell divides ($m \rightarrow m/2$), A decreases back to 0, and the control system returns to its starting condition.

In this simple model, the irreversible transitions of the cell cycle (Start and Finish) are the abrupt jumps of the hysteresis loop, at the saddle-node bifurcation points. The **G1** → **S-G2-M** transition is driven by cell growth, and

the reverse transition is driven by chromosome alignment on the mitotic spindle.

Activation of Cdh1/APC at Anaphase

To fill out the picture in the previous section, we must identify the activator of Cdh1/APC and describe why A increases abruptly at the metaphase \rightarrow anaphase transition and decreases in G1 phase. The form of eqn (2) suggests that A is a phosphatase, removing from Cdh1 the inhibitory phosphate groups placed there by CycB/Cdk. Recent experimental evidence in budding yeast (Jaspersen *et al.*, 1999; Visintin *et al.*, 1998) identifies A as the product of the *CDC14* gene. At the metaphase \rightarrow anaphase transition, Cdc14 phosphatase is activated indirectly by Cdc20/APC, which destroys an inhibitor of Cdc14. To keep our model as simple as possible, we assume that $A \propto [\text{Cdc14}] \propto [\text{Cdc20}]$ and write a differential equation for the production of Cdc20:

$$\frac{d[\text{Cdc20}_T]}{dt} = k'_5 + k'_5 \frac{([\text{CycB}]m/J_5)^n}{1 + ([\text{CycB}]m/J_5)^n} - k_6 [\text{Cdc20}_T]. \quad (3)$$

Because Cdc20 is synthesized only in **S-G2-M** phase of the budding yeast cell cycle (Shirayama *et al.*, 1998; Zachariae & Nasmyth, 1999), we have assumed that its transcription factor is turned on by CycB/Cdk according to a Hill function with parameters n and J_5 . (The significance of the subscript T will become clear shortly.)

By supposing that Cdh1 activity responds rapidly to changes in $[\text{CycB}]$ and $[\text{Cdc20}_T]$, we can solve eqn (2) for $[\text{Cdh1}] = G(k'_3 + k'_3 [\text{Cdc20}_T], k_4 m [\text{CycB}], J_3, J_4)$, where G is the Goldbeter-Koshland function described earlier. With this assumption, our control system is still representable by a pair of ODEs, eqns (1) and (3), and by phase plane portraits (Fig. 4). Consider a newborn cell in G1 phase [Fig. 4(a)]. As the cell grows, the CycB nullcline moves to the right and the control system undergoes a saddle-node-loop bifurcation at a critical value of m ($m_{crit} \approx 0.8$). When the **G1** steady state is destroyed by coalescence with the saddle point, $[\text{CycB}]$ starts to increase, see the dotted trajectory in Fig. 4(b)

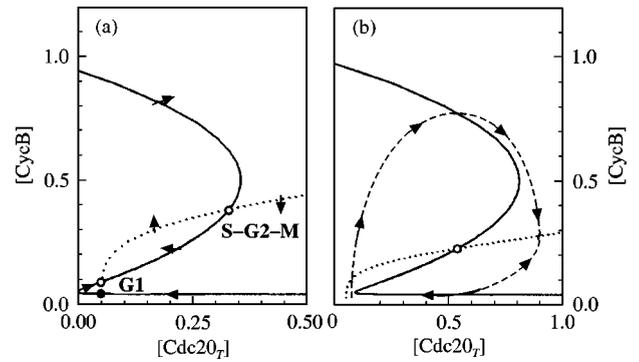


FIG. 4. Phase plane portrait for eqns (1) and (3). $[\text{Cdh1}]$ is computed from $[\text{CycB}]$ by solving for the steady state of eqn (2). Parameter values given in Table 1. (a) $m = 0.5$; (b) $m = 1$. Solid curve: CycB nullcline. Dotted curve: Cdc20 nullcline. Dashed curve: trajectory. The control system undergoes a saddle-node-loop bifurcation at $m \approx 0.8$.

CycB-dependent kinase activity drives the cell into S phase and mitosis, and it turns on synthesis of Cdc20. Notice that the **S-G2-M** steady state in Fig. 4(b) is unstable: as Cdc20 accumulates, the control system loops around the unstable steady state. Cdh1/APC is activated by Cdc20, CycB is destroyed, and the cell exits mitosis. At cell division, m is reduced two-fold, and the nullclines readopt the configuration in Fig. 4(a). The control system is captured by the stable **G1** steady state, until cell size, $m(t)$, once more increases to m_{crit} .

In this picture, cells exit from mitosis “automatically” a certain time after Start (the time required to make enough Cdc20 to activate APC); there is no connection between alignment of replicated chromosomes on the metaphase plate and the transition to anaphase. In budding yeast, the connection is established through further controls on Cdc20 (see bottom part of Fig. 6). Newly synthesized Cdc20 is inactive. A Cdc20-activating signal derives indirectly from CycB/Cdk; some intermediate steps between CycB synthesis and Cdc20 activation assure a minimum time lag for DNA synthesis and chromosome alignment to be completed before anaphase commences. If they are not completed on time, a Cdc20-inactivating signal is imposed by the *MAD*-family of spindle checkpoint genes (Hwang *et al.*, 1998). To take these additional

feature into account, we write

$$\begin{aligned} \frac{d[\text{Cdc}20_A]}{dt} &= \frac{k_7[\text{IEP}]([\text{Cdc}20_T] - [\text{Cdc}20_A])}{J_7 + [\text{Cdc}20_T] - [\text{Cdc}20_A]} \\ &\quad - \frac{k_8[\text{Mad}] \cdot [\text{Cdc}20_A]}{J_8 + [\text{Cdc}20_A]} - k_6[\text{Cdc}20_A], \quad (4) \\ \frac{d[\text{IEP}]}{dt} &= k_9m[\text{CycB}](1 - [\text{IEP}]) - k_{10}[\text{IEP}]. \quad (5) \end{aligned}$$

Here, $[\text{Cdc}20_A]$ is the concentration of “active” Cdc20, and $[\text{Cdc}20_T]$ is the total concentration of both active and inactive forms. From now on, we set $A = [\text{Cdc}20_A]$ in eqn (2). We treat $[\text{Mad}]$ as a parameter; $[\text{Mad}] = 1$, if chromosome alignment is completed on schedule, and = some large number, if not. $[\text{IEP}]$ is the concentration of the active form of a hypothetical “intermediary enzyme”, whose total concentration is scaled to 1. IEP is put in the model to create a time lag (as observed) between the rise in CycB/Cdk activity and the activation of Cdc20. Because the molecular basis of this time lag has yet to be identified, we must be content with this fictional component.

