The molecular machinery of cell cycle control is known in more detail for budding yeast, *Saccharomyces cerevisiae*, than for any other eukaryotic organism. In recent years, many elegant experiments on budding yeast have dissected the roles of cyclin molecules (Cln1–3 and Clb1–6) in coordinating the events of DNA synthesis, bud emergence, spindle formation, nuclear division, and cell separation. These experimental clues suggest a mechanism for the principal molecular interactions controlling cyclin synthesis and degradation. Using standard techniques of biochemical kinetics, we convert the mechanism into a set of differential equations, which describe the time courses of three major classes of cyclin-dependent kinase activities. Model in hand, we examine the molecular events controlling “Start” (the commitment step to a new round of chromosome replication, bud formation, and mitosis) and “Finish” (the transition from metaphase to anaphase, when sister chromatids are pulled apart and the bud separates from the mother cell) in wild-type cells and 50 mutants. The model accounts for many details of the physiology, biochemistry, and genetics of cell cycle control in budding yeast.

INTRODUCTION

To reproduce itself, a cell must duplicate all its components and separate them, more or less evenly, to two daughter cells, so that each daughter has the information and machinery necessary to repeat the process (Murray and Hunt, 1993; Alberts *et al.*, 1994, chap. 17). In general, eukaryotic cells replicate and partition their genetic material in two distinct, coordinated processes. During S phase, the DNA molecule in each chromosome is precisely replicated to form two identical sister chromatids that are held together by cohesins (tethering proteins). During M phase, the cell builds a mitotic spindle, condenses its replicated chromosomes, aligns them on the midplane of the spindle, and then, at anaphase, removes the cohesins and separates sister chromatids to opposite poles of the spindle (Biggins and Murray, 1998; Zachariae and Nasmyth, 1999). Shortly after anaphase, the cell divides into two daughter cells, each one containing a complete set of chromosomes. S and M phases are usually separated temporally by gaps (G1 and G2 phases).

It is crucial that each DNA molecule be replicated once and only once per cycle in eukaryotes. Were this not the case, then each chromosome would contain multiple sister chromatids, and segregation of the correct balance of DNA molecules to the spindle poles would be a difficult affair. This requirement is imposed by a set of proteins called licensing factors (Mcm2–7 and Cdc6). In the gap between the end of mitosis and the beginning of S phase, licensing factors bind to and prime the origins of replication. At the G1/S boundary, several cyclin-dependent protein kinases (CDKs) become active and initiate replication at licensed origins. In the process, the CDKs apparently incapacitate the license at each origin that fires. As long as CDKs remain active, throughout S, G2, and M, licensing factors remain incapacitated, and rereplication is impossible (Botchan, 1996; Wurray and Nurse, 1996; Leatherwood, 1998).

It is also crucial that the cell does not commence anaphase (sister chromatid separation) until DNA replication is complete and each pair of sister chromatids is properly aligned on the metaphase plate. Completion of DNA synthesis is usually a requirement for entry into M phase, whereas chromosome alignment is required for activation of the anaphase-promoting complex (APC) that initiates degradation of an inhibitor of sister chromatid separation (Amon, 1999; Nasmyth, 1999). At anaphase, the APC also mediates proteolysis of mitotic cyclins, thereby destroying CDK activities and allowing licensing factors to accumulate and origins to be primed for replication.

Abbreviations: APC, anaphase-promoting complex; CDK, cyclin-dependent kinase.
CDK activity is low, and origins are licensed; and the S/M state, in which APC is shut off, CDK activity is high, and origins are fired and incapable of firing again. The G1 state is self-reinforcing because APC destroys S-phase and M-phase cyclins. The S/M state is self-reinforcing, suggested Nasmyth (1996), because CDKs inactivate the APC by phosphorylating some of its components. Although Nasmyth's proposal contradicted conventional wisdom that B-type cyclins activate the APC, recent experiments in budding yeast confirmed his hypothesis (Amon, 1997; Zachariae et al., 1998; Jaspersen et al., 1999). In Nasmyth's view, the budding yeast division cycle is an alternating sequence of “Start” transitions from G1 to S/M and “Finish” transitions from S/M back to G1. Our goals are to show how these two stable cell cycle states (G1 and S/M) arise from the underlying molecular machinery and to reveal the dynamical nature of the transitions (Start and Finish) between them.

To this end, we summarize experimental results from many sources to construct a consensus picture of the molecular signals controlling cell cycle events in budding yeast. The present picture is built on a simpler model of cell cycle controls in budding yeast (Tyson et al., 1995) and on a mathematical description of Nasmyth's alternating-states hypothesis (Novak et al., 1998). (Those models, along with earlier studies and reviews [Novak and Tyson, 1993, 1995, 1997; Tyson et al., 1996, 1997], should be consulted for an introduction to our theoretical methods, strategies, and tools.)

After casting the mechanism into a set of kinetic equations, we study the dynamical properties of the control system by numerical simulations. Experimental data are used to estimate the crucial kinetic parameters in the model. Then the model is compared with the phenotypes of mutant cells in which various components of the control system are knocked out or overexpressed.

The model, which accounts for most of the distinctive characteristics of the budding yeast cell cycle, is valuable in bringing together a huge amount of hard-won experimental data in a convenient mathematical repository. As experimentalists think about yet unknown details around the “edges” of the consensus picture, the model can be used to explore the properties of hypothetical mechanisms. As new advances are made, the model can be extended to give an ever more comprehensive picture of cell cycle controls in budding yeast.

A CONSENSUS PICTURE OF CELL CYCLE CONTROLS IN BUDDING YEAST

Cyclin-dependent Kinase Activities

The roles of these cyclins overlap. All the single mutants are viable and nearly normal, except cln3 mutants, which execute Start at about twice the size of wild-type cells (Dirick et al., 1995). (Notation, for example, wild-type allele = CLN3, recessive mutant allele = cln3, dominant mutant allele = CLN3\(^P\), and gene product = Cln3.) Although the triple-cln mutant, cln1 cln2 cln3, is lethal (Richardson et al., 1989), the cln1 cln2 double mutant is large and viable and able to bud. Apparently any one of the Clns can do the essential jobs of the other two, if the cell is large enough. The double mutant clb3 clb4 is normal (Richardson et al., 1992; Schwob and Nasmyth, 1993), so their roles can be played by other Clbs. Because a clb5 clb6 mutant cell carries out DNA synthesis (although with some delay), whereas a cell with all six CLB genes deleted (clb1–6) does not, Clb1–4 can trigger DNA synthesis in the absence of Clb5–6 (Schwob et al., 1994). Only the Clb1–2 pair is special in the sense that at least one of them is necessary for completing mitosis (Surana et al., 1991). Because of these redundancies, it will be sufficient to consider the interaction of Cdc28 with only four classes of cyclin-dependent kinase (CDK): Clb1, Clb2, Clb5, and Clb6.

**Regulation of Cyclin-dependent Kinase Activities**

Cyclin/Cdc28 activities come and go in a characteristic sequence during the budding yeast cell cycle. Regulation is achieved mainly through the synthesis and degradation of cyclin components and of the Clb-dependent kinase inhibitor Sic1. Cln3 is present at low and nearly constant levels throughout the cell cycle; Cln2 and its associated kinase activity are maximal at Start (Wittenberg et al., 1990; Tyers et al., 1993). The pattern of Clb5 is similar to that of Cln2 (Schwob and Nasmyth, 1993), whereas Clb2 and its associated kinase activity peak ~10 min before anaphase (Surana et al., 1991). Furthermore, Sic1 is present in high concentration in G1 and decreases to low levels after Start (Donovan et al., 1994; Schwob et al., 1994).

In many eukaryotic organisms, Cdk activity is also controlled by inhibitory phosphorylation at a conserved tyrosine in the N terminus of its kinase subunit. Although budding yeast has this tyrosine residue (Tyr-19 in Cdc28) and the kinase and phosphatase (Swel and Mih1) that regulate phosphorylation of this site, tyrosine phosphorylation does not play an important role in regulating Cdk activities during normal vegetative growth (Amon et al., 1992; Sorger and Murray, 1992).

**Transcription Factors**

Expression of the CLN2 gene (Koch et al., 1996) is controlled by the transcription factor SBF (Swi4/Swi6) (Nasmyth and Dirick, 1991), which can be activated by all three Cln-associated as well as Clb5-associated kinases (Cross and Tinkelenberg, 1991; Schwob and Nasmyth, 1993) and inactivated by Clb2-associated kinase (Amon et al., 1993). The transcription factor MBF (Mbp1/Swi6) for the CLB5 gene is activated, like SBF, by the Cln- and Clb5-associated kinases (Koch et al., 1993; Schwob and Nasmyth, 1993) but inactivated in G2 by some yet unknown mechanism other than Clb2/Cdc28 kinase (Amon et al., 1993). Transcription of CLB2 is autocatalytic, because Clb2/Cdc28 activates its own transcription factor (Mcml1/SFF) (Amon et al., 1993; Maher et al., 1995). Finally, SIC1 transcription, regulated by Swi5, peaks at anaphase (Knapp et al., 1996), Swi5 is inactivated by Clb2-dependent phosphorylation, which prevents it from entering the nucleus (Nasmyth et al., 1990). It is activated, on the other hand, by a phosphatase, Cdc14, which is in turn activated indirectly by Cdc20 (Visintin et al., 1998; Jasperson et al., 1999), an ancillary protein for the APC-dependent degradation machinery to be described in the next section.

**Proteolysis**

All cyclins are degraded by proteasomes, which destroy proteins that have been tagged by ubiquitin. Ubiquitin tagging is carried out by complex enzymatic machinery that activates ubiquitin molecules, recognizes appropriate proteins to be destroyed, and transfers activated ubiquitin to these doomed proteins (King et al., 1996; Peters, 1998; Zachariae and Nasmyth, 1999). For cyclins, two ubiquitin-conjugating protein complexes are known: the APC and the SCF.

The APC is composed of a dozen proteins, including Cdc16, -23, and -27 (Zachariae et al., 1996). The SCF is a complex of Skp1, Cdc34, Cdc53, and an F box-containing protein, like Cdc4 or Grr1 (Jackson, 1996; Krek, 1998). The APC is responsible for destruction of Clb2 (Ingrigier et al., 1995), Clb5 (partly) (Ingrigier and Nasmyth, 1997), Cdc20 (Shirayama et al., 1998), and Pds1 (Yamamoto et al., 1996), a protein that promotes sister chromatid cohesion until anaphase. The SCF is responsible for destruction of Cln2 (Deshaies et al., 1995; Willems et al., 1996), Cln3 (Yaglom et al., 1995), and Sic1 (Feldman et al., 1997). Because Clb5 is more stable in skp1 mutants than in wild-type cells (Bai et al., 1996), Clb5 may be partly degraded by SCF.

Both APC and SCF require ancillary proteins, whose job is to recognize appropriate protein substrates and present them to the ubiquitin-conjugating machinery. For example, Cdc4 presents Sic1, and Grr1 presents Cln2 and Cln3 to the APC (Barral et al., 1995; Feldman et al., 1997; Li and Johnston, 1997; Skowrya et al., 1997). In like manner, Hct1 (also called Cdh1) presents Clb2, and Cdc20 presents Pds1 and Clb5 to the APC (Schwab et al., 1997; Visintin et al., 1997).

