PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Model scenarios for evolution of the eukaryotic cell cycle

B. Novak, A. Csikasz-Nagy, B. Gyorffy, K. Nasmyth and J. J. Tyson

Phil. Trans. R. Soc. Lond. B 1998 **353**, 2063-2076
doi: 10.1098/rstb.1998.0352

THE ROYAL SOCIETY

Model scenarios for evolution of the eukaryotic cell cycle

Bela Novak[1], Attila Csikasz-Nagy[1], Bela Gyorffy[1], Kim Nasmyth[2] and John J. Tyson[3*]

[1]*Department of Agricultural Chemical Technology, Technical University of Budapest, Gellert ter 4, Budapest 1521, Hungary* (*bnovak@chem.bme.hu*)
[2]*Institute of Molecular Pathology, Dr Bohr Gasse 7, Vienna 1030, Austria* (*nasmyth@nt.imp.univie.ac.at*)
[3]*Department of Biology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061, USA* (*tyson@vt.edu*)

Progress through the division cycle of present day eukaryotic cells is controlled by a complex network consisting of (i) cyclin-dependent kinases (CDKs) and their associated cyclins, (ii) kinases and phosphatases that regulate CDK activity, and (iii) stoichiometric inhibitors that sequester cyclin–CDK dimers. Presumably regulation of cell division in the earliest ancestors of eukaryotes was a considerably simpler affair. Nasmyth (1995) recently proposed a mechanism for control of a putative, primordial, eukaryotic cell cycle, based on antagonistic interactions between a cyclin–CDK and the anaphase promoting complex (APC) that labels the cyclin subunit for proteolysis. We recast this idea in mathematical form and show that the model exhibits hysteretic behaviour between alternative steady states: a G1-like state (APC on, CDK activity low, DNA unreplicated and replication complexes assembled) and an S/M-like state (APC off, CDK activity high, DNA replicated and replication complexes disassembled). In our model, the transition from G1 to S/M ('Start') is driven by cell growth, and the reverse transition ('Finish') is driven by completion of DNA synthesis and proper alignment of chromosomes on the metaphase plate. This simple and effective mechanism for coupling growth and division and for accurately copying and partitioning a genome consisting of numerous chromosomes, each with multiple origins of replication, could represent the core of the eukaryotic cell cycle. Furthermore, we show how other controls could be added to this core and speculate on the reasons why stoichiometric inhibitors and CDK inhibitory phosphorylation might have been appended to the primitive alternation between cyclin accumulation and degradation.

Keywords: cyclin-dependent kinase; anaphase promoting complex; size control; surveillance mechanisms; checkpoints

1. INTRODUCTION

The cell-division cycle is a coordinated set of processes by which a cell replicates all its components and divides into two nearly identical daughter cells. Most cellular components are synthesized continuously throughout interphase and divided more or less evenly between daughters. Growth and cell separation need not be exact, as long as cells maintain their overall size and internal composition within reasonable bounds. On the other hand, all cells have elaborate mechanisms for initiating DNA synthesis, accurately copying its nucleotide sequence and distributing one complete copy to each sister. How do cells coordinate the continuous process of growth with the periodic cycle of chromosome replication and division? How do cells ensure that each daughter receives its share of the genome, no more and no less? How might the complex regulatory systems of modern eukaryotic cells have evolved from more primitive but still effective precursors?

Nasmyth (1995) has recently considered these questions in light of what we presently know about the phylogeny, physiology and molecular biology of cell division in prokaryotes and eukaryotes. The earliest cells on earth were undoubtedly prokaryotes similar to modern bacteria, having a single circular chromosome with a single origin of replication and a single site of termination. At the onset of DNA replication, sister chromatids are identified by attachment to sites on the bacterial cell membrane. After the replication forks meet at the termination site, the sister chromatids are untangled and segregated to the incipient daughter cells by processes (not yet well understood) involving, respectively, topoisomerases and entropic pressure, and membrane growth and cytoskeletal motor proteins (Wheeler & Shapiro 1997). Machinery of this sort is restricted to a single chromosome with unique initiation and termination sites because, were there multiple sites of initiation and termination, it would be difficult (i) to associate multiply initiated sister chromatid strands with proper membrane-attachment sites, and (ii) to determine when a full round of replication is completed. This restriction puts a lower bound on cell-division time, namely the minimum time taken by a single pair of replication forks to copy the chromosome completely. This severe limitation on cell proliferation is side-stepped by a strategy of overlapping rounds of

*Author for correspondence.

Received 30 June 1997 *Accepted* 25 September 1997

replication initiated at a unique origin (Donachie 1993). Despite multiple replication forks at fast growth rates, modern bacteria retain a single termination site. Replication of this termination site signals completion of a full round of DNA synthesis and triggers the process of cell division. Hence, bacteria seem to be locked into reliance on a single circular chromosome, which may place physical limits on the size of their genome.

The first step towards eukaryotic cells may have been an elaboration of the cytoskeleton and associated motor proteins so that cells could dispense with cell walls and adopt phagocytotic feeding strategies (de Duve 1995; Nasmyth 1995). The cytoskeleton of modern eukaryotes is laid out from the microtubule organizing centre (MTOC), which must be replicated and partitioned to daughter cells during each division cycle. The molecular machinery now typically associated with the eukaryotic chromosome cycle may have originally evolved to control replication and partitioning of MTOCs (Nasmyth 1995). If so, what had been a cytoskeletal-membrane attachment site for chromatid segregation would naturally become associated with microtubules and MTOC partitioning.

With something like a mitotic spindle in place, the single circular chromosome can now fragment and expand into many linear segments with multiple origins of replication, if identification of sister chromatids is displaced from the initiation of DNA synthesis to the last step of the chromosome cycle, when sister chromatids are pulled apart at anaphase. The 'glue' which had identified sister chromatids by attaching them to membrane sites now holds sister chromatids to one another and to microtubules until all preparations for anaphase are completed.

If this scenario is correct, then primitive eukaryotic cells, in return for being liberated from the restriction of a single chromosome, must give up the luxury of overlapping rounds of DNA replication; each origin of replication must fire only once per cycle. Modern eukaryotes have 'licensing' factors that enforce strict alternation between 'Start' (initiation of DNA synthesis) and 'Finish' (separation of fully replicated sister chromatids). In order to fire, replication origins must be 'cocked' (i.e. have a full complement of protein subunits), and then the trigger must be pulled by a cyclin-dependent kinase (CDK). But a CDK, in addition to pulling the trigger, also apparently disables a necessary component of the replication complex (RC). Thus, as long as CDK activity is high after Start, RCs cannot reassemble and hence will not mistakenly fire a second time. When the chromosomes are fully replicated and aligned on the mitotic spindle, a signal activates the anaphase promoting complex (APC), which initiates degradation of both the glue presumed to bind sister chromatids together and the cyclin component of CDK. Sister chromatids separate, RCs can now reassemble and the daughter cells are ready to begin a new cycle. For the next Start to occur, APC activity must be turned off, so that CDK activity can rise again.

Following this line of reasoning, Nasmyth (1995, 1996) proposed that cell-cycle control in the earliest eukaryotes was accomplished by antagonistic interactions between CDK and APC: CDK inactivates the APC, and the APC degrades the cyclin subunit of CDK. The interactions generate two self-maintaining states. In the 'G1' state (chromosomes unreplicated), APC activity is high, CDK activity is low and RCs are cocked. In the 'S/M' state (chromosomes replicated), APC activity is low, CDK activity is high and RCs are fired (uncocked). (We put 'G1' and 'S/M' in quotes because they are not precisely the same concepts as the traditional phases of the modern eukaryotic cell cycle. The crucial distinction is not whether DNA is being synthesized (classical S phase) or chromosomes are being divided (classical M phase), but whether the control system is in a pre-replicative or post-replicative state. Furthermore, there need be no recognizable G2 phase in the primitive eukaryotic cell cycle, since mitotic events may be initiated at Start and proceed simultaneously with DNA synthesis (as in present-day budding yeast cells): the crucial event is the timing of anaphase not prophase.)

The cycle of chromosome replication and segregation is simply the cycle of alternating transitions between these two states. At Start the control system switches from 'G1' to 'S/M' and at Finish it switches back. Since each of these states is self-maintaining, a signal of sufficient magnitude must be generated to switch the cell from one state to the other.

In modern eukaryotes, cells must grow to a minimal size before they can execute Start, and this surveillance mechanism (the molecular details of which are still unknown) coordinates the chromosome replication cycle to accumulation of the continuously synthesized components of the cell. A similar coordinating signal also operates in the prokaryotic cell cycle and must have been present in primitive eukaryotes. Finish is also regulated by a surveillance mechanism (also yet to be unravelled) ensuring that DNA is fully replicated and all chromosomes are properly aligned on the metaphase plate before the signal to activate the APC is generated.

In the following sections we cast this intuitive picture of primitive cell-cycle control into a simple molecular mechanism and express its dynamical properties in terms of non-linear ordinary differential equations (ODEs) for the activities of the APC and CDK. 'G1' and 'S/M' correspond to stable steady-state solutions of the ODEs, and the model reveals that the alternations between these states are accomplished by irreversible transitions around a hysteresis loop. After describing the primitive mechanism of cyclin synthesis and degradation, we consider how and why additional controls seen in modern eukaryotes, based on stoichiometric inhibitors and CDK phosphorylation, might have evolved.

2. THE PRIMITIVE APC–CDK CONTROLLER

Our mechanism for control of the cell cycle (figure 1) postulates an antagonistic interaction between CDKs and APCs: CDK inactivates the APC, whereas the active APC initiates degradation of the cyclin subunit of cyclin–CDK dimers. We assume that the catalytic subunit is stable and maintained in the cell at constant concentration. The interactions between CDK and the APC can be described by a pair of kinetic equations

$$\frac{\mathrm{d}[\mathrm{CDK}]}{\mathrm{d}t} = k_1 \cdot \mathrm{size} - \left[k_2'(1-[\mathrm{APC}]) + k_2'' \cdot [\mathrm{APC}]\right] \cdot [\mathrm{CDK}], \tag{1a}$$