To complete this primitive model of cell cycle controls, we provide a differential equation for cell growth:

$$\frac{dm}{dt} = \mu m \left(1 - \frac{m}{m_*} \right), \quad (6)$$

where m_* is the maximum size to which a cell may grow if it does not divide, and μ is the specific growth rate when $m \ll m_*$. Our model consists of eqns (1)–(6), with the proviso that $m \rightarrow m/2$, whenever the cell divides (i.e. when $[\text{CycB}]$ drops below some threshold level, taken to be 0.1). A typical simulation is presented in Fig. 5.

This simple model fulfills all the requirements of a functional, eukaryotic cell cycle, with two irreversible transitions: Start (dependent on cell growth) and Finish (dependent on chromosome alignment) (Nasmyth, 1995). Although this picture may represent the primitive control system

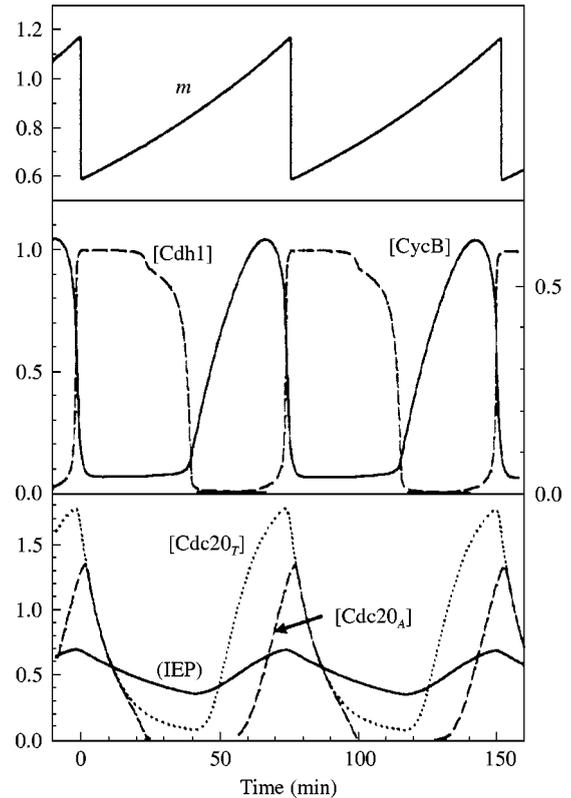


FIG. 5. Simulation of eqns (1)–(6), with parameter values in Table 1. Middle panel: CycB_T (solid curve) scale to the right. Cell division occurs when $[\text{CycB}_T]$ crosses 0.1 from above.

in the earliest eukaryotes, as they evolved from prokaryotic progenitors (Novak *et al.*, 1998a, b), all present-day organisms that have been studied in detail have more complicated mechanisms of cell cycle regulation.

Cell Cycle Controls in Yeast

To get a reasonable model of cell cycle controls in modern yeasts, we need to add a cyclin-dependent kinase inhibitor (CKI) to the picture (Fig. 6). CKI binds to CycB/Cdk to form inactive trimers. The existence of trimers changes slightly the interpretation of eqn (1):

$$\begin{aligned} \frac{d[\text{CycB}_T]}{dt} &= k_1 - (k_2 + k_2''[\text{Cdh}1] \\ &\quad + k_2'''[\text{Cdc}20_A])[\text{CycB}_T], \quad (1') \end{aligned}$$

where $[\text{CycB}_T] = [\text{CycB}] + [\text{Trimer}]$. We also need a kinetic equation for total CKI:

$$\frac{d[\text{CKI}_T]}{dt} = k_{11} - (k'_{12} + k''_{12}[\text{SK}] + k'''_{12}m[\text{CycB}])[\text{CKI}_T]. \quad (7)$$

In eqn (7), the rate of CKI degradation depends on CycB/Cdk activity, because CycB-dependent phosphorylation of CKI renders it unstable. (In budding yeast, this CKI is called Sic1; its kinetic properties are described in Mendenhall & Hodge, 1998). Thus, CKI and CycB/Cdk are mutual antagonists. The model (Fig. 6) postulates a “starter” kinase (SK) that phosphorylates CKI in the absence of CycB/Cdk activity. (In budding yeast, the starter-kinase role is played by Cln-dependent kinases; see Mendenhall & Hodge.) Notice also, in eqn (1'), that we have given Cdc20 some ability to degrade cyclin B, as indicated by experiments (Irniger *et al.*, 1995; Visintin *et al.*, 1997).

We assume that CKI/CycB/Cdk trimers are always in equilibrium with CKI monomers and CycB/Cdk dimers: $[\text{Trimer}] = K_{eq}[\text{CycB}][\text{CKI}] = K_{eq}([\text{CycB}_T] - [\text{Trimer}])([\text{CKI}_T] - [\text{Trimer}])$, or

$$[\text{Trimer}] = \frac{2[\text{CycB}_T][\text{CKI}_T]}{[\text{CycB}_T] + [\text{CKI}_T] + K_{eq}^{-1} + \sqrt{([\text{CycB}_T] + [\text{CKI}_T] + K_{eq}^{-1})^2 - 4[\text{CycB}_T][\text{CKI}_T]}}.$$

To understand how the CKI part of the control system works, let us consider the $[\text{CycB}_T]$ - $[\text{CKI}_T]$ phase plane (Fig. 7) defined by eqn (1') and (7). In these equations, $[\text{Cdh1}] = G(k'_3 + k''_3[\text{Cdc20}_A], k_4m[\text{CycB}], J_3, J_4)$, $[\text{CycB}] = [\text{CycB}_T] - [\text{Trimer}]$, with $[\text{Trimer}]$ given by the equation directly above, and $[\text{SK}]$, $[\text{Cdc20}_A]$ and m are treated as parameters. The CKI_T -nullcline is N-shaped because of the antagonism between CycB and CKI, and the CycB_T -nullcline is N-shaped because of the antagonism between CycB/Cdk and Cdh1/APC.