The SCF seems to be active at all times in the cell cycle. Degradation of its target proteins is controlled by the phosphorylation state of the target (Willems et al., 1996). For example, in G1 phase, Sic1 is unphosphorylated and stable, even though the SCF is active. When Cln2-associated kinase activity rises at Start, Sic1 is phosphorylated, and Sic1P is rapidly presented by Cdc4 to the SCF for ubiquitination and subsequent proteolysis (Verma et al., 1997). Likewise, Cln2 must be phosphorylated before it is recognized by Grr1 (Barral et al., 1995; Li and Johnston, 1997).

APC-dependent proteolysis, on the other hand, is controlled by phosphorylation of the ubiquitination machinery itself, rather than the target proteins. There is evidence in clam oocyte extract (Lahav-Baratz et al., 1995; Sudakin et al., 1995), Xenopus egg extract (Felix et al., 1990; Peters et al., 1996), and mammalian cells (Kotani et al., 1998) that the APC core is activated by phosphorylation and that CDKs may be involved in this activation indirectly via a polo-like kinase (whose homologue in budding yeast is Cdc5) (Descombes and Nigg, 1998; Kotani et al., 1998). But such effects are not
yet well established in budding yeast, so we do not try to model them in the present paper.

Rather, we focus on the ancillary proteins, which seem to exist in active and inactive forms. For the Hct1-dependent degradation machinery, Amon (1997) showed that, in vivo, cyclin proteolysis can be turned off by ectopic expression of Cln2 (and back on again by expression of Sic1). Recent experiments (Zachariae et al., 1998; Jaspersen et al., 1999) show that, in vitro, CDKs can phosphorylate Hct1, rendering it incapable of interaction with the APC core. Together, these findings confirm Nasmuth’s (1996) hypothesis that CDK activity and Cln2 proteolysis are antagonistic events: CDK inactivates APC by phosphorylation, whereas APC destroys CDK activity by degradation of cyclin components. The phosphatase that opposes CDK (and thereby activates Hct1) is Cdc14. Notice that the kinase-phosphatase pair, CDK-Cdc14, regulates not only the activity of Hct1 but also the synthesis (Swi5) and degradation (phosphorylation state) of Sic1 (Visintin et al., 1998; Jaspersen et al., 1999).

The Cdc20-dependent degradation machinery is more complicated still. As cells exit from mitosis, it is responsible for degradation of Pds1, which restrains the dissociation of cohesions by binding to and inhibiting Esp1, a protein essential for sister chromatid separation (Ciosk et al., 1998). Cdc20 is also responsible for loss of an inhibitor of Cdc14 (Novak et al., 1999), leading to activation of Hct1 and Swi5 (Visintin et al., 1997; Lim et al., 1998; Shirayama et al., 1998). The RENT complex, recently identified by Shou et al. (1999) and Visintin et al. (1999), may inhibit Cdc14 by reversible sequestration.

Mitotic Checkpoint

It has been shown (Hwang et al., 1998) that Cdc20 is a likely target for signals from unaligned chromosomes, unreplicated DNA, and damaged DNA, all of which keep Cdc20 in its inactive form. Unreplicated DNA, in addition to keeping Cdc20 inactive, seems to impinge on the APC-activating pathway as well (Hwang et al., 1998; Kotani et al., 1998). The end result is that, when DNA replication is complete and all chromosomes are in tension on the metaphase plate, APC is phosphorylated, and Cdc20 is activated, leading to degradation of Pds1 (hence, dissolution of cohesions) and to activation of Hct1 (hence, destruction of Cln2).

KINETIC MODEL

From these facts we construct a consensus picture (Figure 2) of cell cycle controls in budding yeast. Using standard principles of biochemical kinetics, we cast the molecular mechanism into a set of nine, nonlinear, ordinary differential equations governing the temporal changes of cyclins and their regulatory proteins, plus four auxiliary differential equations describing cell growth and CDK-induced events (activation of DNA replication origins, bud emergence, and spindle assembly), plus three algebraic equations determining the activities of SBF, Mcm1, and Swi5 transcription factors (Table 1). About 50 parameters enter into the definitions of these equations, and their values (for wild-type cells) are specified in Table 2. Appendix A, describes how these parameter values were estimated.

The model involves a number of specific kinetic assumptions that are introduced either to simplify the model or to explain specific characteristics of wild-type and mutant cell cycles, as we shall describe. Here we list these assumptions for easy reference.

1) Cell size is coupled to the CDK engine by assuming that the synthesis of each cyclin is proportional to mass, a variable representing overall cell “size.” (For simplicity, we assume that mass increases exponentially.) We have in mind that cyclins are synthesized in the cytoplasm, where ribosome number increases throughout the cycle, and accumulate in the nucleus, whose volume does not change much. Thus, the concentrations of cyclins in the nucleus, [Cln2], [Cln2], etc., tend to increase as mass increases. Although many experiments demonstrate that budding yeast division cycles are controlled by cell size (Carter, 1981) through effects on CDK activities (Baroni et al., 1994; Tokiwa et al., 1994; Polymenis and Schmidt, 1997), the molecular mechanism whereby cells measure their nucleocytoplasmic ratio has not yet been elucidated. Our hypothesis, although speculative, is the simplest way to couple growth and division.

2) Transcription of CLB5 is controlled by MFB, but the signal that inactivates MFB is unknown at present, so our picture is incomplete. Because MFB and SBF turn on and off at similar times in the cell cycle, under most conditions (Koch and Nasmuth, 1994; Cho et al., 1998; Spellman et al., 1998), we assume for the time being that [MBF] = [SBF]. When MFB regulation is better understood, this part of the model can be easily improved.

3) The activation and inactivation of transcription factors (SBF, Mcm1, and Swi5) are modeled as Goldbeter–Koshland (1981) ultrasensitive switches, as described in Appendix B. We could have represented the sigmoidal behavior of these switches by simpler functions, but the Goldbeter–Koshland function is particularly suitable for the phosphorylation–dephosphorylation reactions typical of cell cycle controls.

4) Bck2 cooperates with Cln3 in activating SBF at Start.

5) At high dosage, the activity of Cln3-dependent kinase plateaus.

6) We assume first-order kinetics for degradation of Cln2 and Cln5 by SBF. We are aware that SBF-catalyzed ubiquitination depends on prior phosphorylation of its substrates, most likely by CDKs themselves. Nonetheless, we choose simple first-order kinetics for cyclin degradation in the present model. Later versions can be improved in this regard, if necessary.

7) To describe how CDK activities drive DNA synthesis, bud emergence, and mitotic events, we introduce three “target” variables: OR1, BUD, and SPN. These targets are phosphorylated by CDKs, and the associated physiological events occur when their cumulative level of phosphorylation reaches a threshold (1 in each case).

8) In the present model, Cln2-dependent kinase stimulates the synthesis of Cdc20 (Prinz et al., 1998) and indirectly activates it by driving [SPN] toward 1. The function of [SPN] is to provide a time delay between the appearance of Cln2 and the activation of Cdc20. To model the effect of nocodazole, we block the activation of Cdc20.

9) Metaphase checkpoint controls are the most primitive part of the model. We assume that Cdc20 is kept inactive until all chromosomes are properly aligned on the mitotic spindle ([SPN] = 1). After it is activated, Cdc20 helps activate Hct1 and Swi5, presumably by degrading some inhibitor of Cdc14 (Novak et al., 1999). In a later model, we will track the kinetics of Cdc14 and its sequestration in RENT complexes, but for now we simply allow Cdc20 to activate Hct1 and Swi5 directly.

10) Cdc20 degrades Cln2, to some extent. Intuitively, the diagram in Figure 2 seems appealing, but the hand-waving arguments used to justify it are not entirely convincing. Exactly what experiments can this model account for and what does it leave unexplained? The only way to address this question is to study the mathematical model (Table 1) thoroughly and rigorously, comparing its solution with the physiology of real cells. Where there is a correspondence between the model and reality, we can have some confidence that our understanding of the budding yeast cell cycle is adequate. Where the model fails will point to aspects of the control system that need further study.
RESULTS

Wild-Type Cell Cycle

Figure 3 presents a numerical solution of the kinetic equations (Table 1), using a basal set of rate constants (Table 2), suitable for wild-type division cycles (see Appendix A for a justification of the parameter values). In this case, the mass-doubling time ($T_d$) of the culture is 120 min (specific growth rate $\mu = 0.693 / T_d = 0.005776 \text{ min}^{-1}$). Because division is asymmetrical, we must distinguish between mother and daughter cells. The smaller daughter cells (Table 3, line 1) have a longer cycle time (146 min from birth to division), because they require more time to grow to the critical size when SBF turns on. (In our model, SBF is turned on abruptly by Cln3 when mass $= 1.1$; see Appendix B.) Mother cells have a cycle time of 100 min, because they turn on SBF more quickly after division. On the other hand, the budded phases of mother and daughter cells are quite similar (~60 min).

Start and Finish. Two major transitions characterize wild-type cell cycles (Figure 3). At Start, a series of events is initiated in rapid succession: SBF turns on, Cln2 and Clb5 levels rise, Sic1 disappears, Hct1 turns off, and DNA synthesis and bud emergence commence. Shortly thereafter, Clb2 level rises and a spindle starts to form. At Finish, Cdc20 and Hct1 turn on, Clb2 is destroyed, and Sic1 makes a comeback. In simulations of various mutant strains, we will see how these chains of events can be dissociated.

Modeling the Budding Yeast Cell Cycle


**Table 1. Mathematical model of the budding yeast cell cycle**

Equations governing cyclin-dependent kinases

\[
\frac{d}{dt}(\text{Cln}2) = (k_{c2} + k_{c2}^\text{SBF}) \cdot \text{mass} - k_{d2} \cdot (\text{Cln}2)
\]

\[
\frac{d}{dt}(\text{Clb}2) = (k_{c2} + k_{c2}^\text{Mcm}) \cdot \text{mass} - V_{A,b2} \cdot (\text{Clb}2)_p \cdot V_{d2} = k_{d2} \cdot (\text{Hct}1) - k_{c2} + k_{d2} \cdot (\text{Cdc}20)
\]

\[
\frac{d}{dt}(\text{Clb}5) = (k_{c5} + k_{c5}^\text{MBF}) \cdot \text{mass} - V_{A,b5} \cdot (\text{Clb}5)_p \cdot V_{d5} = k_{d5} + k_{d5} \cdot (\text{Cdc}20)
\]

\[
[D_\text{Cdc}20] = [\text{Bck2}] \cdot \text{mass}, \quad [\text{Cln}3]^* = [\text{Cln}3]_{\text{mass}} \cdot \frac{\text{mass}}{D_{\text{tot}} + D_{\text{Cdc}20} \cdot \text{mass}}
\]

\[
[D_{\text{Clb}2}] = [\text{Clb}2] + [\text{Clb}2/\text{Sic}1], \quad [D_{\text{Clb}5}] = [\text{Clb}5] + [\text{Clb}5/\text{Sic}1]
\]

\[
[D_{\text{Sic}1}] = [\text{Sic}1] + [\text{Clb}2/\text{Sic}1] + [\text{Clb}5/\text{Sic}1]
\]