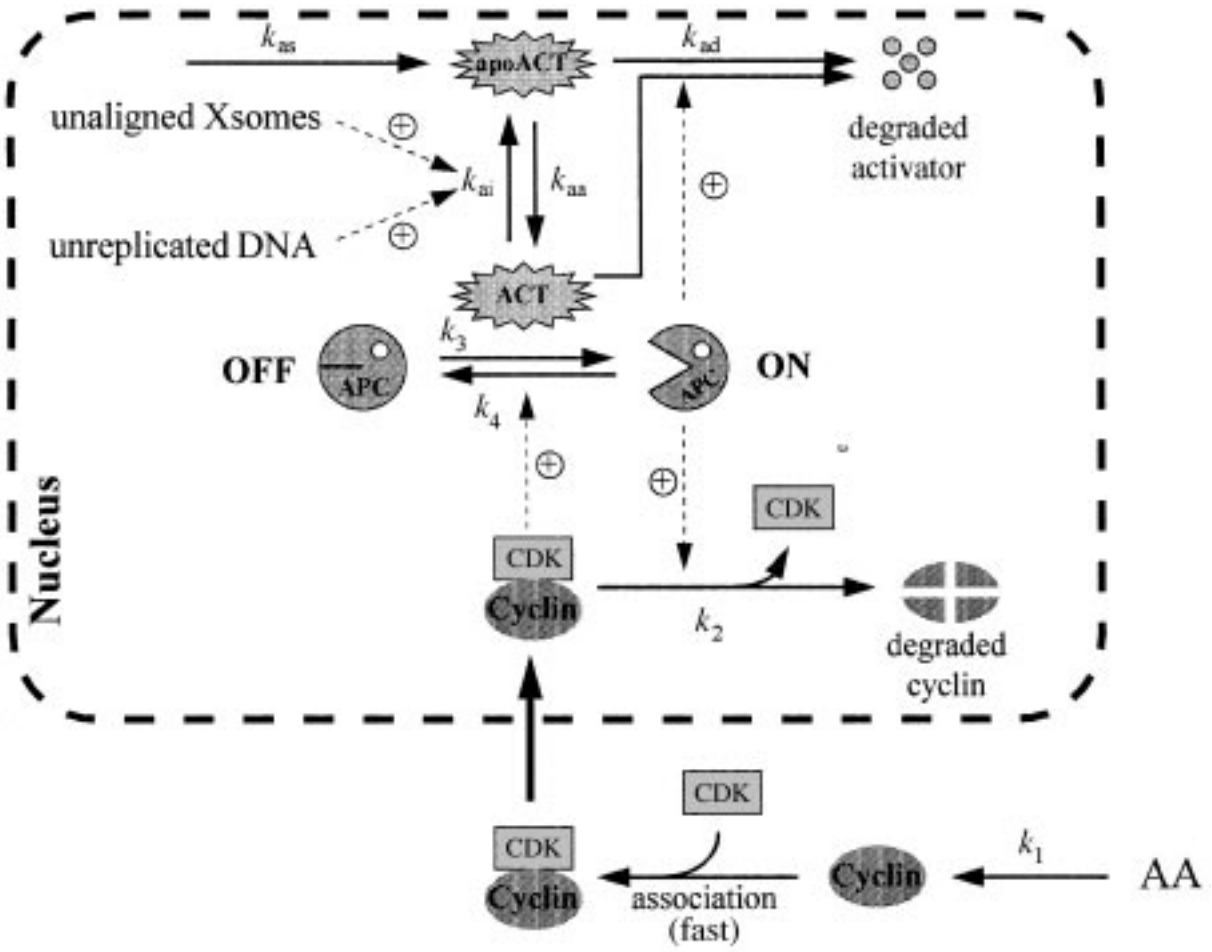

Figure 1. The primitive APC–CDK controller. Cyclin subunits (ovals) are synthesized on ribosomes in the cytoplasm (step 1) and bind rapidly and irreversibly to CDK subunits (rectangles) to form active dimers, which are immediately sequestered in the nucleus. The cyclin subunit is degraded (step 2) by the APC (Pac Man icon), releasing inactive CDK monomers. The APC is inactivated by cyclin–CDK (step 4), and reactivated (step 3) by a hypothetical 'activator'. The activator is synthesized continuously (step as) and degraded periodically by the APC (step ad). Newly synthesized activator must be converted to an active form (step aa), whereas unreplicated DNA and misaligned chromosomes generate signals that keep ACT in its less active form (step ai). The symbols (k_is) representing reaction rates are, for the most part, functions of the dynamic variables. For example, $k_2 = k_2'[\text{inactive [APC]}] + k_2''[\text{active [APC]}]$, where k_2' and k_2'' are enzyme turnover numbers. Values used for all rate constants are listed in table 1.

$$\frac{\mathrm{d}[\mathrm{APC}]}{\mathrm{d}t} = \frac{(k_3' + k_3'' \cdot [\mathrm{ACT}])(1 - [\mathrm{APC}])}{J_3 + 1 - [\mathrm{APC}]} - \frac{(k_4' + k_4'' \cdot [\mathrm{CDK}]) \cdot [\mathrm{APC}]}{J_4 + [\mathrm{APC}]}, \qquad (1b)$$

where $[\mathrm{CDK}](t)$ is the concentration of cyclin–CDK dimers in the nucleus and $[\mathrm{APC}](t)$ is the fraction of total APC that is active. In these equations k_1, k_2', etc., are rate constants, and size refers to some appropriate measure of cell size (e.g. ribosome number). For the time being, regard [ACT] as another parameter, a hypothetical 'activator' of the APC.

Although a convincing body of evidence from unicellular eukaryotic organisms indicates that overall cell size regulates passage through the chromosome replication–segregation cycle, very little is known about molecular mechanisms of size control in present day eukaryotes. We propose a simple mechanism for size control in this model (see also, Futcher 1996). Cyclin molecules are synthesized in the cytoplasm at a rate proportional to the total protein synthetic capacity of the cell ($k_1 \cdot$ size), bind rapidly to free kinase subunits (in excess) and the dimers are rapidly sequestered in the nucleus. Assuming that nuclear volume remains constant as the cell grows, the nuclear concentration of cyclin–CDK dimers increases as cell size increases. When CDK activity in the nucleus reaches a certain critical value (due to an increase in total cell size), Start is initiated and the cell becomes committed to divide.

CDK activity is lost by cyclin degradation at a rate dependent on the distribution of APC between its two forms: k_2' and k_2'' are the enzymatic turnover numbers characterizing the less- and more-active forms, respectively. APC activation and inactivation are described by Michaelis–Menten rate laws: k_3'' and k_4'' are turnover numbers for activation catalyzed by ACT and for inactivation catalyzed by CDK, and k_3' and k_4' are $V_{\max}$ values for the background rates of activation and inactivation. The total concentration of APC is scaled to 1 unit, and the Michaelis constants (J_3 and J_4) are assumed to be small relative to [total APC], so that APC activity behaves as an 'ultrasensitive switch' (Goldbeter & Koshland 1981).

A pair of non-linear ODEs like (1*a*) and (1*b*) are conveniently studied by phase-plane techniques. The phase

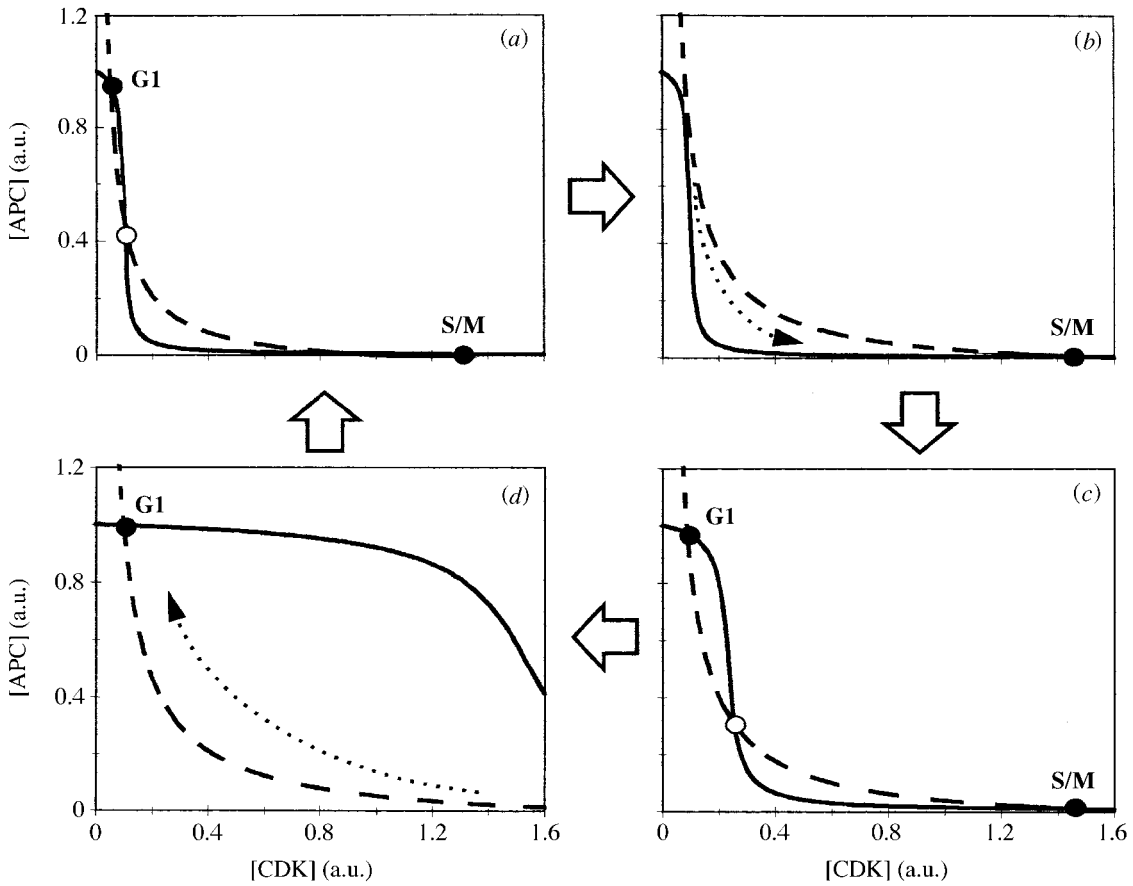

Figure 2. Phase-plane portraits for the primitive APC–CDK controller, equations (1*a*) and (1*b*). In each panel we plot the CDK balance curve, the hyperbolic curve (dashed) defined by equation (2*a*), and the APC balance curve, the sigmoidal curve (solid) defined by equation (2*b*). Steady states, either stable nodes (●) or unstable saddles (○), occur wherever the balance curves intersect. Parameter values are given in table 1, column 1, with size, [ACT], and k_{ai} fixed at the values below. (*a*) At the beginning of the cycle, the cell is arrested at **G1**; size = 1; [ACT] = 0.05; k_{ai}=0.5. (*b*) At Start, **G1** is lost by a saddle-node bifurcation, and the cell moves along the dotted trajectory to **S**/**M**; size = 1.6; [ACT] = 0.05; k_{ai}=0.5. (*c*) After DNA synthesis but before chromosome alignment, the cell is still arrested at the **S**/**M** steady state; size = 1.75; [ACT] = 1; k_{ai} = 7. (*d*) After chromosome alignment, **S**/**M** is lost by a saddle-node bifurcation and the cell moves along the dotted trajectory back to **G1**; size = 2; [ACT] = 1.5; k_{ai}=0.5.

plane is a Cartesian coordinate system spanned by [CDK](*t*) and [APC](*t*). Each biochemically realistic combination of numbers ([CDK], [APC]) defines an instantaneous state of the control system and corresponds to a point on the phase plane. At each point the ODEs tell us how fast the CDK and APC activities are changing. In geometric terms, the ODEs attach an arrow to each point on the phase plane; this set of arrows is called the 'vector field' of the kinetic equations. Wherever the rate of cyclin synthesis is exactly balanced by the rate of cyclin degradation, we have d[CDK]/dt=0, so the vector field is vertical. The locus of such points in the phase plane, called the CDK 'balance curve' (also known as a 'nullcline'), is given by

$$[\mathrm{CDK}] = \frac{k_1 \cdot \mathrm{size}}{k_2'\big(1-[\mathrm{APC}]\big) + k_2'' \cdot [\mathrm{APC}]}. \qquad (2a)$$

Similarly, wherever the rate of activation of APC is exactly balanced by its rate of inactivation, the vector field is horizontal. In this case, the 'APC balance curve' is given by

$$[\mathrm{CDK}] = \frac{\big(k_3' + k_3'' \cdot [\mathrm{ACT}]\big)\big(1-[\mathrm{APC}]\big)}{k_4'' \cdot [\mathrm{APC}]} \times \frac{J_4 + [\mathrm{APC}]}{J_3 + 1 - [\mathrm{APC}]} - \frac{k_4'}{k_4''}. \qquad (2b)$$

These two balance curves are plotted in figure 2. Wherever the balance curves intersect, we have a steady state of the control system. A steady state is called 'stable' if, in response to any small perturbation of [CDK] and [APC], the system returns to the steady state; otherwise, it is 'unstable'.