For proper choice of parameters (Table 1), the control system exhibits bistability, with a stable **G1** state (CycB low, Cdh1 active, CKI abundant) and a stable **S-G2-M** state (CycB high, Cdh1

inactive, CKI missing). Exit from mitosis (**S-G2-M** \rightarrow **G1**) is carried out by Cdc20, as described in the previous section. To start the chromosome cycle (**G1** \rightarrow **S-G2-M**), we assume that synthesis of the “starter kinase” is turned on when the cell reaches a characteristic size. As $[\text{SK}]$ increases, the local maximum of the CKI_T -nullcline is depressed (Fig. 7), destroying the **G1** steady state by a saddle-node bifurcation.

To complete the picture, we need a dynamical equation for the time course of $[\text{SK}]$. Again, we take our hint from budding yeast, where two cyclins, Cln1 and Cln2, in combination with Cdc28 (the catalytic subunit) phosphorylate CKI at Start, permitting B-type cyclins (Clb1–6) to accumulate and drive the cell through S and M phases. Synthesis of Cln1–2 is controlled by a transcription factor (TF; called SBF in budding yeast) that is activated by Cln/Cdc28 and inhibited by Clb/Cdc28, so we write

$$\begin{aligned} \frac{d[\text{SK}]}{dt} &= k_{13}[\text{TF}] - k_{14}[\text{SK}], \\ [\text{TF}] &= G(k'_{15}m + k'_{15}[\text{SK}], k'_{16} \\ &\quad + k''_{16}m[\text{CycB}], J_{15}, J_{16}), \end{aligned} \quad (8)$$

where $G(\dots)$ is the Goldbeter–Koshland function, as usual. Size control at Start enters this model through the term $k'_{15}m$ in the first argument of G ; when the cell gets sufficiently large, $k'_{15}m \approx k'_{16}$, it begins to synthesize SK. Increasing $[\text{SK}]$ activates its own transcription, eqn (8), and destroys CKI, eqn (7). We also assume that SK phosphorylates Cdh1, although not as efficiently as CycB/Cdc28; that is, in eqn (2) we replace $k_4m[\text{CycB}][\text{Cdh1}]$ by $(k'_4[\text{SK}] + k_4m[\text{CycB}])([\text{Cdh1}]$, with $k'_4 \ll k_4$.

With these changes, the system of equations (1'), (2)–(8) accounts for many characteristic features of wild type and mutant budding yeast cells. Wild-type cells have a long G1 (unbudded period) and short S-G2-M (budded period); see

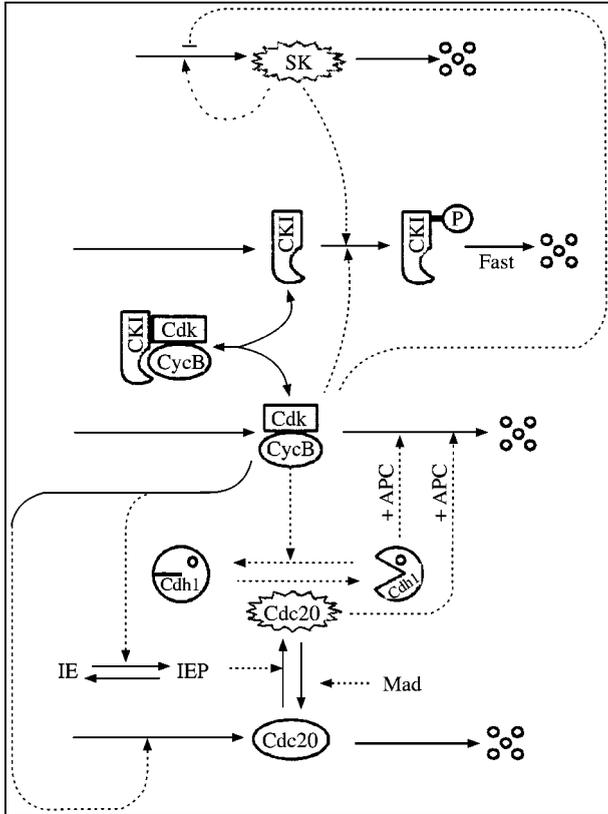


FIG. 6. The basic cell cycle engine in eukaryotic cells. The generic components in this mechanism correspond to specific gene products in well-studied organisms (see Table 2). Dynamical properties of this mechanism are determined by a set of kinetic equations (1'), (2)–(7), with $A = [Cdc20_A]$. A basal set of parameter values, suitable for yeast cells, is given in Table 1.

Fig. 8(a). Throughout G1, SK (Cln) level steadily rises, causing CKI (Sic1) level to fall. In late G1, several events occur in close succession. First, TF is activated and [SK] rises sharply, causing rapid

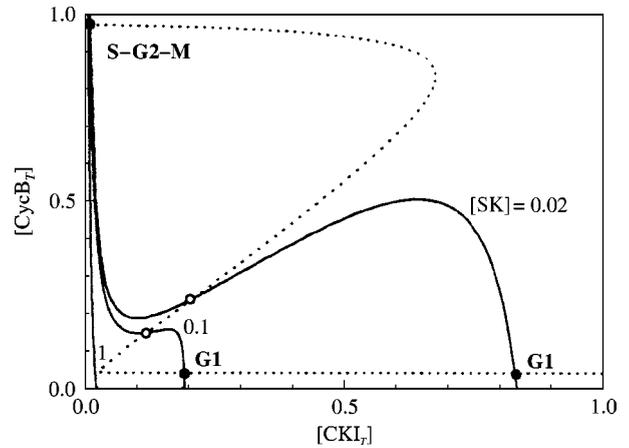


FIG. 7. Phase plane portrait for eqns (1') and (7). $[Cdh1]$ is computed from $[CycB]$ by solving for the steady state of eqn (2). Parameter values in Table 1, plus $[Cdc20_A] = 0$, $m = 1$, $[SK]$ adjustable. Dotted curve: CycB nullcline. Solid curves: CKI nullclines, for $[SK] = 0.02, 0.1$ and 1.0 . ● = stable steady state, ○ = unstable steady state. The control system has a saddle-node bifurcation at $[SK] \approx 0.9$.