Equations governing the inhibitor of Clb-dependent kinases

\[
\frac{d}{dt}(\text{Clb2/Clb1}) = k_{c1} \cdot [\text{Sic}1] + \frac{V_{d1}}{[\text{Clb2}]} \cdot [\text{Clb2/Clb1}]
\]

\[
\frac{d}{dt}(\text{Clb5/Clb1}) = k_{c5} \cdot [\text{Sic}1] + \frac{V_{d5}}{[\text{Clb5}]} \cdot [\text{Clb5/Clb1}]
\]

\[
V_{d1} = k_{d1} \cdot [\text{Clb2/Clb1}] + k_{d1} \cdot [\text{Clb5/Clb1}]
\]

Equations governing the Clb degradation machinery

\[
\frac{d}{dt}(\text{Cdc}20) = (k_{c2} + k_{c2}^\text{SBF}) \cdot \text{mass} - k_{d2} \cdot (\text{Cdc}20)
\]

\[
V_{d2} = \begin{cases} k_{d2}, & \text{for END_M \times 12 \text{ min} < t < \text{START_S}} \\ k_{d2}, & \text{for START_S < t < END_M} \end{cases}
\]

\[
\frac{d}{dt}(\text{Hct1}) = \frac{k_{c5} + k_{c5}^\text{MBF} \cdot (\text{Hct}1) - (\text{Hct}1)}{[\text{Hct1}] + k_{c5} + k_{d5} \cdot (\text{Cdc}20)} - \frac{V_{d2} \cdot (\text{Hct}1)}{[\text{Hct1}] + k_{c5} + k_{d5} \cdot (\text{Cdc}20)}
\]

Equations for growth, DNA synthesis, budding and spindle formation

\[
\frac{d}{dt}(\text{mass}) = \mu \cdot \text{mass}, \quad \frac{d}{dt}(\text{ORI}) = k_{e,\text{ori}} \cdot [\text{Clb5}] + k_{d,\text{ori}} \cdot [\text{Clb2}] - k_{d,\text{ori}} \cdot [\text{ORI}]
\]

\[
\frac{d}{dt}(\text{BUD}) = k_{e,bud} \cdot [\text{Clb2}] + k_{e,bud} \cdot [\text{Clb5}] + k_{d,bud} \cdot (\text{BUD}) - k_{d,\text{bud}} \cdot (\text{ORI}) - k_{d,\text{bud}} \cdot (\text{SPN})\]

\[
\frac{d}{dt}(\text{SPN}) = k_{e,\text{spn}} \cdot [\text{Bck2}] - k_{d,\text{spn}} \cdot (\text{SPN})
\]

Equations governing transcription factors

\[
[D_{\text{SBF}}] = [\text{MBF}] = G.V_{d1} \cdot k_{d1} \cdot [\text{Clb2}] + k_{d1} \cdot [\text{Clb2}] \cdot [\text{Clb2}] + k_{d1} \cdot [\text{Clb5}] + k_{d1} \cdot [\text{Clb5}]
\]

\[
[D_{\text{Mcm1}}] = G.V_{d1} \cdot k_{d2} \cdot [\text{Clb2}] \cdot [\text{Clb2}] \cdot [\text{Clb2}] \cdot [\text{Clb2}] \cdot [\text{Clb2}]
\]

Symbols, \( V \) = rate functions, \( k \) = rate constant, \( f \) = Michaelis constant. Subscripts, \( s \) = synthesis, \( d \) = degradation, \( a \) = activation, \( i \) = inactivation, as = association, di = dissociation, T = total.

\( k_{d,\text{ori}} \) refers to SCF-mediated degradation of Clb5, and \( k_{d,\text{ori}} \) refers to its APC-mediated degradation.

\( d_{\text{dC20}} \) refers to the degradation of unphosphorylated Sic1. \( d_{\text{dClb}} \) refers to the rate of phosphorylation of Sic1 by cyclin-dependent kinases, assuming that phosphorylated Sic1 is rapidly proteolyzed, releasing active Cdc28/Cib dimers.

\( \text{START_S} \) is the time when [ORI] = 1, and END_M when [SPN] = 1. For \( \text{START_S} < t < \text{END_M} \), there is a strong inhibitory signal on Cdc20 (\( V_{d2} = 10 \)). Once the cell reaches metaphase (\( t = \text{END_M} \)), \( V_{d2} \) drops linearly from 10 to 0.1 over 12 min. Thereafter, \( V_{d2} = 0.1 \) until the start of the next S phase.

\( \text{Cell division occurs, we assume, when [Clb2] drops below a threshold (0.3). At this time, we divide mass between mother and daughter cells as follows: mass of daughter cell at birth} = f \times \text{(mass at cell separation)}, \text{mass of mother cell at birth} = (1 - f) \times \text{(mass at cell separation)}, \text{with} \ f = e^{-10D} \text{where} \ D = (1.026/\mu) \times 32 \text{is the observed daughter cell cycle time} \ (\text{Lord and Wheelis, 1980}). \text{(See text for a discussion of the rule for asymmetric division.) At division, we also reset BUD and SPN to zero; however, we reset ORI to zero (for licensing factor to reappear after mitosis) only when [Clb2] + [Clb5] drop below a different threshold (0.2).}

\( G(V_{d1}, V_{d2}, I_{d1}, I_{d2}) \) is the Goldbeter–Koshland function described in Appendix B.

eter values, Cln3 and Bck2 contribute about equally to the activation of SBF.

Finish (exit from mitosis) is triggered by activation of Cdc20. After cells pass Start and begin to synthesize Clb2, Cdc20 accumulates at an increasing rate. But it remains inactive because of inhibitory signals from unreplicated DNA and unaligned chromosomes. When those events are completed and the inhibitory signals disappear, Cdc20 is
found a point of no return shortly before the onset of S phase. (i.e., assuming a 10-min delay for signal transduction). We activated. Active Cdc20 turns on Hct1 by overwhelming the inhibition exerted on Hct1 by Clb2 (presumably by degrading some inhibitor of Cdc14). When Hct1 turns on, Clb2 is degraded, and the control system switches to the G1 state, in which the enemies of Clbs (Hct1 and Sic1) are active.

Response to α-Factor. When an asynchronously population of budding yeast cells is exposed to α-factor (mating pheromone), pre-Start cells are blocked in G1, but post-Start cells finish DNA replication, divide, and stop in the next G1 phase. α-factor initiates a signal transduction pathway that ultimately eliminates all Cln-dependent kinase activities (Chang and Herskowitz, 1990; Peter and Herskowitz, 1994; Wittenberg and Reed, 1996). To simulate α-factor treatment, we set the catalytic efficiencies of Cln2- and Cln3-dependent kinases to zero, 10 min after the time of α-factor addition (i.e., assuming a 10-min delay for signal transduction). We found a point of no return shortly before the onset of S phase.

Dependence of Cell Cycle Time on Growth Rate and Birth Size. Figure 1 shows how certain characteristics of wild-type cell cycles depend on mass-doubling time, as reported by Lord and Wheals (1980) and Hartwell and Unger (1977). As $T_d$ increases (specific growth rate, $\mu$, decreases), cell division becomes increasingly asymmetrical, daughter size at birth decreases, and the duration of its unbudded phase increases. The unbudded phase of mother cells also increases slightly with $T_d$.

To fit the model to these data, we must adopt a rule for partitioning cell size to mother and daughter at cell separation. The simplest rule ("Rule 1") would be to give to the daughter cell all growth from bud emergence to cell division and let mother retain the mass it had when the bud emerged. However, in this case, the mother would be able to bud soon after its birth; hence it should not have an appreciable unbudded period, which is in contradiction to the observations of Lord and Wheals (1980) and Hartwell and Unger (1977). For the mother cell to have an unbudded period, its birth size must be smaller than the critical size for bud emergence. Furthermore, the calculated daughter cycle times at various growth rates are longer than the observed values. Both results reflect that Rule 1 is inadequate, it gives too much mass to mothers and too little to daughters.

Therefore, we adopt a different rule (Rule 2). Let $f = \frac{\text{mass given to the daughter at cell division}}{\text{mother cycle time}}$, choose $f$ to give the observed daughter cycle time ($D$) at any particular growth rate ($\mu = 0.693/T_d$). From our assumption that cells grow exponentially, (mother size at division) = (daughter size at birth) $\times e^{\mu D}$, so $f = \text{(daughter size at birth)/(mother size at division)} = e^{-\mu D}$. By using the empirical formula for daughter cycle time, $D = 1.48 T_d - 32.3$ (Lord and Wheals, 1980, their Table 2) to calculate $f$, we ensure that the model fits the data for $D$ as a function of $T_d$ (Figure 1, top line).

Furthermore, (mother size at division) = (mother size at birth) $\times e^{\mu P}$, where $P = \text{mother cycle time}$, and (mother size at division) = (mother size at birth) + (daughter size at birth). Therefore, $1 = e^{\mu P} + e^{\mu D}$. This relation was originally derived by Hartwell and Unger (1977) in a more complicated manner and shown by them to be consistent with the data. Consequently, by choosing $f$ to fit $D$ as a function of $T_d$, we also ensure a good fit to $P$ as a function of $T_d$ (Figure 1, middle line).

The dependence of budded period on growth rate (Figure 1, bottom line) is unconstrained, so its fit to the data is a valid test of the model. Progression through the budded part of the cycle is slightly dependent on growth rate but not nearly so much in the model as in experiments.
Under Rule 2, simulations show that mother cells become slightly larger each cycle for the first few cycles (Table 3, line 1) but not as much as the observed 25% increase (Hartwell and Unger, 1977; Johnston et al., 1979); furthermore, mother cells soon reach a steady size. This discrepancy is tied inevitably (in our model) to the prominent unbudded phase of mother cells (Figure 1). Under Rule 2, mother cell birth size is less than the critical mass for SBF activation. Hence, mother cells have an extended unbudded phase, as they grow to this critical mass, and mother cell cycles, like daughter cell cycles, are size regulated. Consequently, mother size at division does not increase steadily with each generation. Under Rule 1, size control is absent from mother cells, and they do grow larger each generation, as observed, but Rule 1 does not fit the observations in Figure 1. We do not know how to resolve this problem.

Similar to the observation of Lord and Wheals (1980), Johnston et al. (1977) reported that the smaller a cell is at birth the longer its unbudded interval is. These data support the proposal that smaller cells need more time to grow to a critical size for bud initiation. Our simulations (Figure 4) agree closely with these observations.

## Analysis of Mutants

### Dependence of Cell Size on CLN3 Gene Dosage.

That Cln3 plays a major role in size control of budding yeast is suggested by the strong dependence of mean cell size on CLN3 gene dosage (Cross, 1988; Nash et al., 1988; Dirick et al., 1995; Yaglom et al., 1995). Figure 5 presents the model’s simulation of this effect. The fact that cells approach a minimal size as CLN3 dosage increases suggests that the activity of Cln3-dependent kinase plateaus at high concentration (assumption 5). The parameter \( f_{n3} \) determines how fast [Cln3], the kinase activity of Cln3, saturates with increasing CLN3 dosage, \( D_{n3} \).

### Role of the Positive Feedback Loop.

Experimental evidence clearly shows that SBF can be activated by Cln1–2 and Clb5–6 as well as Cln3 (Cross and Tinkelenberg, 1991; Schwob and Nasmyth, 1993), hence the appearance of all three CDK activities in \( V_{a,bsh} \) (Table 1). In wild-type cells Clb5 can play no role in SBF activation at Start, because any Clb5 present in G1 phase will be tied up in inactive trimers, Sic1/Clb5/Cdc28. However, some active Cln2-dependent kinase is likely present in G1, and it could cooperate with Cln3 and Bck2 in activating SBF. This positive feedback loop (SBF turns on Cln2 synthesis, and Cln2/Cdc28 activates SBF) could potentially play a major role in the activation of SBF at Start.