In figure 2*a*, the parameters of the model are chosen so that the balance curves intersect at three steady states: a stable steady state with the APC on and CDK off; a stable steady state with the APC off and CDK on; and an unstable steady state with intermediate activities of the APC and CDK. In mathematical parlance, the two stable steady states are called 'nodes' and the unstable steady state is a 'saddle point'. The two nodes correspond to the 'G1' and 'S/M' states mentioned in §1. In what follows, we will use boldface **G1** and **S**/**M** to refer specifically to these stable steady states. Their stability with respect to local fluctuations gives precise meaning to the idea that these

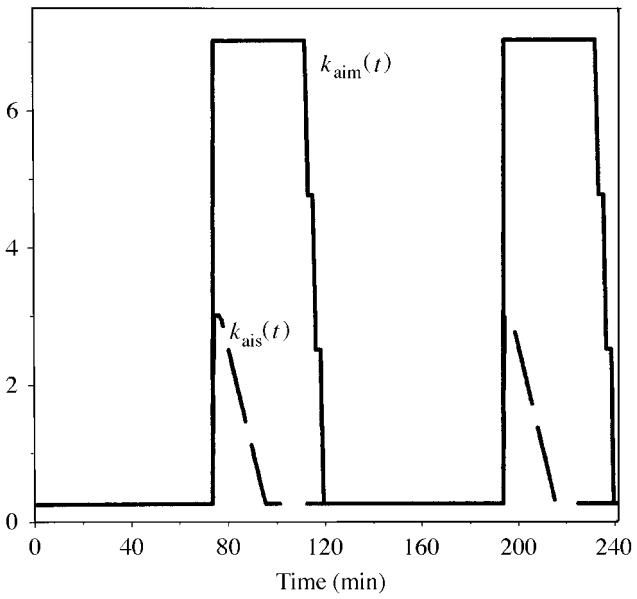

Figure 3. Rules for the mitotic spindle surveillance mechanism and cell division. The factor k_{ai} in equation (1*e*) has two contributions, $k_{ai} = k_{ais}(t) + k_{aim}(t)$, which are illustrated here. $k_{ais}(t)$ represents the inactivation of ACT by DNA replication forks. It is a 'sawtooth' function that increases abruptly at the onset of DNA synthesis and then decreases steadily back to its basal value. $k_{aim}(t)$ expresses the inactivatory effect of misaligned chromosomes. It is a 'stair-step' function that increases abruptly when cells enter M phase, and then decreases stepwise as chromosomes come into alignment on the mitotic spindle. No great significance is attached to the specific details of the time-courses chosen for $k_{ais}(t)$ and $k_{aim}(t)$. It is only important that $k_{ai}(t)$ be sufficiently large just after Start and then drop back to a low value as the cell progresses through the S/M phase of the cycle. To complete the rules, we must define the onset of DNA synthesis (the moment when [APC](*t*) decreases through 20%), the onset of mitosis (the moment when [CDK](*t*) increases through 1), and cell division (the moment when [APC](*t*) increases through 20%). The specific numerical values of these three thresholds are not particularly significant; they merely represent three convenient landmarks in the cell cycle.

are self-maintaining states. The saddle point, being unstable, is not directly observable, but, as we shall shortly see, it plays an essential role in cell-cycle transitions.

Depending on how the regulatory system in figure 2*a* is initially prepared, it will quickly approach one or the other of the two stable steady states and stay there until some major change occurs: either a large fluctuation in one of the variables or a large change in a parameter. In our scenario, Start is the crucial event that pushes a cell from **G1** to **S**/**M**, and this is accomplished by cell growth. As size increases, the CDK balance curve moves up, causing the **G1** node and the saddle point to coalesce and disappear (figure 2*b*). Mathematicians call this a 'saddle-node' bifurcation; in this case size is the bifurcation parameter, the critical size for Start is the value of size at the point of coalescence, and for $\text{size} > \text{size}_{\text{critical}}$ there exists only one stable steady state, **S**/**M**, to which the system must tend. When the cell grows large enough, it is forced to execute Start (APC turns off and CDK turns on) because the **G1** state disappears and **S**/**M** is the only stable steady state available to the control system.

Finish is the event that pushes a cell from **S**/**M** back to **G1**, by moving the APC balance curve to the right. We propose that this is accomplished by an 'activator' (ACT in figure 1) that opposes the inhibitory effect of CDK on APC. We suppose that the activator is synthesized continuously and degraded by the APC. Newly synthesized activator is a non-functional 'apo' form that must be post-translationally modified to its functional form by a pair of opposing reactions labelled 'aa' and 'ai' in figure 1. ApoACT accumulates in **S**/**M** (because the APC is off) but remains non-functional as long as DNA replication or repair is still in progress, or chromosomes are not yet under tension on the mitotic spindle (figure 2*c*). When 'all systems are go' for division, apoACT is converted to the ACT and turns on the APC by overwhelming CDK. In the phase plane (figure 2*d*) this corresponds to a large shift of the APC balance curve to the right, causing the **S**/**M** state to be lost by a saddle-node bifurcation. The control system must now switch back to **G1**. As the cell divides ($\text{size} \rightarrow \text{size}/2$) and ACT is destroyed by APC, the APC balance curve moves back into position 2*a*, and the cycle repeats itself.

To simulate the full cycle of growth and division, we supplement equations (1*a*) and (1*b*) with

$$\frac{\mathrm{dsize}}{\mathrm{d}t} = \mu \cdot \text{size}, \tag{1c}$$

$$\frac{\mathrm{d}[\mathrm{ACT}]_{\mathrm{T}}}{\mathrm{d}t} = k_{\mathrm{as}} - \left[k'_{\mathrm{ad}}\left(1 - [\mathrm{APC}]\right) + k''_{\mathrm{ad}} \cdot [\mathrm{APC}]\right] \cdot [\mathrm{ACT}]_{\mathrm{T}}, \tag{1d}$$

$$\frac{\mathrm{d}[\mathrm{ACT}]}{\mathrm{d}t} = k_{\mathrm{aa}}\left([\mathrm{ACT}]_{\mathrm{T}} - [\mathrm{ACT}]\right) - k_{\mathrm{ai}} \cdot [\mathrm{ACT}] - \left[k'_{\mathrm{ad}}\left(1 - [\mathrm{APC}]\right) + k''_{\mathrm{ad}} \cdot [\mathrm{APC}]\right] \cdot [\mathrm{ACT}], \tag{1e}$$

where $[\mathrm{ACT}]_{\mathrm{T}}(t) = [\mathrm{ACT}] + [\mathrm{apoACT}]$ and k_{ai} is a function describing the inactivating effects of unreplicated DNA and unaligned chromosomes (figure 3).

Figure 4 shows a typical oscillatory solution of equations (1*a*)–(1*e*) supplemented by the rules in figure 3. This numerical solution to the full set of ODEs confirms the qualitative picture in figure 2. Further simulations (not shown) at different values of the growth rate constant μ demonstrate that the interdivision time for the cell cycle is always equal to the mass doubling time, $(\ln 2)/\mu$, which must be true for cells experiencing balanced growth and division. The model has this essential property because the Start transition is regulated by cell size. Because a mother cell divides precisely in half at Finish, the time between successive Start events must be exactly the mass doubling time.

It is instructive to associate the primitive eukaryotic cell cycle with progress around a hysteresis loop. Hysteresis occurs in systems with multiple steady states and refers to the fact that the observed state of the system depends not only on its parameter values but also on its history (how the system is prepared). In our case, the system will find itself in **G1** if it has most recently passed

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

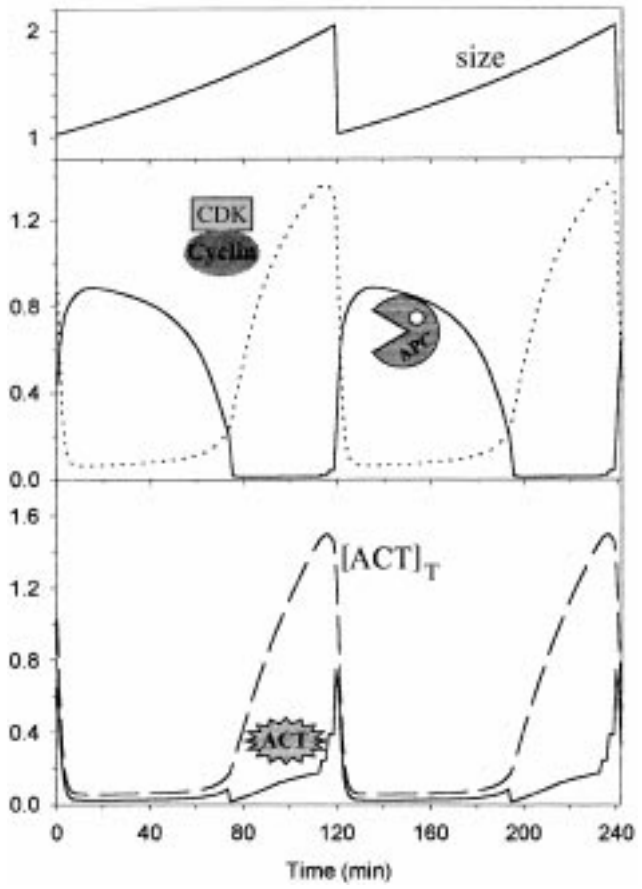

Figure 4. Growth-controlled division cycles in the primitive APC–CDK mechanism (figure 1). Solution of equations (1*a*)–(1*e*) with parameter values given in table 1, column 1. Specific growth rate $=\mu=0.0058\ \mathrm{min}^{-1}$, mass doubling time $=120$ min. The ODEs were solved numerically by a fourth-order Runge–Kutta algorithm, using the software package TimeZero (Kirchner 1990).