destruction of the remaining CKI. When $[CKI]$ drops below $[CycB_T]$, active CycB/Cdc28 dimers make their appearance, helping SK to inactivate Cdh1. As Cdh1 activity drops off rapidly, CycB level rises further. Newly produced CycB/Cdc28 initiates DNA synthesis at about the same time that Cln/Cdc28 initiates a new bud. Meanwhile, CycB/Cdc28 turns off SK production and SK level drops. CKI and Cdh1 do not make a comeback because CycB/Cdc28 keeps these proteins phosphorylated. Persistent CycB/Cdc28 activity drives the cell into M phase. After a delay of about 45 min [during which IE is activated—not shown in Fig. 8(a)], Cdc20 is activated and

TABLE 1
Parameter values

Component	Rate constants (min^{-1})	Dimensionless constants
CycB	$k_1 = 0.04, k'_2 = 0.04, k''_2 = 1, k'''_2 = 1$	$[CycB]_{\text{threshold}} = 0.1$
Cdh1	$k'_3 = 1, k''_3 = 10, k'_4 = 2, k_4 = 35$	$J_3 = 0.04, J_4 = 0.04$
Cdc20 _T	$k'_5 = 0.005, k''_5 = 0.2, k_6 = 0.1$	$J_5 = 0.3, n = 4$
Cdc20 _A	$k_7 = 1, k_8 = 0.5$	$J_7 = 10^{-3}, J_8 = 10^{-3}, [\text{Mad}] = 1$
IE	$k_9 = 0.1, k_{10} = 0.02$	
CKI	$k_{11} = 1, k'_{12} = 0.2, k''_{12} = 50, k'''_{12} = 100$	$K_{eq} = 10^3$
SK	$k_{13} = 1, k_{14} = 1, k'_{15} = 1.5, k''_{15} = 0.05, k'_{16} = 1, k''_{16} = 3$	$J_{15} = 0.01, J_{16} = 0.01$
M	$\mu = 0.01$	$m_* = 10$

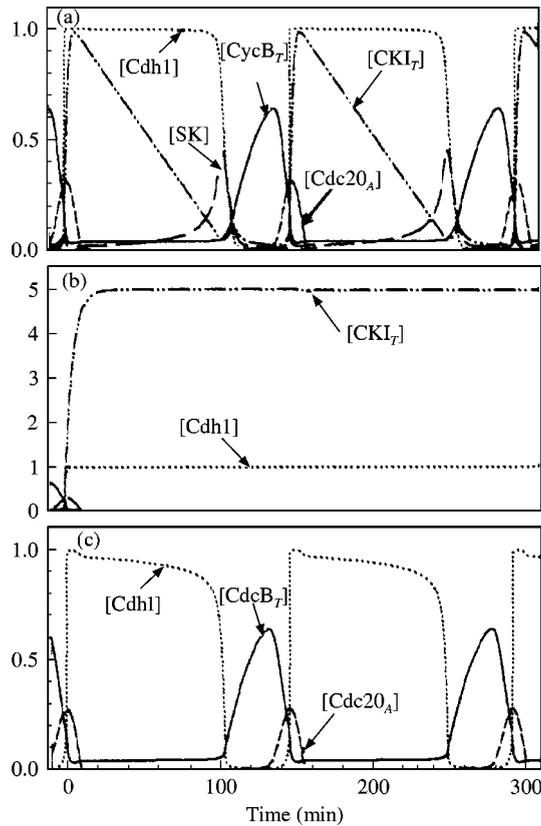


FIG. 8. The budding yeast cell cycle. (a) Wild-type cells: simulation of eqns (1'), (2)–(8), with parameter values in Table 1, except $\mu = 0.005 \text{ min}^{-1}$. (b) Mutant cells lacking SK ($k_{13} = 0$) block in G1 with copious CKI and active APC. (c) Mutant cells lacking SK and CKI ($k_{11} = k_{13} = 0$) are viable.

destroys enough CycB to allow Cdh1 to reactivate and CKI to reaccumulate. When CycB drops low enough, the cell divides. [In Fig. 8(a), we assume symmetric division for simplicity,

although budding yeast cells typically divide asymmetrically.]

Mutant cells lacking SK ($k_{13} = 0$) block in G1 with abundant CKI and active Cdh1 [Fig. 8(b)], which is the phenotype of cells in which all Cln-cyclins are deleted ($cln1\Delta \ cln2\Delta \ cln3\Delta$) (Richardson *et al.*, 1989). (Although Cln3 plays a different role than Cln1–2, it can serve as their backup; so it too must be deleted to see the expected phenotype.) Because the only essential job of the Cln-cyclins is to remove Sic1 (CKI), the quadruple-deletion mutant $cln1\Delta \ cln2\Delta \ cln3\Delta \ sic1\Delta$ is viable (Tyers, 1996); see Fig. 8(c). For a more thorough analysis of the budding yeast cell cycle, consult Chen *et al.* (2000).

Properly interpreted (Table 2), eqns (1'), (2)–(8) also provide a reasonable description of cell cycle controls in fission yeast cells lacking Wee1 ($wee1\Delta$ mutants; see (Novak *et al.*, 1998a, b). To describe wild-type fission yeast, we must add a new level of control to Fig. 6, involving tyrosine phosphorylation and dephosphorylation to Cdk subunits, as in Novak & Tyson (1995) and Svecizer *et al.* (2000).