Dirick et al. (1995) and Stuart and Wittenberg (1995) addressed the role of positive feedback by comparing cell size at SBF activation in two mutant strains, \( cln1 cln2 \) and \( cln3 \). Each strain was made artificially small at birth by ectopic expression of a wild-type \( CLN \) gene carried on a plasmid. Dirick et al. (1995) used \( CLN2 \) under the control of a methionine-repressible promoter. In the presence of methionine (plasmid-borne \( CLN2 \) not expressed), both strains are larger than normal, and SBF is activated soon after cell division. Cell size in this case is not indicative of the minimum size necessary to activate SBF. To assay the minimum size, cells were grown in the absence of methionine (plasmid-borne \( CLN2 \) expressed), so that they grow and divide, like wild type, at a much smaller size. The smallest newborn cells (volume ~10 fl) were selected by centrifugal elutriation and resuspended in medium containing methionine. In this case, the cells needed to grow awhile before SBF was activated. Cells of the \( cln1 cln2 MET-CLN2 \) strain activated SBF at 19 fl, exactly the same size as control cells (\( CLN1 CLN2 CLN3 MET-CLN2 \)). On the other hand, cells lacking Cln3 (the \( cln3 MET-CLN2 \) strain) activated SBF at ~45 fl (Dirick et al. et al., 1995, their Figure 2A).

These remarkable results indicate that 1) positive feedback does not play a determinative role in the activation of SBF at Start (because cell size at SBF activation is unchanged when the positive feedback loop is broken); and 2) there must be some component (other than Cln1–3 and Clb5,6) that turns on SBF in the absence of Cln3, albeit at a larger size. (Clb5,6 are excluded because deletion mutants have size similar to that of wild-type cells, not twice as big.)

To capture these features with our model, we assume that Cln3 is much more efficient than Cln2 in activating SBF (\( \alpha_{a,bsh} > 1 \)), and that Cln3 is assisted by Bck2 (reasons to be described later). Because the \( cln3 \) mutant is about twice the size of wild type, we choose parameters so that Bck2 and

![Figure 3](image-url)  
*Figure 3.* Wild-type cell cycle in daughter cells. Computed from equations and parameter values in Tables 1 and 2.
Table 3. Properties of wild-type cells and cln mutants

<table>
<thead>
<tr>
<th></th>
<th>Mass at birth</th>
<th>Mass at SBF 50%</th>
<th>Mass at DNA repl.</th>
<th>Mass at bud ini.</th>
<th>Mass at division</th>
<th>T_{c1} (min)</th>
<th>Changed parameter</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>wild type</td>
<td>0.71</td>
<td>1.07</td>
<td>1.15</td>
<td>1.15</td>
<td>1.64</td>
<td>84</td>
<td>CT 146 min</td>
</tr>
<tr>
<td></td>
<td>(daughter)</td>
<td>(71')</td>
<td>(84')</td>
<td>(84')</td>
<td>(146')</td>
<td></td>
<td></td>
<td>(time of occurrence of event)</td>
</tr>
<tr>
<td>1st parent</td>
<td>0.93</td>
<td>1.07</td>
<td>1.17</td>
<td>1.16</td>
<td>1.67</td>
<td>39</td>
<td></td>
<td>CT 101 min</td>
</tr>
<tr>
<td>2nd parent</td>
<td>0.95</td>
<td>1.08</td>
<td>1.17</td>
<td>1.17</td>
<td>1.68</td>
<td>37</td>
<td></td>
<td>CT 99 min</td>
</tr>
<tr>
<td>3rd parent</td>
<td>0.96</td>
<td>1.08</td>
<td>1.17</td>
<td>1.17</td>
<td>1.69</td>
<td>36</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>cln3</td>
<td>1.24</td>
<td>2.04</td>
<td>2.20</td>
<td>2.15</td>
<td>2.88</td>
<td>99</td>
<td>Dirick, 1995, Fig. 3, size 1.7 × WT</td>
</tr>
<tr>
<td></td>
<td>sic1</td>
<td>1.09</td>
<td>1.85</td>
<td>1.28</td>
<td>1.90</td>
<td>2.54</td>
<td>28</td>
<td>G1 short, size 1.5 × WT, smaller than cln3</td>
</tr>
<tr>
<td>3</td>
<td>cln3 GAL-CLN3</td>
<td>0.43</td>
<td>0.43</td>
<td>0.46</td>
<td>0.50</td>
<td>0.99</td>
<td>14</td>
<td>D_{n3} = 20</td>
</tr>
<tr>
<td>4</td>
<td>CLN3D</td>
<td>0.44</td>
<td>0.45</td>
<td>0.49</td>
<td>0.52</td>
<td>1.02</td>
<td>17</td>
<td>D_{n3} = 8</td>
</tr>
<tr>
<td>5</td>
<td>cln3 GAL-CLN3</td>
<td>0.42</td>
<td>0.42</td>
<td>0.45</td>
<td>0.49</td>
<td>0.98</td>
<td>12</td>
<td>D_{n3} = 20</td>
</tr>
<tr>
<td>6</td>
<td>cln1 cln2</td>
<td>1.46</td>
<td>1.47</td>
<td>2.47</td>
<td>2.58</td>
<td>3.39</td>
<td>91</td>
<td>Dirick, 1995, Fig. 3, size 3.2 × WT</td>
</tr>
<tr>
<td>7</td>
<td>cln1 cln2 sic1</td>
<td>0.81</td>
<td>1.12</td>
<td>0.97</td>
<td>1.29</td>
<td>1.89</td>
<td>31</td>
<td>Dirick, 1995, Fig. 4, size between cln1 cln2 and WT</td>
</tr>
<tr>
<td>8</td>
<td>cln1 cln2 clb5 clb6</td>
<td>0.71</td>
<td>1.23</td>
<td>[11.66]</td>
<td>No bud</td>
<td>[14.44]</td>
<td></td>
<td>Schwob, 1993, Fig. 5, G1 arrest</td>
</tr>
<tr>
<td>9</td>
<td>cln1 cln2 GAL-CLN2</td>
<td>0.34</td>
<td>0.34</td>
<td>0.38</td>
<td>0.35</td>
<td>0.78</td>
<td>23</td>
<td>Dirick, 1995, Fig. 3, size 3.2 × WT</td>
</tr>
<tr>
<td>10</td>
<td>cln1 cln2 GAL-CLN2 sic1</td>
<td>0.33</td>
<td>0.33</td>
<td>0.36</td>
<td>0.34</td>
<td>0.76</td>
<td>19</td>
<td>Dirick, 1995, Fig. 6, size small, budding is advanced more than DNA replication when compared with WT</td>
</tr>
<tr>
<td>11</td>
<td>cln1 cln2 MET-CLN2 GAL-SIC1</td>
<td>0.39</td>
<td>0.39</td>
<td>0.48</td>
<td>0.40</td>
<td>0.90</td>
<td>38</td>
<td>SBF activated early, G1 short, cells small.</td>
</tr>
<tr>
<td>12</td>
<td>cln1 cln2 MET-CLN2 clb1 clb2 GAL-CLB2</td>
<td>0.20</td>
<td>No SBF</td>
<td>0.33</td>
<td>0.22</td>
<td>0.47</td>
<td>85</td>
<td>Birth size between GAL-CLN2 and GAL-SIC1 (0.80)</td>
</tr>
</tbody>
</table>

Note: to simulate mutants that synthesize cyclins constitutively, e.g., cln1 cln2 GAL-CLN2, we set the rate constant for regulated synthesis to zero (k_{s,b2} = 0) and the rate constant for unregulated synthesis to a uniform value (k_{s,c1} = 0.1) to represent a constant rate of expression from the GAL promoter. This is a neutral assumption, in the absence of any quantitative data about levels of mRNA expression driven by the GAL promoter.

Cln3 contribute about equally to SBF activation. Simulations of the experiments of Dirick et al. (1995) are presented in Figure 6, A–C.

In the cln1 cln2 strain (Figure 6B), the events that normally occur together at Start are dissociated. First, SBF turns on at mass = 1.2 (volume = 24 fl), slightly larger than in wild-type cells (22 fl). (We use the conversion factor 1 mass unit = 20 fl.) Because Cln1 and Cln2 are missing, Sic1 degradation and Hct1 inactivation are delayed. Cells must grow larger (~35 fl) before the combined effects of Cln3 and Clb5 can remove Sic1 (Cln3 phosphorylates and destabilizes Sic1, whereas Clb5 titrates away Sic1). With Sic1 gone, the rapidly rising Clb5-associated kinase activity initiates DNA synthesis, bud emergence, and Hct1 inactivation. Then, as Clb2 appears, cells enter mitosis.

Start events in the cln3 strain (Figure 6C) occur at even larger size, because these cells rely on Bck2 alone to activate SBF. Once SBF turns on (at ~41 fl), subsequent events of the cell cycle occur normally.

**Properties of cln Mutants.** When cycling, recessive cln3 mutant cells are 75% larger than wild-type cells, whereas dominant CLN3O mutant cells are 40% smaller, and double
recessive cln1 cln2 mutant cells are twice as large, all in agreement with observations (Table 3, lines 2, 4, and 6; Cross, 1988; Nash et al., 1988; Dirick et al., 1995).

In the paper by Dirick et al. (1995), the authors reported an intriguing phenomenon. As previously described, cln1 cln2 mutants, when started very small (10 fl), initiate DNA synthesis and budding at ~40 fl (their Figure 4). However, in cycling cln1 cln2 cells, DNA synthesis and budding is delayed further to 60 fl (their Figure 3). That is, although the birth size of the cycling cells is ~32 fl, they are unable to initiate DNA synthesis and budding at 40 fl but have to grow larger still (60 fl) to do these jobs. Why?

Our simulation (Figure 6D) gives an explanation. In the cycling culture, when cells reach 40 fl (which occurs soon after birth), there is still abundant Sic1, because Swi5 is active in early G1, and Sic1 degradation is slow in the absence of Cln2. It takes ~1 h to remove Sic1 by a combination of Cln3-dependent phosphorylation of Sic1 and Clb5 binding to Sic1. As soon as [Clb5] \textsubscript{T} \approx [Sic1] \textsubscript{T}, Clb5-dependent kinase activity starts to rise and initiates DNA synthesis. In our simulation, cycling cln1 cln2 cells (born at mass = 1.46, 29 fl) begin DNA synthesis at mass = 2.47 (Table 3, line 6, equivalent to 49 fl); whereas for very small cells (born at mass = 0.71, 14 fl), DNA synthesis begins at mass = 1.75, equivalent to 35 fl. These results are in reasonable agreement with the observations of Dirick et al. (1995). Without the help of Clb5, as in the case of the cln1 cln2 clb5 clb6 mutant, Cln3-dependent kinase has a hard time fighting against Sic1 alone, and cells are inviable, blocked in G1 (Table 3, line 8).