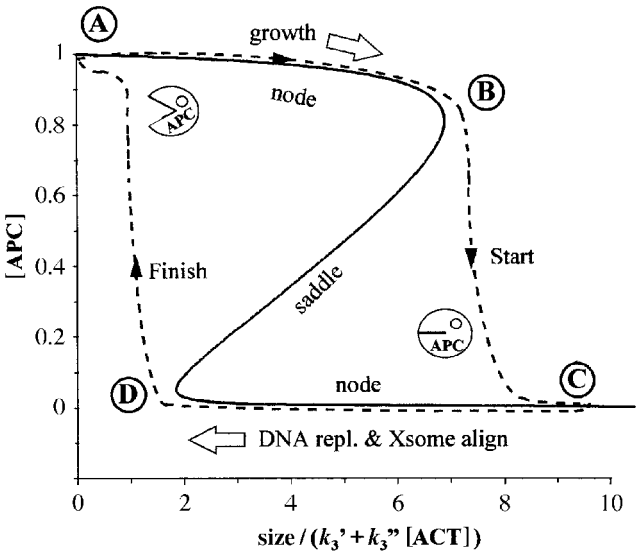

Figure 5. Bifurcation diagram for the primitive APC–CDK controller. Abscissa: bifurcation parameter, $\mathrm{size}/\left(k_3'+k_3''\cdot[\mathrm{ACT}]\right)$. Ordinate: steady state activity of [APC]. Parameters given in table 1. (We may use $\mathrm{size}/\left(k_3'+k_3''\cdot[\mathrm{ACT}]\right)$ as a bifurcation parameter because $k_4'=0$.) For $1.8<\mathrm{size}/\left(k_3'+k_3''\cdot[\mathrm{ACT}]\right)<6.9$, there exist three steady state values of [APC]. At points B and D, the APC-on and APC-off steady states are eliminated by saddle-node bifurcations. As a cell progresses through its division cycle, changes in size(t), [ACT](t) and [APC](t) follow the dashed trajectory, called a hysteresis loop. (In this diagram the hysteresis loop is qualitatively correct but not quantitatively accurate.) Increase in size(t), as the cell grows, drives it through the Start transition, and increase in [ACT](t), as chromosomes replicate and align on the mitotic spindle, drives the cell through the Finish transition. At cell division, size drops abruptly by a factor of two, and the hysteresis loop closes on itself.

Finish, or in **S/M** if it has most recently passed Start. To illustrate this hysteresis loop, we return to the two-component system, equations (1*a*) and (1*b*) and plot the steady-state value of APC activity against a 'bifurcation parameter', $\mathrm{size}/\left(k_3'+k_3''\cdot[\mathrm{ACT}]\right)$, with all other parameter values fixed. An equation for the steady-state value of [APC] can be derived by eliminating [CDK] between equations (2*a*) and (2*b*). Then, for $k_4'=0$, this equation can be solved for $\mathrm{size}/\left(k_3'+k_3''\cdot[\mathrm{ACT}]\right)$ as a function of [APC] at the steady state:

$$\frac{\mathrm{size}}{k_3'+k_3''\cdot[\mathrm{ACT}]}=\frac{\left[k_2'\left(1-[\mathrm{APC}]\right)+k_2''\cdot[\mathrm{APC}]\right]\left(1-[\mathrm{APC}]\right)\left(J_4+[\mathrm{APC}]\right)}{k_1\cdot k_4''\cdot[\mathrm{APC}]\left(J_3+1-[\mathrm{APC}]\right)}.$$

We plot this relationship in figure 5. Over a restricted range of the bifurcation parameter, there are three alternative steady states of APC: two stable nodes separated by a saddle point. Let us imagine that a cell starts in **G1** with its cell-cycle control system at point A in figure 5. (For a newborn cell, [APC] is close to one and size is small.) As size increases, the control system moves along the upper dashed trajectory and the cell stays in **G1** (APC on) until the **G1** steady state is lost at a saddle-node bifurcation (point B). As cell size increases further, past the bifurcation point, the control system must switch over to point C: the APC turns off and CDK turns on. This switch is the Start transition. After Start, as DNA is replicated and the chromosomes congress to the metaphase plate, [ACT] increases (and the bifurcation parameter decreases), but the cell remains in the post-replicative state until **S**/**M** is lost by a saddle-node bifurcation at point D in figure 5. In passing point D, [ACT] has become large enough to overwhelm CDKs and activate the APC. The control system switches back to **G1** (the Finish transition), and the process repeats itself.

The irreversibility of the cell cycle, in this scenario, is intimately related to the unstable steady state (the saddle point) which is an integral part of the hysteresis loop. As $\mathrm{size}/\left(k_3'+k_3''\cdot[\mathrm{ACT}]\right)$ changes due to cell growth, division and chromosome surveillance, the saddle point shuttles back and forth, destroying first the **G1** state, then the **S**/**M** state. The saddle-node bifurcations of the hysteresis loop convert slow incremental changes in size and [ACT] into abrupt and irreversible commitment steps like Start and Finish.

The simple model summarized in equations (1*a*) and 1*b*) and figures 1–5 has all the features of a functional eukaryotic cell cycle: pre-replicative and post-replicative

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

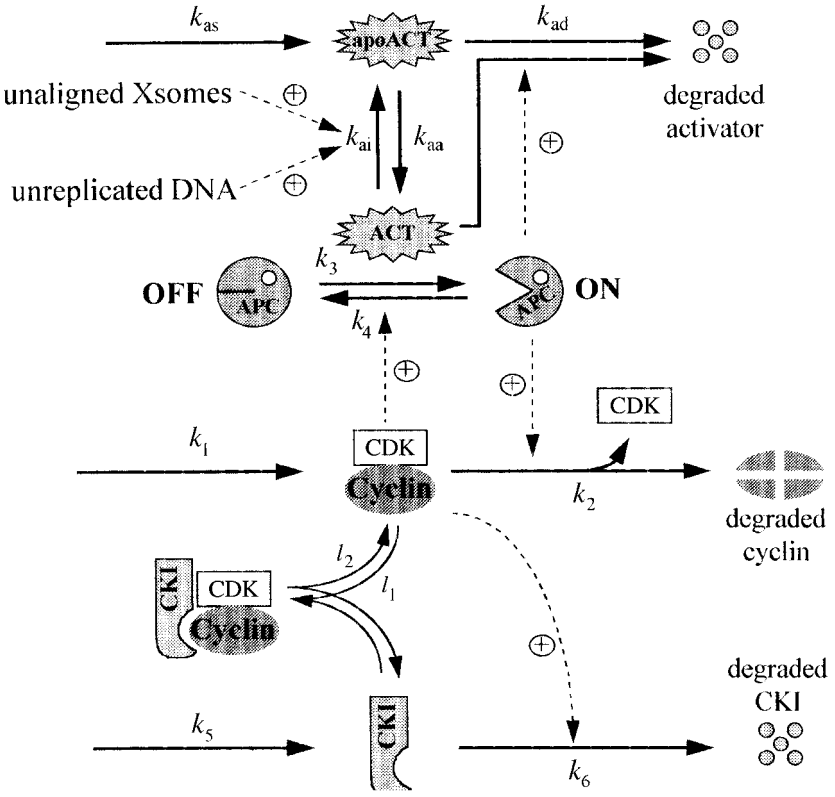

Figure 6. The APC–CDK controller with a CDK-inhibitor (CKI) operative in G1 phase. In addition to the reactions in figure 1, CKI is synthesized at a constant rate, k_5, and degraded by step 6. CKI can be phosphorylated by CDK, and the phosphorylated form is rapidly degraded. CKI binds rapidly and reversibly to cyclin–CDK dimers. The trimer has no kinase activity. The CKI and cyclin subunits of the trimer are still subject to proteolysis (not shown in the diagram).

states, size control over Start, and a surveillance mechanism to link Finish with chromosome replication and alignment. As stressed by Nasmyth (1995), this scenario could have evolved stepwise from bacterial origins and, once in place, would have opened new evolutionary possibilities by allowing the genome to increase in size.

3. ADDING A CDK INHIBITOR

In the primitive APC–CDK controller just described, the chromosome cycle (Start and Finish) is linked to overall cell growth by a minimum size requirement for start. The size requirement stems from our assumption that CDK is sequestered in the nucleus. The newborn cell must grow to a critical size before the APC-inactivating signal from CDK in the nucleus is stronger than the activating signal from ACT.

This mechanism for size control of the primitive cycle is completely hypothetical. In fact, the mechanism of size control in present-day eukaryotes is still unknown. There is some evidence in fission yeast that size at Start is related to the activity of Rum1 (Moreno & Nurse 1994), a cyclin-dependent kinase inhibitor (CKI), which binds strongly to certain cyclin–CDK dimers in G1 phase and blocks their activities (Correa-Bordes & Nurse 1995; Jallepalli & Kelly 1996; Martin-Castellanos *et al.* 1996). In budding yeast an analogous CKI, called Sic1, is operative in G1 phase, inhibiting CDK dimers with B-type cyclins (Schwob *et al.* 1994).

It may be advantageous to hold cells in **G1** with low CDK activity by synthesizing a CKI, because a pool of inactive CDK molecules could accumulate behind CKI in G1 phase without jeopardizing the alternation of pre-replicative and post-replicative states (Nasmyth & Hunt 1993). When the CKI is degraded at Start, pre-formed CDK is unmasked to initiate DNA synthesis.

To study the effects of a CKI on the primitive APC–CDK controller, we expand our model along the lines schematized in figure 6. The new reactions involving CKI and CDK are described by the following kinetic equations:

$$\frac{\mathrm{d}[\mathrm{CDK}]}{\mathrm{d}t} = k_1\cdot\mathrm{size} - \left[k_2'(1-[\mathrm{APC}]) + k_2''\cdot[\mathrm{APC}]\right]\cdot[\mathrm{CDK}] + \left(k_6' + k_6''\cdot[\mathrm{CDK}]\right)\cdot[\mathrm{TRI}] - l_1\cdot[\mathrm{CDK}]\cdot[\mathrm{CKI}] + l_2\cdot[\mathrm{TRI}], \tag{3a}$$

$$\frac{\mathrm{d}[\mathrm{CKI}]}{\mathrm{d}t} = k_5 + \left[\nu_2(1-[\mathrm{APC}]) + \nu_2''\cdot[\mathrm{APC}]\right]\cdot[\mathrm{TRI}] - \left(k_6' + k_6''\cdot[\mathrm{CDK}]\right)\cdot[\mathrm{CKI}] - l_1\cdot[\mathrm{CDK}]\cdot[\mathrm{CKI}] + l_2\cdot[\mathrm{TRI}], \tag{3b}$$

$$\frac{\mathrm{d}[\mathrm{TRI}]}{\mathrm{d}t} = -\left[\nu_2'(1-[\mathrm{APC}]) + \nu_2''\cdot[\mathrm{APC}]\right]\cdot[\mathrm{TRI}] - \left(k_6' + k_6''\cdot[\mathrm{CDK}]\right)\cdot[\mathrm{TRI}] + l_1\cdot[\mathrm{CDK}]\cdot[\mathrm{CKI}] - l_2\cdot[\mathrm{TRI}], \tag{3c}$$

[CDK], [CKI] and [TRI] denote the concentrations of cyclin–CDK dimers, CKI monomers and cyclin–CDK–CKI trimers, respectively. We arrange that CKI binding protects cyclin subunits from degradation by the APC by choosing ν_2'' (the rate constant for degradation of cyclin from trimers) to be less than k_2'' (see table 1).