Checkpoints and Surveillance Mechanisms

The job of the basic cell cycle engine (Fig. 6) is to coordinate the events of DNA synthesis and mitosis with overall cell growth. We have seen how cell size (m) might feed into the engine to ensure balanced growth and division. If cells are too small, the engine stops at the stable steady state (G1 in Fig. 7). Only when m exceeds some critical value is this stable steady state lost

TABLE 2
Cell cycle regulatory proteins in yeasts and vertebrates

Component	Budding yeast	Fission yeast	Frog egg	Mammalian cell
Cdk	Cdc28	Cdc2	Cdc2	Cdk1
CycB	Clb1–6	Cdc13	Cyclin B	Cyclin B
Cdh1	Cdh1	Ste9	Fizzy-related	Cdh1
Cdc20	Cdc20	Slp1	Fizzy	p55cdc
IE	Cdc5?	Plp1?	Plx1?	Plk1?
CKI	Sic1	Rum1	Xic1	p27 ^{Kip1}
SK	Cln1–2	Cig2	Cyclin E?	Cyclin D, E

by coalescence with an unstable steady state (saddle-node bifurcation). (In some cases, the stable G1 state might be lost by a change of stability—a Hopf bifurcation; e.g. Fig. 3 in Novak & Tyson, 1997.) When the G1 attractor is lost, the cell can proceed into S phase. In this way, Start can be controlled by cell size. In some species (e.g. fission yeast), size control operates at the G2 → M transition by the same principle: a stable G2 steady state is lost when cells grown beyond a critical size (Novak & Tyson, 1995).

We believe that this is a general principle of cell cycle control. A checkpoint corresponds to a stable steady state of the cell cycle engine (no further progress). The checkpoint is lifted by changes in crucial parameters, carrying the control system across a bifurcation. The crucial parameters are controlled by surveillance mechanisms that monitor the internal and external milieu of the cell. For instance, if DNA synthesis stalls for any reason, an inhibitory signal suppresses mitosis until the genome is fully replicated. If DNA is damaged in G1 or G2 phases, other surveillance mechanisms suppress entry into S phase or M phase, respectively. If chromosome alignment on the metaphase plate is delayed for any reason, a signal inactivates Cdc20 and blocks progression from metaphase to anaphase.

Spontaneous Oscillations in Fertilized Eggs

Although the proliferation of most eukaryotic cells is controlled by checkpoints, as described, cell division during early embryogenesis is not. The fertilized frog egg, for example, undergoes 12 rapid, synchronous, mitotic cell divisions, which are unconstrained by the usual requirements for growth and DNA integrity (Murray & Hunt, 1993). In the fertilized egg, the checkpoints (stable steady states) are missing, and the cell cycle engine exhibits its capacity for free-running oscillation.

The cyclin/Cdk network controlling cell divisions in early embryos is a stripped-down version of Fig. 6, lacking CKI and Cdh1. (With CKI missing, SK has no role to play in the model.) The only remaining components are CycB, IEP and

Cdc20, described by the following equations:

$$\frac{d[\text{CycB}]}{dt} = k_1 - (k'_2 + k''_2 [\text{Cdc20}_A])[\text{CycB}], \quad (9)$$

$$\frac{d[\text{IEP}]}{dt} = k_9[\text{CycB}](1 - [\text{IEP}]) - k_{10}[\text{IEP}], \quad (10)$$

$$\frac{d[\text{Cdc20}_A]}{dt} = \frac{k_7[\text{IEP}]([\text{Cdc20}_T] - [\text{Cdc20}_A])}{J_7 + [\text{Cdc20}_T] - [\text{Cdc20}_A]} - \frac{k_8[\text{Mad}][\text{Cdc20}_A]}{J_8 + [\text{Cdc20}_A]}. \quad (11)$$

For simplicity, we assume that Cdc20 (fizzy) is a stable protein in the early embryo and set $[\text{Cdc20}_T] = 1$ in eqn (11). Furthermore, $[\text{Mad}] = 1$, because the spindle assembly checkpoint is inoperative. Cell size (m) does not appear in these equations, because the embryo is not growing.

The system of equations (9)–(11) is a classical negative-feedback oscillator; see Goldbeter (1991). As shown in Fig. 9, it has limit-cycle solutions, corresponding to spontaneous oscillations in activity of CycB/Cdk (usually called MPF in the frog-egg literature). After 12 rapid, synchronous divisions, the frog egg undergoes an abrupt reorganization of the cell cycle (called the midblastula transition). Expression of zygotic genes provides the missing components of the cell cycle checkpoints. Consequently, the pace of cell division slows, as the checkpoint controls come into play.

Discussion

Kohn (1999) has recently summarized our knowledge of the molecular signals controlling the cell cycle in mammals. The “wiring” diagram extends in fine print over four journal pages, and most people would agree that we are only beginning to unravel the details. How are we to make sense of a control system of such complexity? Where casual verbal arguments will not suffice, reliable quantitative tools are needed. To this end, we have been developing analytical and computational methods to study the molecular machinery of cell cycle regulation.

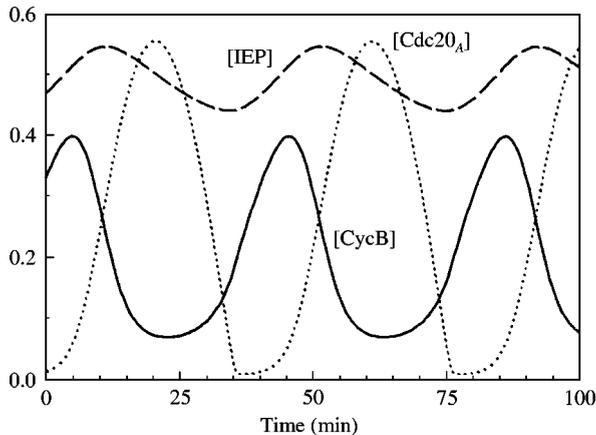


FIG. 9. Spontaneous oscillations of CycB/Cdk activity in early embryonic cells. Simulation of eqns (9)–(11), with parameter values in Table 1. Period = 41 min.