When Cln2 is synthesized constitutively (cln1 cln2 GAL-CLN2; Table 3, line 9), cells are smaller than normal. Because Cln2-dependent kinase activity is always high, Start occurs shortly after division, and size control at the G1/S transition is lost. Nonetheless, this strain is perfectly viable, and its cell cycle is still size regulated. As pointed out by Futcher (1996), this observation implies a size control mechanism in M phase that is cryptic as long as size control at Start is operating. In our model, this stems largely from the mass dependence of the positive feedback loop that activates Clb2 transcription. In small cells, the rise of Clb2-dependent kinase activity is delayed, which lengthens the duration of M phase. Bypassing transcriptional control of Clb2 can test this explanation: cells constitutively expressing both Cln2 and Clb2 (cln1 cln2 MET-CLN2 clb1 clb2 GAL-CLB2) should be considerably smaller than cells constitutively expressing Cln2 alone (cln1 cln2 MET-CLN2) (Figure 7 and Table 3, lines 9 and 12). Such small cells may be inviable.

**Rescue of Triple-cln Mutant.** Especially noteworthy is the inviable triple-cln mutant cln1 cln2 cln3 (Table 4, line 2). SBF is activated by Bck2 (at a larger than normal size), but no other events of Start occur, because they all require CDK activity (the Clns are all missing, and the Clbs are all inhibited by Sic1). In our simulations, the cell eventually grows large enough for the low, G1 level of Clb5 to turn off Hct1 and Sic1 and then to initiate DNA synthesis and progress toward mitosis, but S/M commences at such a large size, 5 times larger than in wild-type, that the cell, we assume, has already died.

Clearly, the triple-cln mutant can be rescued by supplying CLN2 or CLN3 on a plasmid with a GAL promoter (Table 4, lines 3 and 4). It can also be rescued by GAL-CLB5 (Table 4, line 6) or simply by providing an extra genomic copy of CLB5 (Table 4, line 6), as observed (Epstein and Cross, 1992). In the latter case, Clb5 is made twice as fast, so cells are able to turn off Sic1 and Hct1 at a reasonable size. In addition, triple cln can be rescued by deleting the genomic copy of SIC1 (Schneider et al., 1996; Tyers, 1996) (see Figure 6F and Table 4, line 8; the mechanism for the rescue will be described later).

However, triple-cln mutants cannot be rescued by GAL-CLB2 (Table 4, line 7); cells remain arrested in G1 because active Hct1/APC keeps Clb2 level low (Amon et al., 1994). Nor can it be rescued by hct1 or apc mutations (Table 4, lines 9 and 10), as observed (Irniger and Nasmyth, 1997; Schwab et al., 1997); here DNA synthesis can be initiated, but cells cannot exit from mitosis (more on this later).

**Role of Bck2.** Bck2 has not received much attention from molecular biologists, but what is known (Epstein and Cross, 1994; Di Como et al., 1995) is consistent with the role given
to Bck2 in the model (assumption 4). As for the case of CLN3 mutants, cells overexpressing BCK2 are smaller than normal (66%), and bck2 loss-of-function mutants are larger than normal (180%) (Table 5, lines 1b and 1c). Although the triple mutant cln1Δ cln2Δ bck2 (line 2b) is viable and a little larger than cln1Δ cln2Δ, the double mutant cln3Δ bck2 (line 3b) is inviable: SBF is never activated, and cells arrest in G1. The inviable cln3Δ bck2 cell can be rescued, just like the triple-cln mutant, by GAL-CLN2, GAL-CLB5, or sic1 (lines 4a–4c). However, because SBF is not activated in this case, it takes more copies of genomic CLB5 (10 copies vs. 2) for its rescue (line 4d). Modest overproduction of Bck2 rescues triple-cln mutants (line 5b), provided both Swi4 and Swi6 are present, suggesting that Bck2 works through SBF.

**Regulation of Clb Proteins.** Because Start represents the commitment of a budding yeast cell to a new round of DNA synthesis and division, it is important that B-type cyclins (which drive S phase and mitosis in budding yeast) be inoperative before Start occurs. The Clbs are kept out of the picture in G1 by three mechanisms: 1) CLB mRNA transcription is repressed, 2) Clb proteolysis by the APC is active, and 3) a Clb-dependent kinase inhibitor, Sic1, is abundant. In this section we explore the interrelations of
Table 4. Mutations that rescue the \( cln \) \( cln \) strain

<table>
<thead>
<tr>
<th></th>
<th>Mass at birth</th>
<th>Mass at SBF 50%</th>
<th>Mass at DNA repl.</th>
<th>Mass at bud ini.</th>
<th>Mass at division</th>
<th>( T_{G1} ) (min)</th>
<th>Changed parameter</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 wild type</td>
<td>0.71</td>
<td>1.07</td>
<td>1.15</td>
<td>1.15</td>
<td>1.64</td>
<td>84</td>
<td></td>
<td>CT 146 min</td>
</tr>
<tr>
<td></td>
<td>(daughter)</td>
<td>(71')</td>
<td>(84')</td>
<td>(84')</td>
<td>(146')</td>
<td></td>
<td></td>
<td>Richardson, 1989, inviable</td>
</tr>
<tr>
<td>2 cln1 cln2 cln3</td>
<td>0.71</td>
<td>2.47</td>
<td>6.20</td>
<td>[6.48]</td>
<td>[8.11]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 cln1 cln2 cln3</td>
<td>0.65</td>
<td>0.65</td>
<td>0.75</td>
<td>0.95</td>
<td>1.50</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 cln1 cln2 cln3</td>
<td>0.34</td>
<td>0.34</td>
<td>0.39</td>
<td>0.35</td>
<td>0.79</td>
<td>25</td>
<td>k _s,n2 = 0.1</td>
<td></td>
</tr>
<tr>
<td>5 cln1 cln2 cln3</td>
<td>2X CLB5</td>
<td>2.81</td>
<td>2.83</td>
<td>4.96</td>
<td>5.15</td>
<td>6.52</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>6 cln1 cln2 cln3</td>
<td>1.23</td>
<td>2.07</td>
<td>2.05</td>
<td>2.14</td>
<td>2.86</td>
<td>88</td>
<td>k _b,o,0 = 0.04</td>
<td></td>
</tr>
<tr>
<td>7 cln1 cln2 cln3</td>
<td>0.71</td>
<td>2.57</td>
<td>5.49</td>
<td>[5.64]</td>
<td>[7.38]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 cln1 cln2 cln3</td>
<td>1.30</td>
<td>2.12</td>
<td>1.50</td>
<td>2.24</td>
<td>3.01</td>
<td>24</td>
<td>k _s,1 = 0.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>sic1</td>
<td>(85')</td>
<td>(24')</td>
<td>(94')</td>
<td>(146')</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9 cln1 cln2 cln3</td>
<td>0.71</td>
<td>2.48</td>
<td>4.62</td>
<td>5.03</td>
<td>No mit</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>hct1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 cln1 cln2 cln3</td>
<td>0.71</td>
<td>2.48</td>
<td>4.16</td>
<td>4.60</td>
<td>No mit</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Figure 7. Simulation of MET-CLN2 GAL-CLB2 (no transcriptional control of Cln2 or Clb2 synthesis). In the absence of both methionine and galactose, cells are smaller than wild-type, because Cln2 is synthesized constitutively. When galactose is added (arrow), cells get smaller still, eventually dividing at \(-30\%\) of wild-type size. Such small cells may be inviable.

(K.C. Chen et al.)

These three effects by simulating mutants that knock out the components singly and in combinations.

First of all, any one of these component processes is expendable. For instance, cells that synthesize Clb2 or Clb5 get smaller still, eventually dividing at \(-30\%\) of wild-type size. Such small cells may be inviable.

(Molecular Biology of the Cell)
evidence support this assumption (10). Experiments of Irriger et al. (1995, their Figure 4) show that Clb2 is partially degraded in *cdc15* telophase-arrested cells when (we presume) Cdc20 is active and Hct1 is not. Visintin et al. (1997, their Figure 2) showed that Clb2 is degraded by both Hct1 and Cdc20 in nocodazole-arrested cells. Finally, it is well known that sister chromatid separation and B-type cyclin degradation are mediated by a single Cdc20 homologue during early embryogenesis of *Drosophila* and *Xenopus* (Dawson et al., 1995; Sigrist et al., 1995; Lorca et al., 1998).

The ability of Sic1 to bring Hct1-deficient cells out of mitosis depends on cell size: in the presence of α-factor, hct1 cells grow very large, replicate their DNA, and block in mitosis (Schwab et al., 1997). Our simulation of *cni cln2 cln3* hct1 behaves similarly (Table 6, line 9). By the time the mitotic checkpoint is satisfied and cells are ready to divide, they are so big and their accumulated Clb2 level is so high that Sic1 is unable to win over Clb2.

Although *sic1* is sick (having many deficiencies in chromosome dynamics), it is still a viable mutant (Table 6, line 6, and Figure 8, bottom panel). The only major change in the timing of cell cycle events is the advancement of DNA synthesis (relative to bud emergence) by ~35 min (Schneider et al., 1996, their Figure 4). These authors show that *sic1* cells initiate DNA synthesis at a much smaller size than wild-type cells.

Because in wild-type cells DNA synthesis and SBF activation occur almost concurrently, and SBF activation depends mainly on the actions of Cln3 and Bck2 (not inhibited by Sic1), it follows that in *sic1* mutants initiation of DNA synthesis is well ahead of SBF activation. That is, the small amount of Clb5, synthesized by an MBF-independent pathway, is able to initiate DNA synthesis at a small size (because there is no Sic1 present to inhibit it), well before the cell is large enough to activate SBF.

In contrast to the viability of single mutants (*GAL-CLBs, hct1*, and *sic1*), double mutants are all inviable (Table 6, lines 9–13). hct1 sic1 cells, being unable to eliminate Clb2-dependent kinase, cannot exit mitosis (Visintin et al., 1997). *clb5 GAL-CLB5 sic1* cells complete one cycle after adding galactose but die in the second cycle because they cannot replicate their DNA (they cannot resynthesize licensing factor, because Clb-dependent kinase activity stays high after mitosis) (Schwob et al., 1994). *clb2 GAL-CLB2 sic1* cells die after adding galactose, because they cannot bud (SBF is kept off by high Clb2-dependent kinase activity after mitosis). For the same reason, *clb2 GAL-CLB2 hct1* and *clb5 GAL-CLB5 hct1* cells are inviable.

**Properties of SIC1** Mutants. Twofold overexpression of Sic1 is tolerated (Verma et al., 1997), but (roughly) 10-fold overexpression is deleterious (Nugroho and Mendenhall, 1994): some 20% of the cells have elongated buds and fail to divide. Our simulations of *sic1 GAL-SIC1* (Table 6, line 7, with $k_{s,c1}^*=0$ and increasing $k_{s,c1}$ up to fivefold from 0.1 to 0.5) give viable cells with increasing G1 period and larger sizes, but a sixfold increase is lethal (DNA synthesis commences at mass > 5). This behavior is consistent with the experimental observations, provided cells in a population have a distribution of levels of Sic1 production. Similarly, cells with the phosphorylation sites of Sic1 removed (protein stable) never enter S phase (Table 6, line 7), as observed (Verma et al., 1997).