In figure 7 we report a simulation of the full system in figure 6, described by equations (1*b*)–(1*e*) and (3*a*)–(3*c*). As for the more primitive model (figure 4), these cycles

Table 1. *Parameter values*[*]

figures 2 and 4	figures 7 and 8	figures 10 and 11
rate constants (min^{-1}):	same as column 1:	same as column 1:
$k_1 = 0.05$	k_1, k_2', k_2'', k_3'',	k_1, k_2', k_2'', k_4',
$k_2' = 0.05$	k_4', k_4'', k_{as}, k_{ad}'	k_4'', k_{as}, k_{ad}', k_{ad}''
$k_2'' = 1$	k_{ad}'', k_{aa}, $\mathcal{J}_3$, $\mathcal{J}_4$	
$k_3' = 0.1$		rate constants (min^{-1}):
$k_3'' = 3$	rate constants (min^{-1}):	$k_3' = 0.02$
$k_4' = 0$	$k_3' = 0.001$	$k_3'' = 2$
$k_4'' = 2$	$\nu_2' = 0.05$	$k_{aa}' = 0.001$
$k_{as} = 0.05$	$\nu_2'' = 0.15$	$k_{aa}'' = 1$
$k_{ad}' = 0.005$	$k_5 = 0.15$	$k_w = k_{25} = 0.5$
$k_{ad}'' = 1$	$k_6' = 0.15$	$k_{wr} = k_{25r} = 0.2$
$k_{aa} = 1$	$k_6'' = 9$	$\nu_{wee}' = 0.01$
	$l_1 = 200$	$\nu_{wee}'' = 0.8$
Michaelis constants:	$l_2 = 1$	$\nu_{c25}' = 0.02$
$\mathcal{J}_3 = \mathcal{J}_4 = 0.05$		$\nu_{c25}'' = 0.5$
		Michaelis constants:
		$\mathcal{J}_3 = \mathcal{J}_4 = 0.01$
		$\mathcal{J}_w = \mathcal{J}_{wr} = \mathcal{J}_{25}$
		$= \mathcal{J}_{25r} = 0.1$
		other:
		$\alpha = 0.06$

[*]All concentration variables are scaled to dimensionless values of order one. The rate constant values were chosen to illustrate the dynamical behaviour we desire.

are growth controlled: interdivision time = mass doubling time = $(\ln 2)/\mu$. In this case, size control over start can be attributed either to the inhibition of the APC by CDK or to the degradation-inducing phosphorylation of CKI by CDK. Choice of parameter values will determine which critical size is operative and which is cryptic. For instance, if a growing cell first reaches the critical size necessary to destabilize the CKI–CDK interaction, then that size control will be operative and the APC–CDK size control will be cryptic. During the course of evolution, it may be that the locus of size control shifted from the APC–CDK interaction to the CKI–CDK interaction, and then it may have been lost completely during later modifications of the APC–CDK subsystem. For this reason, we would not expect that present-day growth-surveillance mechanisms are indicative of the original size control that we ascribe to the APC–CDK hysteresis loop.

As before, it is informative to visualize the control system in the phase plane, but first we must reduce the full system of seven ODEs to only two. In the appendix we show how to describe this model in terms of two composite variables: $[CDK]_T = [CDK] + [TRI]$ = total concentration of cyclin subunits, and $[CKI]_T = [CKI] + [TRI]$ = total concentration of inhibitor subunits, governed by equations (A3*a*) and (A3*b*). In the $[CKI]_T - [CDK]_T$ phase plane (figure 8*a*), when the cell is small, the balance curves intersect in three steady states:

(i) **G1** (stable node): $[CKI]_T$ large, $[CDK]_T$ small, and APC on.
(ii) **S/M** (stable node): $[CKI]_T$ small, CDK active, and APC off.

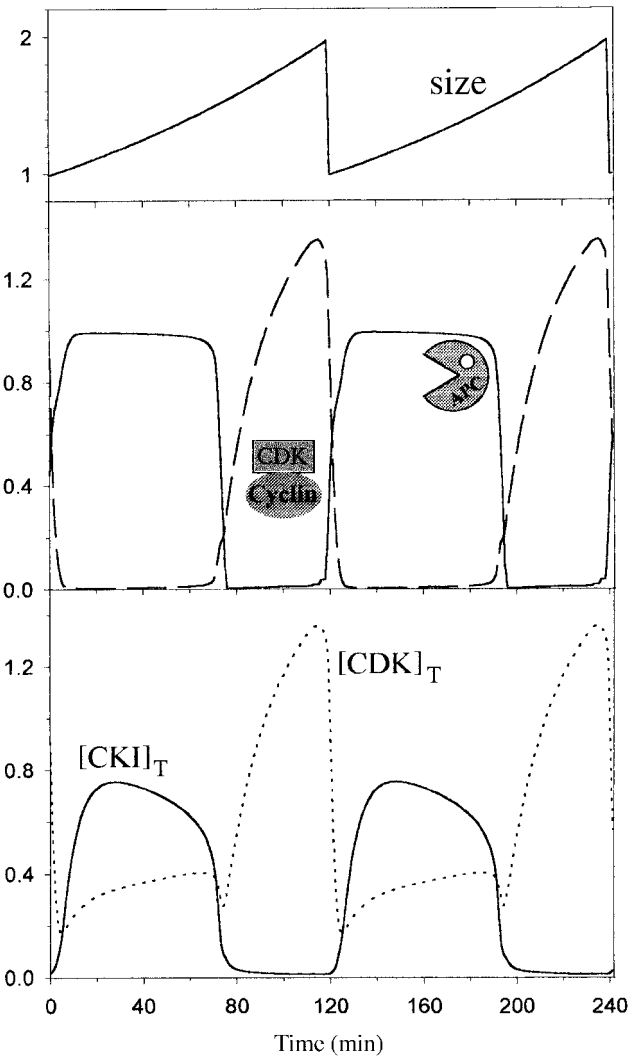

Figure 7. Growth-controlled division cycles in the primitive APC–CDK–CKI mechanism (figure 6). Solution of equations (1*b*)–(1*e*) and (3*a*)–(3*c*) with parameter values given in table 1, column 2.

(iii) Intermediate (saddle): $[CKI]_T \approx [CDK]_T$, and APC on.

As size increases, the **G1** state is eliminated by a saddle-node bifurcation (figure 8*b*), and the CDK–CKI subsystem must switch to the **S/M** state, in which CKI is removed, the APC is switched off, and CDK accumulates. ApoACT accumulates (figure 8*c*) until the chromosomes are fully replicated and aligned, when it is converted to ACT (figure 8*d*) and the **S/M** steady state disappears. CDK is destroyed as the APC turns on (dashed trajectory in figure 8*d*). When CDK activity drops low enough, CKI makes a comeback. Meanwhile, after cell division and degradation of ACT during **G1**, the CKI–CDK subsystem re-establishes the portrait in figure 8*a*.

4. A ROLE FOR CDK PHOSPHORYLATION

Returning to the primitive APC–CDK controller, we recall that no distinction is made between initiation of S and M phases. To separate S and M phases and introduce a G2 phase, it is necessary to delay the onset of mitosis

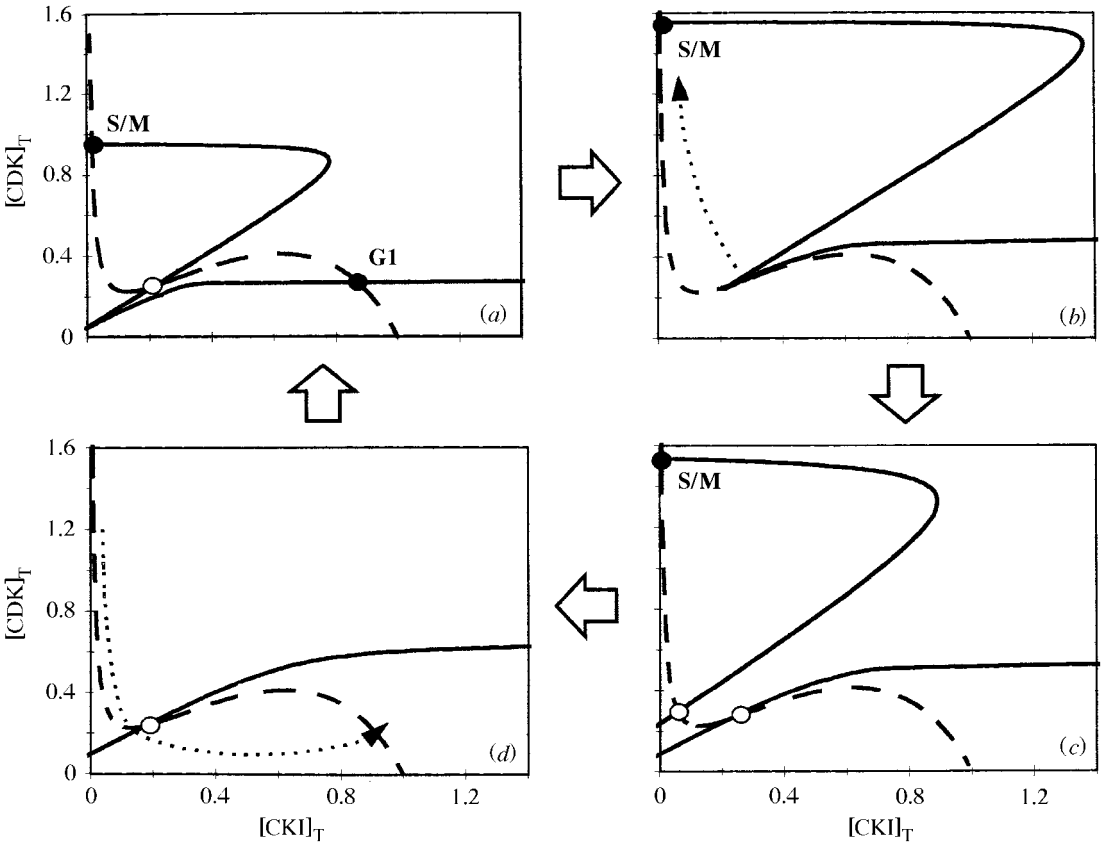

Figure 8. Phase-plane portraits for the CKI–CDK subsystem, equations (A3*a*) and (A3*b*). The CDK balance curve is defined by the solid line, and the CKI balance curve defined by the dashed line. Parameter values given in table 1, column 2, with size, [ACT], and k_{ai} fixed at the values printed below. (*a*) At the beginning of the cycle, the cell is arrested at **G1**; size = 1; [ACT] = 0.05; $k_{ai} = 0.5$. (*b*) At start, **G1** is lost by a saddle-node bifurcation, and the cell moves along the dotted trajectory to **S**/**M**; size = 1.6; [ACT] = 0.05; $k_{ai} = 0.5$. (*c*) After DNA synthesis but before chromosome alignment, the cell is still arrested at the **S**/**M** steady state; size = 1.75; [ACT] = 1.1; $k_{ai} = 7$. (*d*) After chromosome alignment, **S**/**M** is lost by a saddle-node bifurcation and the cell moves along the dotted trajectory back toward the 'G1 region' ($[CKI]_T$ high, $[CDK]_T$ low); size = 2; [ACT] = 1.5; $k_{ai} = 0.5$.