Following the reductionist approach of experimentalists, we have broken down the theoretical problem into manageable pieces. By understanding the dynamical properties of restricted parts of the network first, we are able to put the pieces together into ever more comprehensive, computational models of intact control systems in specific organisms (Marlovits *et al.*, 1998; Novak *et al.*, 1998a, b; Novak & Tyson, 1997). In this paper we summarize what we have learned over the years about bistability and oscillations in the control system, and their relations to checkpoints and surveillance mechanisms in living cells.

THE CELL CYCLE ENGINE AS A HYSTERESIS LOOP

We subscribe to the opinion of Nasmyth (1996) that the eukaryotic cell cycle is an alternation between two self-maintaining states: G1 (unreplicated DNA) and S–G2–M (DNA replication and mitosis). The cell switches between these states by the production and destruction of cyclin-dependent kinase activities. In G1, the cyclin degradation machinery is active and cyclins are scarce. At “Start,” the cyclin degradation machinery is turned off, and cyclins accumulate, driving the cell from G1 into S–G2–M. At “Finish,” the cyclin degradation machinery is turned back on, cyclins disappear, the cell divides and its progeny enter G1.

From a dynamical point of view, these two self-maintaining states of the cell cycle are stable steady states of the kinetic equations describing the interactions between cyclin-dependent kinases (Cdk) and the cyclin degradation machinery (APC). The control system is bistable because of a fundamental antagonism: APC destroys Cdk activity (by labeling its cyclin partner for proteolysis), and Cdk turns off the APC (by inactivating one of its components, Cdh1). As expected for a dynamical system of this sort, bistability is observed only within a restricted region of parameter space; the boundaries of this region are parameter values where saddle-node bifurcations occur (e.g. Fig. 3). The control system can be driven from one state to the other by parameter changes that carry the system across the boundary of saddle-node bifurcation points. Because the stable state initially occupied by the cell (G1) is lost at the saddle-node bifurcation, the cell is forced to make an irreversible transition (Start) to the other stable state (S–G2–M). In general, the opposite transition (Finish) can only be induced by parameter changes that carry the system across a different boundary, where the S–G2–M state is lost and the system jumps irreversibly to G1. When traced out in a diagram like Fig. 3, these parameter changes and state transitions create a “hysteresis loop”. Our line of reasoning suggests that the irreversible transitions of the cell cycle are intimately connected to the molecular antagonism of Cdk and APC. The connection is established by classical ideas from dynamical systems theory: bistability, saddle-node bifurcation, and hysteresis.

The phenomenon of hysteresis, upon which our whole notion of cell cycle control depends, is not a special feature of eqns (1) and (2) but rather a general property of dynamical systems with antagonism. Indeed, the establishment of G1 phase is not a simple matter of antagonism between Cdk and APC. On the contrary, in most cells, proteins called cyclin-dependent kinase inhibitors (CKIs) play significant roles in stabilizing the G1 state. Any realistic model of cell cycle transitions must take these CKIs into account. However, because CKI and Cdk are also antagonistic proteins, the generic property of hysteresis is maintained in more comprehensive models with more state variables and parameters.

Figures 2 and 3 provide convenient illustrations of the basic ideas that we believe will be generally applicable to cell cycle models of any complexity.

The “parameter” changes that drive cells through Start and Finish are carried out by additional components of the cell cycle control system, which we have called “helper” molecules. The role of “starter kinases” is to destroy CKIs so that the cell can leave G1, and the role of Cdc20 is to activate Cdh1 and stabilize CKIs so that the cell can reenter G1. The helpers do not participate in the antagonistic interactions: starter kinases are not inhibited by CKIs and not degraded by APC, and Cdc20 is not inhibited by Cdk. Helper activity is only transient: it rises to induce a transition, but then falls back down in preparation for the reverse transition. Were the helper activity to stay high, it would impede the reverse transition. Mutations that interfere with the rise or fall of helper proteins are usually inviable or severely compromised in progress through the cell cycle. For a thorough discussion of such mutants in budding yeast, and a presentation of the experimental evidence for multiple stable steady states in the yeast cell cycle engine, see Chen *et al.* (2000).

IS THE CELL CYCLE ENGINE A LIMIT-CYCLE OSCILLATOR?

We have been arguing that progress through the cell cycle consists of a series of irreversible transitions from one stable steady state to another of the underlying molecular regulatory system; cell reproduction is a periodic process because these steady states are connected in a ring. In the simplest case (budding yeast), there are two stable steady states (**G1** and **S–G2–M**) and the cell flips back and forth between them at Start and Finish. In higher eukaryotes, there are at least three checkpoints, in G1, S–G2, and M (Alberts, 1994), and the cell cycles through them in order. Instead of this “domino-like” theory of the cell cycle (Murray & Kirschner, 1989b), would not it be simpler, more elegant, and perhaps more accurate to describe periodic cell division by a limit-cycle oscillator (LCO)? Indeed, many theoreticians have put forward LCO models of cell cycle regulation (Norel & Agur, 1991; Obeyesekere, 1999; Hatzimanikatis

et al., 1999), assuming that interdivision time = period of an LCO. We do not think that LCOs, in general, provide a useful picture of cell cycle regulation, for the following reasons.

Generally speaking, the period of an LCO is a complicated function of all the kinetic parameters in the differential equations. However, the period of the cell division cycle depends on only one kinetic property of the cell: its mass-doubling time. That is, the period of the chromosome cycle (DNA synthesis, mitosis and cell division) is always equal to the period of the growth cycle (the time it takes for the cell to duplicate all its other components: total protein, membranes, etc.). If the chromosome cycle ran faster than the growth cycle, cells would get smaller at each division and eventually die. If the chromosome cycle ran slower than the growth cycle, cells would become larger at each division, which is also eventually fatal. Hence, in the long run, growth and division must be balanced, and the period of the cell division cycle must depend only on the growth rate of the culture [the parameter μ in eqn (6) in this paper]. For this reason, cell cycle period is insensitive to large changes in all other kinetic parameters of the control system. For example, by mutating cyclin genes or APC-related genes, one can modify the rate constants for cyclin synthesis or degradation many-fold without altering the balance between cell division time and mass-doubling time (see the literature citations and simulations in Chen *et al.*, 2000). Our models are consistent with this fundamental property of the cell cycle because they take into account the role played by cell growth in driving the Start transition of the cell cycle. (In wild-type fission yeast cells, size control operates at the G2 → M transition, but the basic principle is the same.) In our opinion, to understand balanced growth and division, it is more profitable to think of progress through the cell cycle as a domino-like sequence of irreversible transitions from one checkpoint to the next than as a clock-like sequence of events driven by an underlying LCO.