The reason that cells tolerate high levels of Sic1 expression (i.e. $5 \times GAL-SIC1$) and still go through DNA synthesis and mitosis is because of the role played by CLN2. SBF activation is unaffected by Sic1; however, high levels of Sic1 inhibit Clb2 activity, so SBF inactivation is delayed. Cln2 will be synthesized at a high rate for longer time, and eventually

**Table 5. Properties of bck2 mutants**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Observed</th>
<th>Simulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Epstein</td>
<td>Di Como</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1a</td>
<td>CLN1 CLN2 CLN3 BCK2</td>
<td>1</td>
</tr>
<tr>
<td>b</td>
<td>bck2</td>
<td>1.3</td>
</tr>
<tr>
<td>c</td>
<td>+ low-copy BCK2</td>
<td>1.5</td>
</tr>
<tr>
<td>2a</td>
<td>cln1 cln2 CLN3 BCK2</td>
<td>1.5</td>
</tr>
<tr>
<td>b</td>
<td>bck2</td>
<td>1.7</td>
</tr>
<tr>
<td>3a</td>
<td>CLN1 CLN2 cln3 BCK2</td>
<td>1.7</td>
</tr>
<tr>
<td>b</td>
<td>bck2</td>
<td>Inviable</td>
</tr>
<tr>
<td>4a</td>
<td>CLN1 CLN2 cln3 bck2 + GAL-CLN2</td>
<td>Inviable</td>
</tr>
<tr>
<td>b</td>
<td>+ GAL-CLB5</td>
<td></td>
</tr>
<tr>
<td>c</td>
<td>+ sic1</td>
<td></td>
</tr>
<tr>
<td>d</td>
<td>+ low-copy CLB5</td>
<td></td>
</tr>
<tr>
<td>5a</td>
<td>cln1 cln2 cln3 BCK2</td>
<td>Inviable</td>
</tr>
<tr>
<td>b</td>
<td>+ low-copy BCK2</td>
<td>Rescued</td>
</tr>
</tbody>
</table>

$^a$ Epstein and Cross (1994).

$^b$ Di Como et al. (1995).

$^c$ Dirick et al. (1995).
they will be able to phosphorylate Sic1, causing it to degrade and Cib kinases to win.

Initiation of DNA Synthesis in the sic1 Mutant. As described in the previous section, when compared with wild-type cells, sic1 mutants initiate S phase at a much smaller size, whereas cln1 cln2 mutants initiate it at a much larger size (Dirick et al., 1995). What will happen if the two mutations are combined?

Because, in sic1 mutants, initiation of DNA synthesis is driven mainly by the small amount of Cib5 present in early G1 cells, deletion of Clns should have little effect on its timing. Hence, the multiple mutants cln1 cln2 sic1 or cln1 cln2 cln3 sic1 all should initiate DNA synthesis at about the same size as the sic1 single mutant, at a size smaller than wild type.

Our simulation (Figure 6F) of cln1 cln2 cln3 sic1 agrees with observations of Schneider et al. (1996, their Figure 4B). Without Sic1 inhibition, Cib5 is able to initiate DNA synthesis early and

### Table 6. Properties of clb, sic1, and hct1 mutants

<table>
<thead>
<tr>
<th></th>
<th>Mass at birth</th>
<th>Mass at SBF 50%</th>
<th>Mass at DNA repl.</th>
<th>Mass at bud ini.</th>
<th>Mass at division</th>
<th>T&lt;sub&gt;G1&lt;/sub&gt; (min)</th>
<th>Changed parameter</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>wild type</td>
<td>0.71</td>
<td>1.07</td>
<td>1.15</td>
<td>1.15</td>
<td>1.64</td>
<td>84</td>
<td>CT 146 min</td>
</tr>
<tr>
<td>2</td>
<td>clb1 clb2</td>
<td>0.71</td>
<td>1.07</td>
<td>1.16</td>
<td>1.16</td>
<td>1.64</td>
<td>84</td>
<td>CT 146 min</td>
</tr>
<tr>
<td>3</td>
<td>clb1 clb2 1X GAL-CLB2</td>
<td>0.65</td>
<td>1.10</td>
<td>1.19</td>
<td>1.19</td>
<td>1.64</td>
<td>105</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>clb1 clb2 4X GAL-CLB2</td>
<td>0.71</td>
<td>No SBF</td>
<td>1.50</td>
<td>No bud</td>
<td>inviable</td>
<td>(k_{b,c} = 0)</td>
<td>(k_{b,c} = 0)</td>
</tr>
<tr>
<td>5</td>
<td>sic1</td>
<td>0.71</td>
<td>1.07</td>
<td>1.30</td>
<td>1.17</td>
<td>1.70</td>
<td>99</td>
<td>Sic1 mutant</td>
</tr>
<tr>
<td>6</td>
<td>sic1 GAL-SIC1</td>
<td>0.80</td>
<td>1.07</td>
<td>1.38</td>
<td>1.17</td>
<td>1.86</td>
<td>94</td>
<td>Sic1 mutant</td>
</tr>
<tr>
<td>7</td>
<td>sic1 GAL-SIC1db</td>
<td>0.71</td>
<td>1.07</td>
<td>No repl</td>
<td>1.17</td>
<td>No mit</td>
<td>(k_{b,c} = 0)</td>
<td>(k_{b,c} = 0.1)</td>
</tr>
<tr>
<td>8</td>
<td>hct1</td>
<td>0.73</td>
<td>1.08</td>
<td>1.17</td>
<td>1.18</td>
<td>1.69</td>
<td>82</td>
<td>hct1 mutant</td>
</tr>
<tr>
<td>9</td>
<td>sic1 hct1</td>
<td>0.71</td>
<td>1.07</td>
<td>1.02</td>
<td>1.04</td>
<td>1.51</td>
<td>(k_{b,c} = 0)</td>
<td>(k_{b,c} = 0)</td>
</tr>
<tr>
<td>10</td>
<td>sic1 GAL-CLB5</td>
<td>0.71 (1st)</td>
<td>0.74</td>
<td>0.73</td>
<td>0.76</td>
<td>1.20</td>
<td>(k_{b,c} = 0)</td>
<td>(k_{b,c} = 0)</td>
</tr>
<tr>
<td>11</td>
<td>hct1 GAL-CLB5</td>
<td>0.71</td>
<td>0.72</td>
<td>0.77</td>
<td>0.77</td>
<td>No mit</td>
<td>(k_{b,c} = 0)</td>
<td>(k_{b,c} = 0)</td>
</tr>
<tr>
<td>12</td>
<td>sic1 GAL-CLB2</td>
<td>0.71</td>
<td>0.81</td>
<td>0.81</td>
<td>0.81</td>
<td>No mit</td>
<td>(k_{b,c} = 0)</td>
<td>(k_{b,c} = 0)</td>
</tr>
<tr>
<td>13</td>
<td>hct1 GAL-CLB2</td>
<td>0.71</td>
<td>0.83</td>
<td>0.83</td>
<td>0.83</td>
<td>No mit</td>
<td>(k_{b,c} = 0)</td>
<td>(k_{b,c} = 0)</td>
</tr>
<tr>
<td>14</td>
<td>sic1 clb5 clb6</td>
<td>0.74</td>
<td>1.08</td>
<td>1.32</td>
<td>1.18</td>
<td>1.72</td>
<td>99</td>
<td>Behaves very much like clb5 clb6</td>
</tr>
</tbody>
</table>

(time of occurrence of event)
to help Bck2 turn on SBF and MBF, causing more Clb5 accumulation, Hct1 inactivation, and eventually progression toward mitosis. However, for cln1 cln2 sic1, the simulation (Figure 6E) does not agree with observations. Dirick et al. (1995, their Figure 4) reported that DNA synthesis occurs at about the same size for the mutant as for wild-type cells. If the observation of Schneider et al. (1996) is true, then how can the addition of a functional CLN3 gene (giving cln1 cln2 sic1) delay DNA synthesis to a larger mass, when Cln3 is a helper to Clb5? Thus, we are unable to fit all these observations with our present understanding of the control system, and we believe that the experimental observation of Dirick et al. (1995) requires closer investigation.

DISCUSSION

In Figure 2, we propose a realistic mechanism for regulating the cell division cycle in budding yeast. Its components are Cln1 and 2 (lumped together), Cln3 and Bck2, Clb1 and 2 (lumped), Clb5 and 6 (lumped), Sic1, Hct1 (=Cdhl), and Cdc20. (Cdc28, the kinase subunit that combines with the cyclins, is present in excess, so we need not keep track of its fluctuations.) In addition, the model tracks the relative activities of three transcription factors, Swi4/Swi6 (=SBF), Mcm1/SFF, and Swi5, which determine the rates of synthesis of Cln2, Clb2, and Sic1, respectively. At present, we assume that MBF, the transcription factor for Clb5, is regulated coordinately with SBF. In the model, overall cell growth is exponential, and the basic events of the yeast division cycle (DNA synthesis, budding, and spindle assembly) are driven by the integrated activities of cyclin-dependent kinases. These assumptions lead to a mathematical model (Table 1) consisting of 10 nonlinear, ordinary differential equations (for mass, the cyclins, and their consorting proteins), three algebraic functions for transcription factors, three “integrators” to trigger DNA synthesis, budding, and spindle assembly, and a simple rule for separating mother and daughter cells at division.

Table 2 is in no sense an optimal parameter set, nor can we quantify how robust is the system, although our experience suggests that the model is quite hardy. Currently we are working on computational methods of parameter optimization and sensitivity analysis and hope to address these problems in a later publication.

Bistability and Hysteresis

The crucial idea behind our model of the budding yeast cell cycle is Nasmyth’s (1996) hypothesis that G1 and S/M are alternative, self-maintaining states, generated by mutual antagonism between Clb-dependent kinases and their opponents, Sic1 and Hct1. In theoretical terms, the molecular regulatory system exhibits bistability and hysteresis (Figure 9). In its “neutral” condition (no Cln2 or Cdc20), the control system can persist in either the stable G1 state or the stable S/M state. Transitions between these alternative steady states can be driven by changes in Cln2 and Cdc20 that push the control system past the “fold” points in Figure 9 (Novak et al., 1998).

At Start, Cln2-dependent kinase activity rises abruptly and pushes the cell from G1 to S/M by inactivating Hct1 and promoting Sic1 degradation (Figure 9, stage a). The Clns can drive this transition because they are neither degraded by Hct1 nor inhibited by Sic1. After Clb2 appears, Cln2 is removed, but the cell remains in S/M because the Clbs can now keep Hct1 and Sic1 in abeyance without further help from Clns (stage b). This effect, called hysteresis, makes the Start transition irreversible.

Cdc20, activated at metaphase, pushes the cell from S/M to G1 (Finish) by activating Hct1 and promoting Sic1 accu-
mulation (Figure 9, stage c). Bistability and hysteresis (schematic). Steady-state level of total Clb-dependent kinase activity depends on the expression of CLN2 and CDC20. When [Cln2] is large and [Cdc20] is small, the Clb1–6 regulatory system is in a state of high kinase activity (S/M), whereas in the other extreme, Clb-dependent kinase activity is low (G1). When [Cln2] and [Cdc20] are both small, the regulatory system is in a state of high kinase activity (S/M), whereas in the other extreme, Clb-dependent kinase activity is low (G1).

Direct experimental confirmation of bistability can be sought by holding the control system in neutral (Figure 9, position A/B) and then driving it between G1 and S/M by ectopic expression of Clb5 and Sic1 (Figure 9, vertical arrows). This experiment has been done in part by Dahmann et al. (1995). After arresting cells in mitosis with nocodazole, they induced transition to G1 (without nuclear or cell division) by ectopic expression of Sic1. When ectopic synthesis of Sic1 was repressed, their cells executed a second round of DNA synthesis, because endogenous production of Clns drove the cells through Start. To prevent autonomous reentry into S phase, we suggest that cells be blocked with α-factor as well as nocodazole.

We propose that a synchronous culture of MET-CLB5 TET-SIC1 cells (where MET = methionine-repressible promoter and TET = tetracycline-inducible promoter), about to execute Start and bud, be transferred from ‘growth’ medium (containing methionine) to “arrest” medium (containing methionine, α-factor, and nocodazole). (Notice that the use of α-factor and nocodazole to arrest cells in neutral could be replaced by cln1–Δα and clb1–Δk, respectively.) Those cells that have not yet executed Start when the medium is changed will be kept in G1 phase by α factor (moving from a to G1 in Figure 9), whereas those cells that have already executed Start will be arrested in M phase by nocodazole (moving from b to S/M in Figure 9). The culture is now a mixed population of G1- and S/M-arrested cells, suggesting that, in this neutral position, there coexist two stable steady states of Clb activity. To prove the coexistence of these states, divide the culture into two batches. One batch is subjected to transient Clb5 synthesis by transferring the cells briefly to “Clb5” medium (α-factor + nocodazole) and then back to arrest medium. All cells in this batch are expected to arrest in the S/M state (in Figure 9, cells initially at G1 will be driven to S/M, whereas those initially at S/M will return there). Cells of the other batch, after brief exposure to “Sic1” medium (methionine + tetracycline + α-factor + nocodazole), are expected to arrest uniformly in G1 phase. Furthermore, the duration of the “brief” exposure is important: there should be threshold levels of exposure to Clb5 and Sic1 below which the transitions are not accomplished (see Figure 9).

Note that, at the end of treatment, all cells are of uniform size and are exposed to arrest medium. Nonetheless, if our model is correct, individual cells will be in different phases of the division cycle, depending on how they were perturbed. Those cells initially in G1 will be pushed into S/M by a Clb5 perturbation but not by a Sic1 perturbation (Schwob and Nasmyth, 1993) and vice versa for those cells initially in S/M (Dahmann et al., 1995). This behavior would indicate that two stable states of Clb activity coexist (bistability) when the regulatory system is in neutral. By alternating treatment with Clb5 and Sic1, one should be able to induce multiple rounds of endoreplication in cln1–Δα clb1–Δk cells.

**Reversibility of the SBF Switch**

In contrast to the irreversibility of the Start and Finish transitions, the activation of SBF in our model is a reversible, ultrasensitive switch. To test this feature of the model, one could modify slightly the experimental design of Dirick et al. (1995). The strain cln1Δ cln2Δ cln3Δ MET-CLN2 is grown in
the absence of methionine, so that newborn daughter cells are small. Small cells, transferred to methionine-containing medium at permissive temperature, will activate SBF (measured by expression of PCL1 mRNA, say) at wild-type size, but Sic1 degradation and Hct1 activation will be delayed to a much larger size. If, after SBF activation, the cells are transferred to restrictive temperature, then SBF should inactive (i.e., this event is reversible), and the cells should remain in G1.

**Autonomously Oscillating Versus Checkpoint-controlled Cell Cycles**

Cell division cycles of budding yeast (and somatic cells in general) are blocked by drugs that inhibit DNA replication or spindle assembly (“checkpoint controls”), whereas early embryonic cell divisions are unrestrained by these same drugs (Murray and Hunt, 1993). If somatic and embryonic cells use the same cell cycle control machinery, why do they behave so differently?

In theoretical terms, checkpoints correspond to stable steady states (G1 and S/M in the current model), and drugs that inhibit growth, DNA synthesis, or spindle assembly abort the signals that normally push the cell from one checkpoint to the next. The existence of these checkpoints depends on the mutual antagonism between cyclin B-dependent kinases and their opponents (Sic1 and Hct1 homologues).

In early *Xenopus* embryos, there are no effective antagonists of cyclin B/Cdc2 kinase (also called MPF). 1) The only identified MPF inhibitors are p28Kix1 (Shou and Dunphy, 1996) and p27\(Xic1\) (Su et al., 1995), but both are present at very low levels until the late gastrula stage. Furthermore, neither one inhibits MPF in vitro. 2) X-FZY (the *Xenopus* homologue of HCT1) is not translated before the midblastula transition. Instead, X-FZY (the *Xenopus* homologue of Cdc20) is responsible for cyclin B degradation during early embryonic cell cycles (Lorca et al., 1998). X-FZY, like Cdc20, seems to be activated rather than inhibited by MPF (Felix et al., 1990). 3) Even the antagonistic relationship between MPF and Wee1 (a tyrosine kinase that inhibits Cdc2) seems to be ineffective, because Cdc2 shows very little tyrosine 15 phosphorylation during the early cycles of intact embryos (Ferrell et al., 1991).

Without effective antagonists in early embryonic cells, MPF cannot establish the alternative steady states characteristic of checkpoint controls. The only remaining control is a time-delayed negative feedback loop, whereby MPF activates X-FZY, which degrades cyclin B and thereby destroys MPF activity. The sufficiency of this mechanism to generate autonomous oscillations in MPF activity was shown first by Goldbeter (1991) and later by Novak and Tyson (1993). Because the early embryo lacks cyclin B antagonists, it supports rapid MPF oscillations that are insensitive to errors in DNA replication and spindle assembly; apparently the early embryo has sacrificed accuracy for speed. However, in frog egg extracts, checkpoint control can be elicited if a sufficient amount of unreplicated sperm DNA is added (Dasso and Newport, 1990; Smythe and Newport, 1992), which creates an effective antagonist by activating Wee1. If our hypothesis is right, one may be able to elicit checkpoint responses in *Xenopus* embryos before the midblastula transition by injecting *XFZR* mRNA (or protein) into the fertilized egg.

**Models as Tools in Molecular Biology**

Undoubtedly the genetic regulatory system of cell division in yeast and higher eukaryotes is even more complex than Figure 2. To understand regulatory systems of such complexity, we need analytical tools that can handle realistic biochemical control mechanisms. Our work confirms that modern methods of kinetic theory and computation are capable of connecting a realistic, multilayered, regulatory mechanism to the complex physiological behavior of cells.

In addition to its role in synthesizing molecular and physiological details about cell division, the model is a predictive tool. The rate constant estimates in Table 2 can be tested by more direct kinetic measurements. Tables 3–6 specify many quantitative properties of mutant cells that have never been reported, and they predict phenotypes of several mutants yet to be examined.

One can learn as much from the failures of the model as from its successes. Where there are inconsistencies between the model and experiment, we are prompted, first of all, to look for a better parameter set. If that fails, we consider slight changes in the mechanism, which might bring the model in accord with observations. If that fails, and if the experimental community is convinced that the observations are reliable and significant, then we have identified an area that deserves closer scrutiny to resolve the discrepancies. If the mechanism proves insufficient, that does not invalidate our approach. Mathematical modeling, as a tool, is no more “falsifiable” than gel electrophoresis. The tool tells us what a mechanism can and cannot explain. When the model fails, the fault lies with the mechanism, not the tool.

The molecular mechanism of cell cycle control in budding yeast is an evolving hypothesis that must be continually examined, revised, and improved as new observations tell us more about the control system. We intend to extend the model in several directions. First, we will provide a more detailed description of Finish (Novak et al., 1999), including roles for Cdc14 (Visintin et al., 1998; Jaspersen et al., 1999), RENT complexes (Shou et al., 1999), and Pds1/Esp1 interactions (Ciosk et al., 1998; Cohen-Fix and Koshland, 1999; Tinker-Kulberg and Morgan, 1999). The next step will be to connect mathematical representations of surveillance mechanisms to the underlying cell cycle engine. For instance, the mating factor pathway connects pheromone binding at the cell surface, through a protein kinase cascade, to the inhibition of Cln kinases, which arrests cells before Start (Wittenberg and Reed, 1996; Posas et al., 1998). Another important signal transduction pathway, through mad and bop gene products, arrests cells in mitosis, if the mitotic spindle is improperly assembled (Alexandru et al., 1999; Taylor, 1999).

**APPENDIX A: ESTIMATION OF RATE CONSTANTS**

First some definitions:

1) A Michaelis constant \(J\) (in our notation) carries units of concentration (nM). In the Michaelis–Menten rate law, \(J\) is the characteristic substrate concentration at which reaction rate is half-maximal.

2) A zero-order rate constant carries units of nM min\(^{-1}\). Examples include rates of synthesis \(k_i\) (in our notation), and \(V_{\text{max}}\) values in Michaelis–Menten rate laws \(k_i\) and \(k_i\) (in our notation).
3) A first-order rate constant carries units of min$^{-1}$. Examples include degradation rate constants ($k_d$) and dissociation rate constants ($k_{da}$).

4) A second-order rate constant, e.g., an association rate constant ($k_a$), carries units of nM$^{-1}$ min$^{-1}$.

In writing the kinetic equations in Table 1, all concentration variables are dimensionless, all zero-, first-, and second-order rate constants carry units of min$^{-1}$ (Table 2).

Rates of Degradation
Because the stability of cyclins at various stages in the budding yeast cell cycle has been studied carefully by many experimental groups, we can estimate the cyclin degradation constants in our model with some confidence. For instance, the half-life of Cln2 is 5–10 min (Salama et al., 1994; Barral et al., 1995; Lanker et al., 1996), implying that $k_{da,2} \approx \ln 2/7 = 0.1$ min$^{-1}$, which is the value we use (Table 2). Because Hct1 is turned off at Start, Clb2 is more stable in S/M than in G1: half-life $\approx 1$ min in G1 and $>1$ h in S/M (Amon et al., 1994; Irie et al., 1995; Seufert et al., 1995). These observations imply that $k_{da,2} \approx \ln 2/70 = 0.01$ min$^{-1}$ and $k_{da,2} \approx \ln 2/1 = 0.7$ min$^{-1}$, which are close to the values in Table 2. Cln5, on the other hand, is only slightly more stable in S/M (half-life $\approx 10$ min) than in G1 (half-life $= 2$ min) (Irie and Nasmyth, 1997). Hence, $k_{da,5} \approx \ln 2/10 = 0.07$ min$^{-1}$, and $k_{da,5} + k_{da,5} \approx \ln 2/2 = 0.35$ min$^{-1}$ (see Table 2).

From the observed half-life of Cdc20, 15 min (Shirayama et al., 1998), we estimate that $k_{da,20} = 0.05$ min$^{-1}$.

Sic1 disappears at Start over a time scale of $\sim 20$ min (Schwob et al., 1994). Because we use a Michaelis–Menten rate law for Sic1 phosphorylation (the rate-limiting step for Sic1 degradation at Start), it is not so obvious how to estimate our rate parameters, but the values $J_{d2,1} = \ll 1$ and $k_{d2,1} = 0.3$ min$^{-1}$ give reasonable agreement to Schwob’s observation.