until some time after DNA replication is complete. In modern fission yeast this is accomplished, in part, by phosphorylating Cdc2, the catalytic subunit of the mitotic kinase, on a tyrosine residue in the ATP-binding site, which inhibits its M-phase promoting activity while S phase is in progress (Gould & Nurse 1989; Nurse 1994). Two tyrosine kinases, Wee1 and Mik1, accomplish this job in fission yeast (Lundgren *et al.* 1991; Russell & Nurse 1987). Some time after DNA replication is complete, the level of tyrosine phosphorylation drops and fission yeast cells enter mitosis (Nurse 1994). Because a single cyclin (Cdc13) is sufficient in fission yeast to drive periodic, alternating S and M phases, the kinase activity of the tyrosine-phosphorylated dimer (Cdc13/Cdc2-Y15P) must be sufficient to initiate S phase, but insufficient to initiate M phase (Fisher & Nurse 1996; Borgne & Meijer 1996). Only after S phase is completed is the tyrosine residue dephosphorylated (by a tyrosine phosphatase, Cdc25) and the cell induced to enter mitosis.

We explore this elaboration of the primitive APC–CDK controller with the mechanism in figure 9. We rewrite equation (1*a*) as

$$\frac{d[CDK]_T}{dt} = k_1 \cdot \text{size} - \left[k_2'(1-[APC]) + k_2'' \cdot [APC]\right] \cdot [CDK]_T \tag{4a}$$

where $[CDK]_T = [CDK]_A + [CDK]_P$, to distinguish between active and inactive (phosphorylated) dimers. The equation for $[CDK]_A\,(t)$ is

$$\frac{d[CDK]_A}{dt} = k_1 \cdot \text{size} - \left[k_2'(1-[APC]) + k_2'' \cdot [APC]\right] \cdot [CDK]_A - k_{wee} \cdot [CDK]_A + k_{c25}\left([CDK]_T - [CDK]_A\right), \tag{4b}$$

where

$$k_{wee} = \nu'_{wee}\left(1 - [Wee1]\right) + \nu''_{wee} \cdot [Wee1]$$
$$k_{c25} = \nu'_{c25}\left(1 - [Cdc25]\right) + \nu''_{c25} \cdot [Cdc25]$$

[Wee1] and [Cdc25] represent the active fractions of these enzymes. Activation and inactivation of Wee1 and Cdc25 are accounted for by

$$\frac{d[Wee1]}{dt} = \frac{k_{wr}\left(1 - [Wee1]\right)}{\mathcal{J}_{wr} + 1 - [Wee1]} - \frac{k_w \cdot MPF \cdot [Wee1]}{\mathcal{J}_w + [Wee1]}, \tag{4c}$$

$$\frac{d[Cdc25]}{dt} = \frac{k_{25} \cdot MPF \cdot \left(1 - [Cdc25]\right)}{\mathcal{J}_{25} + 1 - [Cdc25]} - \frac{k_{25r} \cdot [Cdc25]}{\mathcal{J}_{25r} + [Cdc25]}, \tag{4d}$$

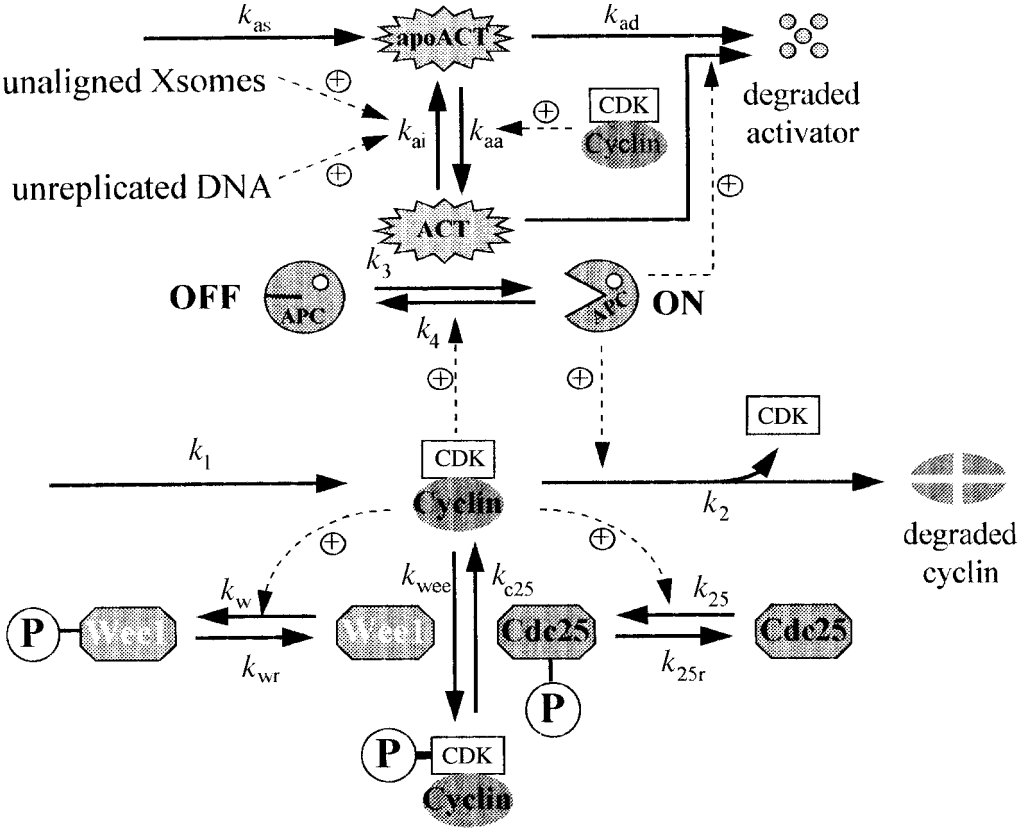

Figure 9. The APC–CDK controller supplemented by tyrosine phosphorylation of the kinase subunit. In addition to the reactions in figure 1, CDK is reversibly phosphorylated by a tryosine kinase–phosphatase pair, Weel and Cdc25. Weel and Cdc25 are themselves phosphoproteins. Phosphorylation of Weel by CDK inhibits Weel, whereas Cdc25 is activated when phosphorylated by CDK.

where $MPF = [\mathrm{CDK}]_A + \alpha \cdot \left([\mathrm{CDK}]_T - [\mathrm{CDK}]_A\right)$, i.e. *MPF* (in an italicised form) is the weighted activity of the unphosphorylated and phosphorylated forms of cyclin–CDK. We use $\alpha = 0.06$ in our simulations. In equations (4*c*) and (4*d*), $\mathcal{J}$ represents the Michaelis constants relative to the total concentrations of Weel and Cdc25, which (we assume) remain constant throughout the cycle.

The full model is now equations (1*b*)–(1*e*) and (4*a*)–(4*d*) with two further changes: in equation (1*b*) '[CDK]' is replaced by '*MPF*', and in equation (1*e*) k_{aa} is replaced by $k'_{aa} + k''_{aa} \cdot MPF$. The latter change introduces CDK as an activator of ACT as well as an inhibitor of the APC. A typical simulation is illustrated in figure 10. In this model, size control can operate either at the transition from **G1** to **S**/**G2** or at the transition from **S**/**G2** to **M**. In figure 10 we have set the parameters so that the G1 size control is cryptic.

In the appendix we show how to reduce the full model to only two components, $[\mathrm{CDK}]_A$ and $[\mathrm{CDK}]_T$, described by equations (4*a*) and (4*b*). Phase plane portraits for this reduced system are illustrated in figure 11. A newborn cell starts near the origin of the phase plane in figure 11*a*, with low $[\mathrm{CDK}]_T$ and, of course, $[\mathrm{CDK}]_A$. However, there is no **G1** steady state to hold it there and the cell starts immediately accumulating CDK in the tyrosine-phosphorylated form. Presumably, this form of CDK has enough activity to trigger S phase but not M phase. The cell is caught at the **S**/**G2** steady state (figure 11*b*) until it grows large enough to eliminate this checkpoint by a saddle-node bifurcation (figure 11*c*). After passing the bifurcation point, the cell activates the store of CDK by tyrosine dephosphorylation and proceeds to the metaphase checkpoint (the M steady state in figure 11*c*). When all chromosomes come into alignment on the metaphase plate, ACT is turned on and the APC is activated, causing the CDK_T balance curve to collapse and the CDK_A balance curve to move up (figure 11*d*). The cell must now transit toward the **G1** steady state. After cell division and ACT degradation, the CDK_T balance curve moves back into position 11*a*, the **G1** state is lost, and the cycle repeats itself.