Nonetheless, there is an LCO “hidden” in the cell cycle regulatory mechanism, and it is revealed under specific circumstances. For instance, it is possible, by mutation, to remove all size controls from the fission yeast cell cycle (the *wee1⁻rum1Δ* mutant of Moreno & Nurse, 1994),

in which case the Cdk-regulatory system exhibits its underlying, free-running, oscillatory mode. These cells divide more rapidly than they can grow, and they soon die. In other words, the LCO mode of control is indeed possible for yeast cells, but it is a lethal mistake! The free-running oscillations must be restrained by checkpoint mechanisms (especially size control) in order to create viable, balanced cycles of growth and division.

There is an important circumstance in which growth and division are unbalanced, namely, the rapid division of the fertilized egg to produce a multicellular blastula. These division cycles seem to be driven by an LCO in the Cdk-regulatory system (Goldbeter, 1991; Novak & Tyson, 1993; Borisuk & Tyson, 1998). All checkpoints are inoperative, and the period of the oscillator is noticeably dependent on kinetic parameters, such as the rate of cyclin synthesis (Murray & Kirschner, 1989a). At the midblastula transition, checkpoints are re-established in the cell cycle, rapid division ceases, and cell division eventually becomes size-regulated again.

Finally, the LCO hypothesis seem inappropriate for cell cycle control because another hallmark of LCOs (Type-1 phase resetting in response to small perturbations) was not evident in the unique experimental setting where it could have been observed, namely, plasmodial fusion experimental in *Physarum polycephalum* (Tyson & Sachsenmaier, 1978; Winfree, 1980, pp. 444–448).

CELL CYCLE CONTROLS IN METAZOANS

Our analysis of cell cycle control shows that the complex molecular regulatory system can be understood in terms of some basic building blocks, whose dynamical features are most evident in the proliferation of yeast cells and other unicellular eukaryotes. Within the complex wiring diagram of mammalian cell cycle controls (Kohn, 1999), we can easily find the same basic building blocks (Novak & Tyson, in preparation). In multicellular organisms, unlike yeast, cell growth and division are under additional “social” constraints, because most somatic cells, though they find themselves bathed in a richly nutritious medium, are restrained from proliferating.

Only if they receive specific “permission” from the body as a whole may these cells grow and divide. The permission slips include growth factors (small polypeptides secreted into the blood stream or interstitial fluids), and signals that reflect cell–cell contacts and adhesion to the extracellular matrix (Alberts *et al.*, 1994). Surveillance mechanisms monitor these signals and hold the cell in a resting state (alive but not proliferating) until conditions permit cell growth and division. If these surveillance mechanisms become mutated so that a cell loses crucial social constraints, it becomes transformed, in stages, to an invasive cancer, whose uncontrolled proliferation eventually interferes with some vital function and kills the organism (Hanahan & Weinberg, 2000). By modeling the dynamical properties of these surveillance mechanisms and considering how they might plug into the cell cycle engine (Fig. 6), we hope eventually to construct a realistic model of mammalian cell cycle controls that will faithfully describe the physiology of normal and cancerous cells and accurately predict the results of pharmaceutical interventions.

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REFERENCES

- AGUDA, B. D. (1999). Instabilities in phosphorylation–dephosphorylation cascades and cell cycle checkpoints. *Oncogene* **18**, 2846–2851.
- ALBERTS, B., BRAY, D., LEWIS, J., RAFF, M., ROBERTS, K. & WATSON, J. D. (1994). *Molecular Biology of the Cell*, 3rd edn New York: Garland Publishers.
- BARTEK, J., BARTKOVA, J. & LUKAS, J. (1996). The retinoblastoma protein pathway and the restriction point. *Curr. Opin. Cell Biol.* **8**, 805–814.
- BORISUK, M. T. & TYSON, J. J. (1998). Bifurcation analysis of a model of mitotic control in frog eggs. *J. theor. Biol.* **195**, 69–85.
- CHEN, K., CSIKASZ-NAGY, A., GYORFFY, B., VAL, J., NOVAK, B. & TYSON, J. J. (2000). Kinetic analysis of a molecular model of the budding yeast cell cycle. *Mol. Biol. Cell* **11**, 369–391.
- ELLEDGE, S. J. (1996). Cell cycle checkpoints: preventing an identity crisis. *Science* **274**, 1664–1672.