Rates of Synthesis
Rate constants for cyclin synthesis can be estimated from degradation rate constants and steady-state concentrations. However, because the concentrations are unknown, we cannot ascribe directly meaningful values to the synthesis rate constants. Instead, we choose $k_s \approx k_{d2}$ so that the maximum concentrations of all variables during a wild-type cycle are $\sim 1$. The chosen values of $k_s$ reflect the relative concentrations of the cyclins, Sic1, and Cdc2, as best we can guess, but the arbitrary units (au) in which each concentration is expressed are unknown until we have better experimental data. For instance, suppose the peak concentrations of Sic1 and Cln2 were measured to be 100 and 30 nM, respectively. Then, from Figure 3, we would assign 1 au of Sic1 concentration = 90 nM and 1 au of Cln2 concentration = 60 nM, and we could predict that $k_{s,c1} = 1.8$ nM min$^{-1}$, $k_{s,c1} = 9$ nM min$^{-1}$ (when Sic1 is fully active), $J_{d2,1} = 4.5$ nM, and $k_{d2,1} = 0.3$ min$^{-1}$ (90/60 nM) = 0.45 min$^{-1}$.

Rates of Association and Dissociation
The rate constant ($k_{da}$) for association of Sic1 with Clb2/Cdc28 and Cln2/Cdc28 is an important parameter in the model. It must be large enough to account for the binding, given reasonable lability of the complexes ($k_{da} = 0.05$ min$^{-1}$, assumed). We choose $k_{da} = 50$ min$^{-1}$, which implies a binding constant of $10^5$. The theory of diffusion-controlled reactions (Tinoco et al., 1978, p. 385) puts an upper limit on second-order rate constants: $k_{max} = 8 \pi N D_{avg}$, where $r$ = encounter distance of a pair of reacting molecules, $N_o$ = Avogadro’s number, and $D_{avg}$ = average diffusion coefficient of the reacting molecules. Assuming $r = 4 \times 10^{-7}$ cm, and $D_{avg} = 2 \times 10^{-6}$ cm$^2$ s$^{-1}$, we estimate that $k_{max} = 10^{10}$ l mol$^{-1}$ s$^{-1}$ = 10 nM$^{-1}$ s$^{-1}$. If the concentration of protein partners is roughly 80 nM, then $k_{max}$ (scaled) $\approx 5 \times 10^4$ min$^{-1}$, which is 1000 times larger than the value assigned to $k_{da}$.

Rates of Activation and Inactivation
The transcription factors SBF, Mcm1, and Swi5 are each described by Goldbeter–Koshland functions, $G(V_a, V_b, J_a, J_b)$. (Please refer to Appendix B for the definition of this function and a detailed description of its properties.) When $J_a$ and $J_b$ are much less than 1, a Goldbeter–Koshland function changes very abruptly in the neighborhood of $G = \frac{1}{2}$. Because the SBF switch turns on abruptly as cells grow (Dirick et al., 1995), we choose $J_{a,0}$ and $J_{b,0}$ to be small (0.01). By contrast, we suppose that Mcm1 turns on and off more smoothly ($J_{a,mcm} = J_{b,mcm} = 1$).

Activation and inactivation of Hct1 is also governed by a Goldbeter–Koshland mechanism, but we do not make a pseudo–steady-state approximation ($d[Hct1]/dt = 0$). Rather,

$$\frac{d}{dt}[Hct1] = \frac{k_{a,0} + k'_{a,0}[Cdc20]}{[Hct1] + J_{a0} + [Hct1]} \left(\frac{[Cdc20]}{[Hct1]} + \frac{[Cdc20]}{[Hct1]} + \frac{[Cdc20]}{[Hct1]} + \frac{[Cdc20]}{[Hct1]}\right)$$

The rates at which Hct1 switches between more and less active forms depend on the rate constants, $k_a$ and $k_i$. We expect they have values of order 1 min$^{-1}$, so that changes occur in minutes rather than seconds or hours. More precise estimates cannot be made from presently available measurements.

To estimate the conditions for turning Hct1 on and off, we notice that $[Hct1] = \frac{1}{2}[Hct1]_I$, when

$$k_{s,i1} + k'_{s,i1}[Cdc20] = k_{s,i1} + k'_{s,i1}[Cln3] + e_{s,i1,cl}[Cln2] + e_{s,i1,cl}[Cln2] + e_{s,i1,cl}[Cln2]$$

(provided $J_{a,11} = J_{b,11}$). In metaphase, when Clb2 plays the major role in keeping Hct1 inactive, the condition for activating Hct1 is $[Cdc20] = k'_{a,11}[Clb2]/k'_{a,11} \approx 0.5$. That is, Hct1 turns on when Cdc20 activity exceeds $0.32 \times Clb2$ activity. The threshold can be adjusted by changing the ratio $k'_{a,11}/k'_{a,11}$. In late G1 phase, when $[Cdc20] = 0.25$, $[Clb2] = 0.5$, $[Cln3] = \ll [Cln2]$, and Clb2 is still inhibited by Sic1, the condition for inactivating Hct1 is $[Cln2] = (k'_{a,11} + k'_{a,11}[Cdc20])/k'_{a,11} \approx 0.2$. With our choice of parameters, Cln2 plays the major role in turning off Hct1 at Start. It may be that Clb5 plays a more important role, in which case the
relative efficiencies ($e_{i,t1,b5}$ and $e_{i,t3,n3}$) would have to be readjusted.

Other Parameters

$[Cln3]_{\text{max}} = 0.02$ is chosen so that the amount of Cln3 at Start, $[Cln3]^* = 0.003$ when mass $\approx 1$, is $\sim 100$ times smaller than the maximum amounts of Cln1 and Cln2 during the cycle (Tyers et al., 1993). Although Cln3 is present in a very small amount, it plays an important role turning on SBF at Start (Dirick et al., 1995; Stuart and Wittenberg, 1995), because $e_{i,\text{ori},n3}$ is very large. $[Bck2]^* = 0.0027$ is chosen to account for the observed size for SBF activation in the $cln3$ mutant.

Efficiencies

Our model contains many other dimensionless “efficiencies” of different CDKs for various phosphorylation reactions. These efficiencies simply represent the fact that budding yeast cyclins have overlapping specificities: they often catalyze the same reactions but with different relative turnover numbers. We adjusted the efficiencies to account for the relative timing of events in mutants where the primary cyclin is missing and the event is triggered by one of its backups.

For instance, in triple-$clb\Delta$ sic1$\Delta$, Clb5 is the primary cyclin responsible for Hct1 inactivation. If $e_{i,t1,b5}$ were too small, then Hct1 would never turn off, and the mutant would be inviable. If $e_{i,t1,b5}$ were too large, then the small amount of uninhibited Clb5 early in the cycle (which drives premature DNA synthesis in this mutant) would inactivate Hct1 too soon. As a result, Clb2 would rise early, and SBF could never be activated, so the mutant would divide at a small size without ever making a bud. In our simulations, $e_{i,t1,b5} \approx 0.5$ gives a correct phenotype to this mutant.

Integrators

Finally, we introduced three “integrators” (ori, bud, and spn), which determine when the events of 1) initiation of DNA synthesis, 2) bud emergence, and 3) chromosome alignment on the mitotic spindle will occur. Experiments show that 1) DNA synthesis can be initiated by Clbs but not by Clns (Schwob et al., 1994); 2) bud emergence can be driven by Clb5 as well as Clns (Schwob and Nasmyth, 1993), and 3) mitosis can be driven to completion only by Clb1 and Clb2 (Surana et al., 1991). These facts determine the forms taken by the integrators. The rate constants ($k_{\text{ori}}, k_{\text{bud}}$, and $k_{\text{spn}}$) and efficiencies are chosen to give the right timing of these events in various mutants.

Note that some parameters are introduced only for notational consistency (e.g., $k_{\text{ori}}, k_{\text{bud}}$, and $k_{\text{spn}}$) and are set equal to 1 or 0 in all computations. Other parameters are constrained to be identical (e.g., $I_{a,b5} = I_{a,n3}$), to limit the scope of the problem. Therefore, we had $\approx 50$ parameters to estimate from the properties of $\sim 50$ different genotypes. Wild-type cells provide us with enough information to estimate more than half of these parameters. The remaining parameters are constrained by the properties of all the mutants we have simulated. Because each viable mutant gives us at least two pieces of information (relative size and G1 duration), we have much more data to fit than parameters to vary. Therefore, because the model accounts for so many facts (in quantitative detail wherever possible), we conclude that the molecular mechanism in Figure 2 is essentially correct.

APPENDIX B: ZERO-ORDER ULTRASENSITIVITY

We model the kinetic behavior of the budding yeast transcription factors (SBF, Mcm1, and Swi5) as zero-order ultrasensitive switches (Goldbeter and Koshland, 1981; Ferrell, 1996). We assume that each transcription factor (TF) exists as either an active or inactive form (let $F = \text{fraction in active form}$), and that transitions between the two forms are enzyme-catalyzed reactions following Michaelis–Menten kinetics,

$$V_a = \frac{\frac{\alpha}{\beta} \cdot V_i \cdot J_i - \frac{\gamma}{\beta} \cdot V_i \cdot J_i}{\frac{\alpha}{\beta} + \frac{\gamma}{\beta} - 4\alpha \gamma},$$

where $a = V_i - V_{a,b}$, $\beta = V_i - V_a + V_{a,b} + V_{a,ab}$, and $\gamma = V_i J_i$. Notice that $G = \frac{1}{2}$ when $V_a/V_i = (2J_{a,1} + 1)/(2J_{a,1} + 1)$. If $J_{a,1}$ and $J_{a,2}$ are both $\ll 1$, then 50% of the TF is active when $V_a/V_i = 1$. Moreover, Goldbeter and Koshland (1981) showed that $G$ is a sharply sigmoidal function of $V_a/V_i$ if $J_{a,1}$ and $J_{a,2}$ are $\ll 1$.

In our model, this behavior shows up as a sharp activation of SBF by Cln3-dependent kinase, as the cell grows through a critical size. To calculate the size at which $[\text{SBF}] = \frac{1}{2}$, we set $V_a = V_i$

$$k_{a,\text{ab}}[[\text{Cln2}] + e_{a,b,\text{n3}}[[\text{Cln3}]^* + [\text{Bck2}]] + e_{a,b,\text{ld}}[\text{Clb5}]] = k_{a,\text{ab}} + k_{a,\text{ld}}[\text{Clb2}]$$

Noting that $[\text{Cln2}] \approx [\text{Clb5}] \approx [\text{Bck2}] \approx 0$ in G1, we find that $[\text{SBF}] = \frac{1}{2}$ when

$$k_{a,\text{ab}} \cdot e_{a,b,\text{n3}} \left( [\text{Cln3}]_{\text{max}} \cdot \frac{D_a \cdot \text{mass}}{J_{a,n3} + D_a \cdot \text{mass}} + [\text{Bck2}]^\gamma \cdot \text{mass} \right) = k_{a,\text{ab}}$$

or, given the parameter values listed in Table 2,

$$\frac{3 \cdot \text{mass}}{6 + \text{mass}} + 0.4 \cdot \text{mass} = 1$$

The solution of this quadratic equation is mass $\approx 1.2$. This is the critical size for SBF activation; in wild-type cells, all other events of Start occur in rapid succession after the cell activates SBF. (In the full model, SBF activates at a slightly smaller size, presumably because of the residual activities of the other kinases in G1, which we neglected in this calculation.)
ACKNOWLEDGMENTS

We benefited greatly at various stages from conversations with Kim Nasmyth, Attila Toth, Jill Sible, Ray Deshaies, and Fred Cross and from the diligence of the anonymous referees. This work was supported by US National Science Foundation grants MCB-9600536 and DBI-9724085, National Science Foundation of Hungary grant T-022182, and Howard Hughes Medical Institute grant 75195-542501.

REFERENCES


Modeling the Budding Yeast Cell Cycle