5. DISCUSSION

We have explored the properties of several candidates for molecular regulation of the chromosome replication–segregation cycle in putatively primitive eukaryotic cells. Our goals are to uncover the minimal requirements for cell-cycle regulation that can handle several linear chromosomes, each with multiple replication origins, and to propose a simple but adequate regulatory mechanism, based on cyclin-dependent kinases, that could plausibly have evolved from bacterial precursors. Our approach is to hypothesize a simple molecular mechanism for cell-cycle control, to convert the mechanism into a set of non-linear ordinary differential equations by standard principles of biochemical kinetics, to reveal the behaviour of the mechanism by graphical techniques (phase-plane analysis, balance curves, bifurcation theory) and by numerical simulations, and to compare the properties of the model with the minimal characteristics of eukaryotic cell division. A rigorous mathematical approach reveals the strengths and limitations of different scenarios and often uncovers features that are not intuitively obvious. In this particular case, our models provide a fresh and informative perspective on some ambiguous ideas in the

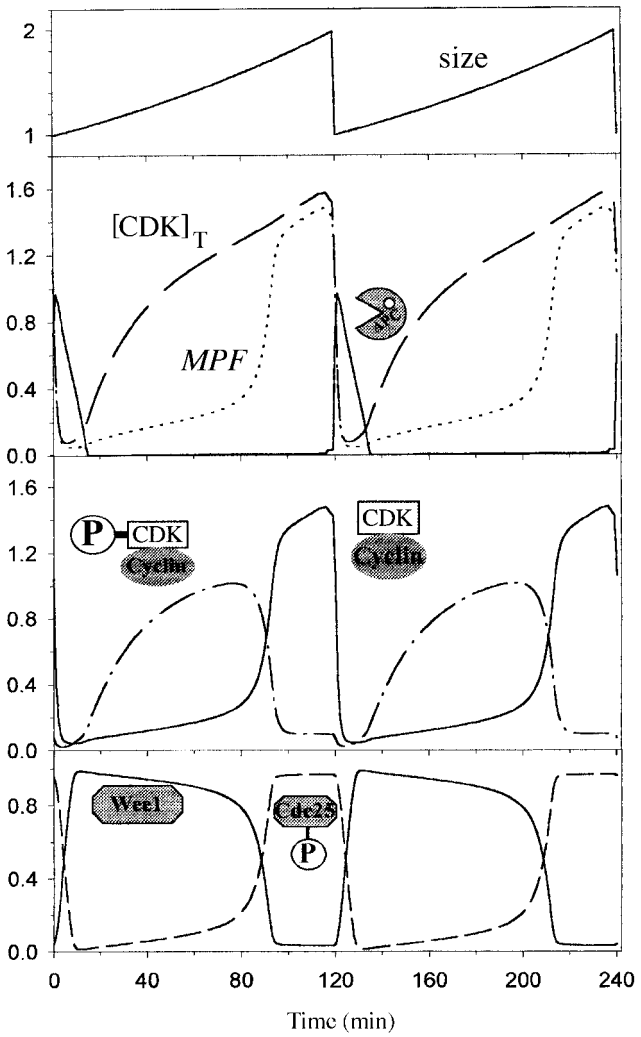

Figure 10. Growth-controlled division cycles in the primitive APC–CDK_T–CDK_A mechanism (figure 9). Solution of equations (1*b*)–(1*e*) and (4*a*)–(4*d*) with parameter values given in table 1, column 3.

cell-cycle literature, in particular: checkpoints, feedback, surveillance and commitment.

The central logic and core mechanism of the eukaryotic cell cycle is a strict alternation of once-only chromosome replication and sister chromatid separation, and the minimal mechanism to enforce this alternation consists of a CDK and the APC that labels cyclin subunits for proteolysis (Nasmyth 1995). The alternation is accomplished by transitions between two self-maintaining states: pre-replicative and post-replicative. In the pre-replicative state, cyclin degradation is rapid, CDK activity is low and RCs are assembled and waiting for the initiation signal. In the post-replicative state, cyclin molecules are more stable, CDK activity is high, RCs have initiated and are not able to reassemble. Granted that this picture is correct, the key issues are: (i) what interactions maintain the stability of the pre- and post-replicative states? (ii) what signals trigger transitions between the states? and (iii) why are these transitions irreversible?

In answer to the first question, Nasmyth's intuitive idea (Nasmyth 1995, 1996) is that CDK must inactivate APC. If CDK activity is high, then APC activity should be low, and therefore CDK is stable. On the other hand, if CDK activity is low, then APC activity is high and CDK activity is kept low by rapid cyclin degradation. Do antagonistic interactions between CDK and APC lead inescapably to alternative self-maintaining states? It is possible, and quite likely, that these antagonistic interactions balance each other at a unique stable state of 'mutual assured destruction'. By casting the idea in precise mathematical terms, we see that Nasmyth's self-maintaining states are actually steady-state solutions of the kinetic equations, that his idea of alternative, coexisting states corresponds to bistability (two stable steady states) in the mechanism, and that bistability is intimately connected to the phenomenon of hysteresis. Furthermore, the mathematical equations uncover the precise relationships among the parameters of the mechanism that ensure bistability and hysteresis.

If the pre-replicative state is a stable, self-maintaining, time-invariant state of the control system, what causes a cell to leave this state and enter the post-replicative state? Nasmyth suggests with good reasons that growth somehow triggers the pre-to-post transition and chromosome alignment the post-to-pre transition, although it is not entirely clear how these transitions happen. The mathematical model reveals that the self-maintaining states can be eliminated or destabilized by certain changes in parameter values that induce 'bifurcations' in the solution structure of the underlying differential equations. In the primitive cycle there were two fundamental transitions. Start occurs at a critical cell size when the pre-replicative steady state is lost by a saddle-node bifurcation: as the cell grows, the pre-replicative steady state merges with an unstable steady state and both disappear. As a result, the control system must switch over to the only remaining steady state, namely, the post-replicative state, for which the APC is inactivated, RCs are fired by increasing CDK activity, DNA is synthesized, a mitotic spindle is constructed and replicated chromosomes congress to the metaphase plate. Finish occurs when all chromosomes are under tension on the metaphase plate and the control system crosses a different saddle-node bifurcation that eliminates the post-replicative state. When this happens, the control system transits irreversibly to the pre-replicative state: the APC is activated, CDK is destroyed and RCs can reform.

In this scenario the important and primordial effect of CDK is to inactivate the APC. This signal generates hysteresis effects in cyclin degradation, and the one-way transitions around the hysteresis loop account for irreversibility of the Start and Finish events of the primitive eukaryotic cell cycle. Nonetheless, the hypothesis that CDK inactivates the APC is a controversial proposal and merits some discussion. Although in budding yeast CDK apparently inactivates the APC (Amon 1997), in frog extracts it is well documented that sufficiently high CDK activity turns on cyclin degradation (Felix *et al.* 1990). Both signals may be operative in modern eukaryotes: Minshull *et al.* (1994) provide evidence in frog eggs for a pathway, through MAP kinase, by which CDK may turn off the APC, as well as the signal established by Felix *et al.* (1990) for high CDK activity to turn on the APC. During early embryonic divisions, when the surveillance mechanisms for DNA replication and chromosome alignment are inoperative, the inactivating signal may be hard

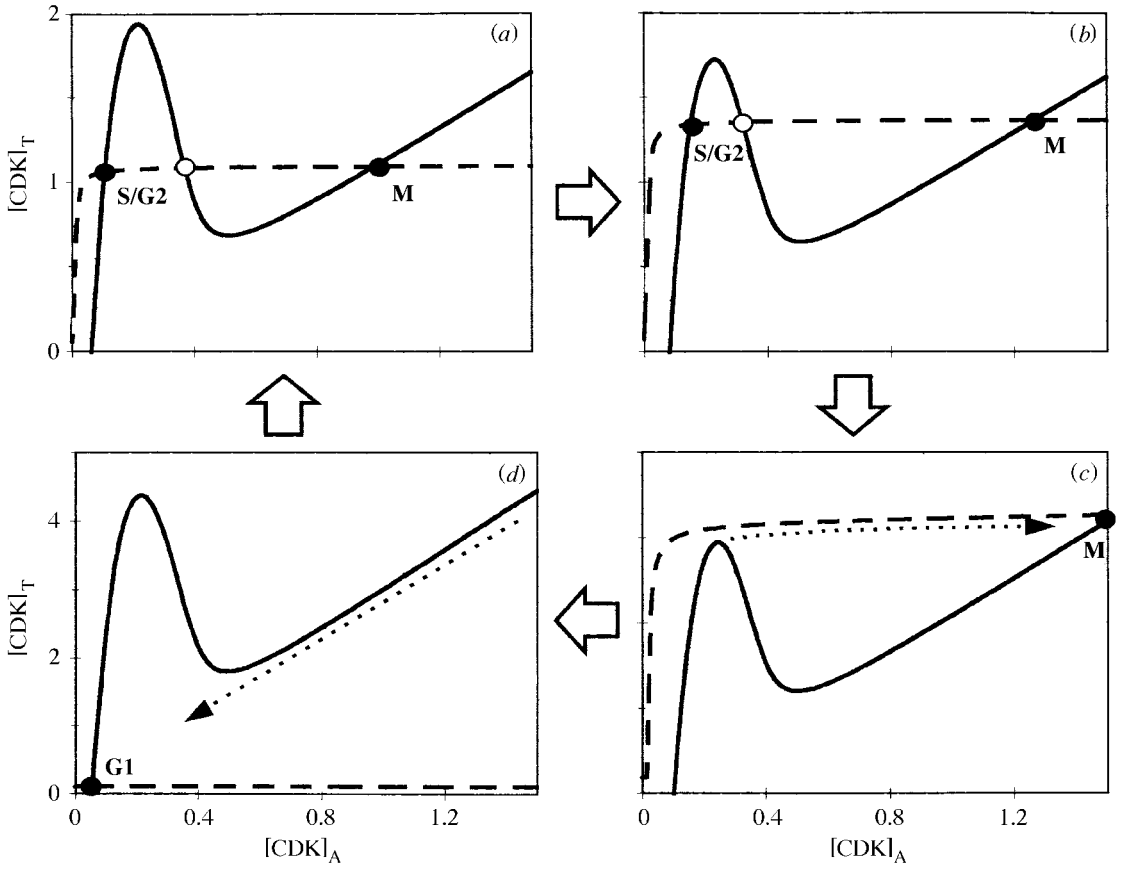

Figure 11. Phase-plane portraits for the CDK_A–CDK_T subsystem, equations (4*a*) and (4*b*). The CDK_A balance curve is defined by the solid line and the CDK_T balance curve defined by the dashed line. Parameter values given in table 1, column 3, with size, [ACT], and k_{ai} fixed at the values below. (*a*) Just after division, the cell is finishing S phase and halting at the G2-arrested state, as in modern fission yeast; size=1; [ACT]=0.05; k_{ai}=0.5. (*b*) As the cell grows, the **S**/**G2** state is about to be lost by a saddle-node bifurcation; size=1.4; [ACT]=1; k_{ai}=7. (*c*) The cell moves along the dotted trajectory to the metaphase checkpoint (**M**); size=1.8; [ACT]=3; k_{ai}=7. (*d*) After chromosome alignment, the **M** state is lost and the cell moves along the dotted trajectory to the pre-replicative **G1** steady state; size=2; [ACT]=3; k_{ai}=0.5.

to detect, whereas the activating signal plays a prominent role in driving embryonic cells out of M phase into the next S phase.

Many authors have pointed out that protein degradation probably plays an important role in making cell-cycle transitions irreversible (Deshaies 1997; King *et al.* 1996). But protein degradation itself does not account for irreversible transitions, because protein synthesis and degradation can always be held in a dynamical balance that is kinetically reversible. Neither can protein phosphorylation and dephosphorylation nor inhibitor binding release generate irreversible transitions in and of themselves. Rather it is the network of regulatory interactions between these components that creates irreversible, ratchet-like behaviour of the system. Hysteresis in our model is based on protein degradation, to be sure, but it is generated by proper choice of the parameters that describe the antagonistic effects of the APC and CDK. As we showed in the extended models, hysteresis can also occur in regulation based on CKI binding or CDK phosphorylation, provided the right sort of antagonistic interactions are present in the mechanism and the parameters are chosen appropriately.