- GOLDBETER, A. (1991). A minimal cascade model for the mitotic oscillator involving cyclin and cdc2 kinase. *Proc. Natl Acad. Sci. U.S.A.* **88**, 9107–9111.
- GOLDBETER, A. & KOSHLAND, D. E. Jr (1981). An amplified sensitivity arising from covalent modification in biological systems. *Proc. Natl Acad. Sci. U.S.A.* **78**, 6840–6844.
- HANAHAH, D. & WEINBERG, R. A. (2000). The hallmarks of cancer. *Cell* **100**, 57–70.
- HATZIMANIKATIS, V., LEE, K. H. & BAILEY, J. E. (1999). A mathematical description of regulation of the G1–S transition of the mammalian cell cycle. *Biotechnol. Bioeng.* **65**, 631–637.
- HWANG, L. H., LAU, L. F., SMITH, D. L., MISTROT, C. A., HARDWICK, K. G., HWANG, E. S., AMON, A. & MURRAY, A. W. (1998). Budding yeast Cdc20: a target of the spindle checkpoint. *Science* **279**, 1041–1044.
- IRNIGER, S., PIATTI, S., MICHAELS, C. & NASMYTH, K. (1995). Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in yeast. *Cell* **81**, 269–277.
- JASPERSON, S. L., CHARLES, J. F. & MORGAN, D. O. (1999). Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14. *Curr. Biol.* **9**, 227–236.
- KOHN, K. W. (1999). Molecular interaction map of the mammalian cell cycle control and DNA repair systems. *Mol. Biol. Cell* **10**, 2703–2734.
- MARLOVITS, G., TYSON, C. J., NOVAK, B. & TYSON, J. J. (1998). Modeling M-phase control in *Xenopus* oocyte extracts: the surveillance mechanism for unreplicated DNA. *Biophys. Chem.* **72**, 169–184.
- MENDENHALL, M. D. & HODGE, A. E. (1998). Regulation of Cdc28 cyclin-dependent protein kinase activity during the cell cycle of the yeast *Saccharomyces cerevisiae*. *Microbiol. Mol. Biol. Rev.* **62**, 1191–1243.
- MORENO, S. & NURSE, P. (1994). Regulation of progression through the G1 phase of the cell cycle by the *rum1*⁺ gene. *Nature* **367**, 236–242.
- MURRAY, A. W. (1995). The genetics of cell cycle checkpoints. *Curr. Opin. Genet. Devel.* **5**, 5–11.
- MURRAY, A. & HUNT, T. (1993). *The Cell Cycle. An Introduction*. New York: W. H. Freeman & Co.
- MURRAY, A. W. & KIRSCHNER, M. W. (1989a). Cyclin synthesis drives the early embryonic cell cycle. *Nature* **339**, 275–280.
- MURRAY, A. W. & KIRSCHNER, M. W. (1989b). Dominoes and clocks: the union of two views of the cell cycle. *Science* **246**, 614–621.
- NASMYTH, K. (1995). Evolution of the cell cycle. *Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci.* **349**, 271–281.
- NASMYTH, K. (1996). At the heart of the budding yeast cell cycle. *Trends Genet.* **12**, 405–412.
- NOREL, R. & AGUR, Z. (1991). A model for the adjustment of the mitotic clock by cyclin and MPF levels. *Science* **251**, 1076–1078.
- NOVAK, B. & TYSON, J. J. (1993). Numerical analysis of a comprehensive model of M-phase control in *Xenopus* oocyte extracts and intact embryos. *J. Cell Sci.* **106**, 1153–1168.
- NOVAK, B. & TYSON, J. J. (1995). Quantitative analysis of a molecular model of mitotic control in fission yeast. *J. theor. Biol.* **173**, 283–305.
- NOVAK, B. & TYSON, J. J. (1997). Modeling the control of DNA replication in fission yeast. *Proc. Natl Acad. Sci. U.S.A.* **94**, 9147–9152.
- NOVAK, B., CSIKASZ-NAGY, A., GYORFFY, B., CHEN, K. & TYSON, J. J. (1998a). Mathematical model of the fission yeast cell cycle with checkpoint controls at the G1/S, G2/M and metaphase/anaphase transitions. *Biophys. Chem.* **72**, 185–200.
- NOVAK, B., CSIKASZ-NAGY, A., GYORFFY, B., NASMYTH, K. & TYSON, J. J. (1998b). Model scenarios for evolution of the eukaryotic cell cycle. *Philos. Trans. R. Soc. Lond. Ser. B: Biol. Sci.* **353**, 2063–2076.
- NOVAK, B., TÓTH, A., CSIKÁSZ-NAGY, A., GYÖRFFY, B., TYSON, J. J. & NASMYTH, K. (1999). Finishing the cell cycle. *J. theor. Biol.* **199**, 223–233.
- OBEYSEKERE, M. N., ZIMMERMAN, S. O., TECARRO, E. S. & AUCHMUTY, G. (1999). A model of cell cycle behavior dominated by kinetics of a pathway stimulated by growth factors. *Bull. Math. Biol.* **61**, 917–934.
- RICHARDSON, H. E., WITTENBERG, C., CROSS, F. & REED, S. I. (1989). An essential G1 function for cyclin-like proteins in yeast. *Cell* **59**, 1127–1133.
- SHIRAYAMA, M., ZACHARIAE, W., CLOSK, R. & NASMYTH, K. (1998). The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerevisiae*. *EMBO J.* **17**, 1336–1349.
- SVEICZER, A., CSIKASZ-NAGY, A., GYORFFY, B., TYSON, J. J. & NOVAK, B. (2000). Modeling the fission yeast cell cycle, especially quantized cycle times in *wee1⁻cdc25Δ* mutant cells. *Proc. Natl Acad. Sci., U.S.A.* **93**, 7772–7776.
- THRON, C. D. (1999). Mathematical analysis of binary activation of a cell cycle kinase which down-regulates its own inhibitor. *Biophys. Chem.* **79**, 95–106.
- TYERS, M. (1996). The cyclin-dependent kinase inhibitor p40^{sic1} imposes the requirement for Cln G1 cyclin function at Start. *Proc. Natl Acad. Sci. U.S.A.* **93**, 7772–7776.
- TYSON, J. J., NOVAK, B., CHEN, K. & VAL, J. (1995). Checkpoints in the cell cycle from a modeler's perspective. *Prog. Cell Cycle Res.* **1**, 1–8.
- TYSON, J. J. & SACHSENMAIER, W. (1978). Is nuclear division in *Physarum* controlled by a continuous limit cycle oscillator? *J. theor. Biol.* **73**, 723–737.
- VISINTIN, R., CRAIG, K., HWANG, E. S., PRINZ, S., TYERS, M. & AMON, A. (1998). The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation. *Mol. Cell* **2**, 709–718.
- VISINTIN, R., PRINZ, S. & AMON, A. (1997). CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis. *Science* **278**, 460–463.
- WINFREE, A. T. (1980). *The Geometry of Biological Time*. New York: Springer-Verlag.
- ZACHARIAE, W. & NASMYTH, K. (1999). Whose end is destruction: cell division and the anaphase-promoting complex. *Genes Devel.* **13**, 2039–2058.