Our scenario for primitive eukaryotic cell-cycle control contains only a few essential components: (i) antagonistic interactions between a cyclin–CDK dimer and the APC to generate two alternative steady states; (ii) a replication licensing factor to ensure that each origin of replication fires only once per division cycle; (iii) a tethering protein to bind sister chromatids together until the APC is activated; and (iv) signal transduction pathways to keep the APC inactive until DNA is fully replicated and chromosomes are perfectly aligned on the metaphase spindle. We suggest that other features of cell-cycle control in modern eukaryotes evolved as later variations on this central theme. CDK inhibitors, such as Rum1, Sic1 and $p27^{Kip1}$, might have evolved to give more flexible control over the G1/S transition. The enzymes that phosphorylate and dephosphorylate Cdc2 at Tyr-15 may have evolved to separate M phase from S phase. Clearly gene duplication has also played a major role in generating redundancy and combinatorial complexity in the modern machinery, with different CDK–cyclin dimers primarily responsible for specific events of the cycle. To construct intelligible, comprehensive models of modern controls, we propose to build outward from the central, ancient control mechanism, the traces of which remain in all eukaryotic cells.

Our research is supported by the National Science Foundations of the USA (MCB-9207160) and Hungary (T-022182), the Howard Hughes Medical Institute (75195-512302) and the

Wellcome Trust (037465/Z/92/Z/MJM/LC). Kathy Chen provided valuable assistance at several stages.

APPENDIX A

Our first job is to reduce the model in figure 6, described by equations (1*b*)–(1*e*) and (3*a*)–(3*c*), to just two ODEs. The first step is to ignore changes in size and [ACT], treating these components as parameters rather than variables. Next we assume that the [APC] is always at a pseudo-steady state level determined by the instantaneous value of [CDK](*t*). We calculate [APC] as a function of [CDK] by inverting equation (2*b*)

$$[\mathrm{APC}] = G\left(k_3' + k_3'' \cdot [\mathrm{ACT}],\ k_4' + k_4'' \cdot [\mathrm{CDK}],\ \mathcal{J}_3, \mathcal{J}_4\right), \tag{A1}$$

where $G(\cdot,\cdot,\cdot,\cdot)$ is the 'Goldbeter–Koshland' function (Goldbeter & Koshland 1981)

$$G(a,b,c,d) = \frac{2ad}{b-a+bc+ad+\sqrt{(b-a+bc+ad)^2-4(b-a)ad}}.$$

This leaves us with just the three equations (3*a*)–(3*c*).

Next we assume that CKI binding to CDK is fast and reversible, i.e., l_1 and l_2 are large with respect to k_6', k_6'', ν_2' and ν_2''. In this case the trimer will always be in equilibrium with its free subunits

$$\begin{aligned}[\mathrm{TRI}] &= L\cdot[\mathrm{CDK}]\cdot[\mathrm{CKI}],\\ &= L\cdot\left([\mathrm{CDK}]_\mathrm{T}-[\mathrm{TRI}]\right)\left([\mathrm{CKI}]_\mathrm{T}-[\mathrm{TRI}]\right),\end{aligned}$$

where $L = l_1/l_2$ = equilibrium binding constant. Solving this equation for [TRI], we find

$$\begin{aligned}[\mathrm{TRI}] &= \left(2L\cdot[\mathrm{CDK}]_\mathrm{T}\cdot[\mathrm{CKI}]_\mathrm{T}\right)/\Big(1+L\cdot[\mathrm{CDK}]_\mathrm{T}+L\cdot[\mathrm{CKI}]_\mathrm{T}\\ &\quad+\sqrt{\left(1+L\cdot[\mathrm{CDK}]_\mathrm{T}+L\cdot[\mathrm{CKI}]_\mathrm{T}\right)^2-4L^2\cdot[\mathrm{CDK}]_\mathrm{T}\cdot[\mathrm{CKI}]_\mathrm{T}}\Big)\end{aligned} \tag{A2}$$

Therefore, we can rewrite our kinetic equations as

$$\begin{aligned}\frac{\mathrm{d}[\mathrm{CDK}]_\mathrm{T}}{\mathrm{d}t} &= k_1\cdot\mathrm{size}\\ &\quad-\left[k_2'\left(1-[\mathrm{APC}]\right)+k_2''\cdot[\mathrm{APC}]\right]\cdot[\mathrm{CDK}]_\mathrm{T}-[\mathrm{TRI}]\big)\\ &\quad-\left[\nu_2'\left(1-[\mathrm{APC}]\right)+\nu_2''\cdot[\mathrm{APC}]\right]\cdot[\mathrm{TRI}],\end{aligned} \tag{A3a}$$

$$\frac{\mathrm{d}[\mathrm{CKI}]_\mathrm{T}}{\mathrm{d}t} = k_5 - \left[k_6'+k_6''\cdot\left([\mathrm{CDK}]_\mathrm{T}-[\mathrm{TRI}]\right)\right]\cdot[\mathrm{CKI}]_\mathrm{T} \tag{A3b}$$

with [TRI] = function of $[\mathrm{CDK}]_\mathrm{T}$ and $[\mathrm{CKI}]_\mathrm{T}$ given by equation (A2), and [APC] = function of $[\mathrm{CDK}]_\mathrm{T}$ and $[\mathrm{CKI}]_\mathrm{T}$ given by equation (A1), with the substitution $[\mathrm{CDK}] = [\mathrm{CDK}]_\mathrm{T}-[\mathrm{TRI}]$. Thus, equations (A3*a*,*b*) form a closed pair of ODEs in the composite variables $[\mathrm{CDK}]_\mathrm{T}$ and $[\mathrm{CKI}]_\mathrm{T}$.

Next we reduce the model in figure 9, described by equations (1*b*)–(1*e*) and (4*a*)–(4*d*), to just two ODEs. As before, we treat size and [ACT] as parameters rather than variables, and now we assume that APC, Weel and Cdc25 are all in pseudo-equilibrium between their active and inactive forms. [APC](*t*) is given by equation (A1), and

$$[\mathrm{Wee1}] = G\left(k_\mathrm{wr},\ k_\mathrm{w}[\mathrm{CDK}]_\mathrm{A},\ \mathcal{J}_\mathrm{wr},\ \mathcal{J}_\mathrm{w}\right) \tag{A4a}$$

$$[\mathrm{Cdc25}] = G\left(k_{25}[\mathrm{CDK}]_\mathrm{A}, k_{25\mathrm{r}},\ \mathcal{J}_{25},\ \mathcal{J}_{25\mathrm{r}}\right) \tag{A4b}$$

With these simplifications, equations (4*a*) and (4*b*) form a closed pair of ODEs in the variables $[\mathrm{CDK}]_\mathrm{T}(t)$ and $[\mathrm{CDK}]_\mathrm{A}(t)$, as desired.

REFERENCES

Amon, A. 1997 Regulation of B-type cyclin proteolysis by Cdc28-associated kinases in budding yeast. *EMBO J.* **16**, 2693–2702.

Borgne, A. & Meijer, L. 1996 Sequential dephosphorylation of $p34^{cdc2}$ on Thr-14 and Tyr-15 at the prophase/metaphase transition. *J. Biol. Chem.* **271**, 27 847–27 854.

Correa-Bordes, J. & Nurse, P. 1995 $p25^{rum1}$ orders S phase and mitosis by acting as an inhibitor of the $p34^{cdc2}$ mitotic kinase. *Cell* **83**, 1001–1009.

de Duve, C. 1995 *Vital dust*. New York: Basic Books.

Deshaies, R. 1997 Phosphorylation and proteolysis: partners in the regulation of the cell division in budding yeast. *Curr. Opin. Gen. Dev.* **7**, 7–16.

Donachie, W. 1993 The cell cycle of *Escherichia coli*. *A. Rev. Microbiol.* **47**, 199–230.

Felix, M.-A., Labbe, J.-C., Doree, M., Hunt, T. & Karsenti, E. 1990 Triggering of cyclin degradation in interphase extracts of amphibian eggs by cdc2 kinase. *Nature* **346**, 379–382.

Fisher, D. L. & Nurse, P. 1996 A single fission yeast mitotic cyclin B $p34^{cdc2}$ kinase promotes S-phase and mitosis in the absence of G1 cyclins. *EMBO J.* **15**, 850–860.

Futcher, B. 1996 Cyclins and the wiring of the yeast cell cycle. *Yeast* **12**, 1635–1646.

Goldbeter, A. & Koshland, D. E., Jr. 1981 An amplified sensitivity arising from covalent modification in biological systems. *Proc. Natn. Acad. Sci. USA* **78**, 6840–6844.

Gould, K. L. & Nurse, P. 1989 Tyrosine phosphorylation of the fission yeast cdc2 protein kinase regulates entry into mitosis. *Nature* **342**, 39–45.

Jallepalli, P. V. & Kelly, T. J. 1996 Rum1 and Cdc18 link inhibition of cyclin-dependent kinase to the initiation of DNA replication in Schizosaccharomyces pombe. *Genes Devel.* **10**, 541–552.

King, R. W., Deshaies, R. J., Peters, J. M. & Kirschner, M. W. 1996 How proteolysis drives the cell cycle. *Science* **274**, 1652–1658.

Kirchner, T. B. 1990 *Time-zero: the integrated modeling environment, version 2*. Fort Collins, CO: Quaternary Software.

Lundgren, K., Walworth, N., Booher, R., Dembski, M., Kirschner, M. & Beach, D. 1991 mik1 and weel cooperate in the inhibitory tyrosine phosphorylation of cdc2. *Cell* **64**, 1111–1122.

Martin-Castellanos, C., Labib, K. & Moreno, S. 1996 B-type cyclins regulate G1 progression in fission yeast in opposition to the $p25^{rum1}$ cdk inhibitor. *EMBO J.* **15**, 839–849.

Minshull, J., Sun, H., Tonks, N. K. & Murray, A. W. 1994 A MAP kinase-dependent spindle assembly checkpoint in Xenopus egg extracts. *Cell* **79**, 475–486.

Moreno, S. & Nurse, P. 1994 Regulation of progression through the G1 phase of the cell cycle by the *rum1*$^{+}$ gene. *Nature* **367**, 236–242.

Nasmyth, K. 1995 Evolution of the cell cycle. *Phil. Trans. R. Soc. Lond.* B **349**, 271–281.

Nasmyth, K. 1996 At the heart of the budding yeast cell cycle. *Trends Genet.* **12**, 405–412.

Nasmyth, K. & Hunt, T. 1993 Dams and sluices. *Nature* **366**, 634–635.

Nurse, P. 1994 Ordering S-phase and M-phase in the cell cycle. *Cell* **79**, 547–550.

Russell, P. & Nurse, P. 1987 Negative regulation of mitosis by *wee1*$^{+}$, a gene encoding a protein kinase homolog. *Cell* **49**, 559–567.

Schwob, E., Bohm, T., Mendenhall, M. D. & Nasmyth, K. 1994 The B-type cyclin kinase inhibitor p40^{Sic1} controls the G1 to S transition in S. cerevisiae. *Cell* **79**, 233–244.

Wheeler, R. T. & Shapiro, L. 1997 Bacterial chromosome separation: is there a mitotic apparatus? *Cell* **88**, 577–579.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES