

# A Mathematical Description of Regulation of the G1-S Transition of the Mammalian Cell Cycle

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**Abstract:** A mathematical model of regulation of the G1-S transition of the mammalian cell cycle has been formulated to organize available experimental molecular-level information in a systematic quantitative framework and to evaluate the ability of this manifestation of current knowledge to calculate correctly experimentally observed phenotypes. This model includes nine components and includes cyclin-cdk complexes, a pocket protein (pRb), a transcription factor (E2F-1), and a cyclin-cdk complex inhibitor. Simulation of the model equations yields stable oscillatory solutions corresponding to cell proliferation and asymptotically stable solutions corresponding to cell cycle arrest (quiescence). Bifurcation analysis of the system suggests changes in the intracellular concentrations of either E2F or cyclin E can activate cell proliferation and that co-overexpression of these molecules can prevent cell proliferation. Further analysis suggests that the amount of inhibitor necessary to prevent cell proliferation is independent of the concentrations of cyclin E and E2F and depends only on the equilibrium ratio between the bound and unbound forms of the inhibitor to the complex. © 1999 John Wiley & Sons, Inc. *Biotechnol Bioeng* 65: 631–637, 1999.

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## INTRODUCTION

The quest to understand development and tumor biology has motivated extensive research on control of transit of the mammalian cell cycle. Many molecular-level interactions involved in regulation of the G1-S and the G2-M transitions or “checkpoints” of the eukaryotic cell cycle have been elucidated (reviewed in Hartwell, 1995; Elledge, 1996; and Fussenegger and Bailey, 1998), and additional components and interactions continue to emerge from ongoing research. Rigorous definition of particular molecular features of the regulatory network involved in cell-cycle control has necessarily relied on careful experimental studies of small subsets of molecules and processes extracted from this network. The assembly of information on network subsets into a picture of the entire network is a critical contribution of such

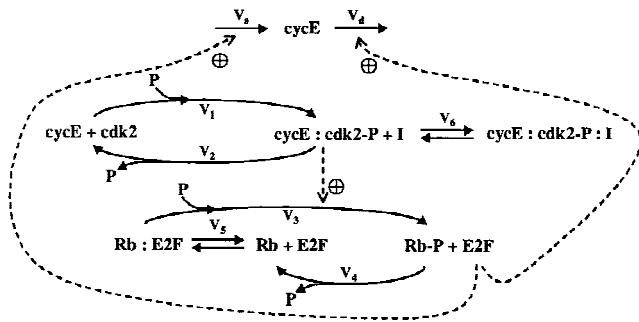
research. However, this picture alone does not completely define how the integrated, dynamic network functions. This can only be achieved by an explicit, unambiguous synthesis of the subset information into a quantitative description of the entire network, and subsequent investigation of this model of the network.

Such an investigation of a network model answers one vital question: Does the existing body of knowledge on cell-cycle regulation, integrated systematically into a larger framework, suffice to describe existing experimental observations of the entire network? Failure indicates error in description of a subsystem, or omission of important components in the overall picture. Success means the model can reasonably be employed to make predictions about the network which suggest new experiments to test the validity of the hypothesized network, or applications such as potential cancer targets or modification of cell culture processes.

At the protein level, there are several important elements involved in regulation of the G1/S transition. Broadly, the G1 cyclins (such as cyclin D1, cyclin E, and others) are known to form phosphorylated complexes with a family of kinases known as cyclin dependent kinases or “cdk’s” (for example cdk2, cdk4, cdk6, and others). These cyclin-cdk phosphorylated complexes, which can be negatively regulated by another class of proteins (cyclin dependent kinase inhibitors or cdi’s), can phosphorylate so-called “pocket proteins” such as p107, pRb, and p300. Phosphorylation can occur at multiple sites and in a temporally distinct manner (Elledge, 1996). In particular, hyperphosphorylation of pRb is nearly coincidental with release of free E2F-1. The free transcription factor is active and able to activate transcription of genes involved in S phase activities including DNA polymerase and thymidine kinase (Sherr, 1996).

The network depicted in Fig. 1 contains representations of some of the components and interactions currently believed to be most important in controlling the G1-S transition. Included in this description are nine components which include cyclin E (Nasmyth, 1996; Lees et al., 1992; LaThangue, 1994; Ohtsubo and Roberts, 1993) which can form phosphorylated complexes with cyclin dependent kinase 2 (cdk2) which can subsequently phosphorylate pocket

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**Figure 1.** Schematic representation of the molecular mechanism considered (see text for explanation).

proteins, represented here by Rb (reviewed in Sherr, 1994; Hartwell and Kastan, 1994; Peeper et al., 1994). Although the cyclin E-cdk2 phosphorylated complex is short-lived in the mammalian cell cycle, it plays an important function at the G1-S checkpoint in that it contributes to pRb phosphorylation which leads to E2F release. This complex is essential for entry into the S phase in mammalian cells which lack a functional pRb (Ohtsubo et al., 1995), and it is essential for completion of G1 in *Drosophila* (Knoblich et al., 1994). To account for this role, the model assumes that cyclin E-cdk2 phosphorylated complex promotes the formation of free E2F. The active cyclin E-cdk2 complex can be inhibited by binding to cdi (*I*) as depicted in Fig. 1 (Sherr and Roberts, 1995). In this work, cyclin E is assumed to be produced at a rate  $V_s$  and to be degraded at a rate  $V_d$ . We further assume that  $V_d$  increases during S phase; thus  $V_d$  increases with increasing E2F concentration. The concentration of E2F can be further used as an indicator for cell cycle position as it peaks at the G1/S transition.

$V_1$  and  $V_2$  are the rates of formation and degradation of the cyclin E-cdk2 phosphorylated active complex (see Fig. 1) where we assume that the phosphorylation (or dephosphorylation) occurs much faster than the association of cycE with cdk2. The cyclin E-cdk2 active complex promotes the reaction with rate  $V_3$  which results in the formation of free E2F.  $V_4$  represents the rate of the set of reactions which dephosphorylates the hyperphosphorylated form of pRb (Rb-P) which leads to the formation of the hypophosphorylated form of pRb (Rb).  $V_5$  represents the reversible binding of E2F to pRb that leads to the formation of the inactive pRb:E2F complex and  $V_6$  represents the reversible binding of the inhibitor to the cyclin E-cdk2 phosphorylated complex.

Formulation of a mathematical embodiment of the regulatory mechanism shown in Fig. 1 is aided by prior research on modeling cell-cycle control (Obeyesekere et al., 1995), most of which has focused on the G2-M transition (Norel and Agur, 1991; Novak and Tyson, 1993a; Goldbeter, 1991). Goldbeter's model reasonably postulates involvement of phosphorylation-dephosphorylation cascades (MacNeill and Fantes, 1995), and special attention to phosphorylation of key species is included in this present G1-S model. Novak and Tyson (1993b) formulated a set of dif-

ferential equations centered around calculation of the level of maturation promoting factor in the cell. The resulting model provides exceptional agreement with experimentally observed phenomena. The model presented here for regulation of the G1-S transition is similarly based on intracellular mass conservation equations for key components of the regulatory network. Both the Goldbeter and the Tyson and Novak studies emphasized thresholds and triggers, concepts examined here in terms of bifurcations of model solutions.

We earlier presented a preliminary G1-S transition model based on these concepts (Hatzimanikatis et al., 1995). There, a simpler regulatory network lacking cdi effects was considered. Also, we introduced there a consideration of expanding cell volume effects in combination with the regulatory network. Consideration of changing cell volume substantially complicates analysis and computation using the model. For this reason, and because localization of the components in Fig. 1, and the changes with time in the pertinent subcellular compartments, are not well known, we consider here a more complex biochemical regulatory network operating in a single well-mixed compartment of time-invariant volume.

## MATHEMATICAL MODEL

Based on the above molecular model we can formulate a mathematical model which consists of the following set of ordinary differential equations that describe the dynamic mass balances of the participating species:

$$\frac{dC}{dt} = V_s - V_1 + V_2 - V_d \quad (1)$$

$$\frac{dK}{dt} = V_2 - V_1 \quad (2)$$

$$\frac{dK_P}{dt} = V_1 - V_2 + V_{6,r} - V_{6,f} \quad (3)$$

$$\frac{dK_{P,I}}{dt} = V_{6,f} - V_{6,r} \quad (4)$$

$$\frac{dR_P}{dt} = V_3 - V_4 \quad (5)$$

$$\frac{dR}{dt} = V_4 - V_3 + V_{5,r} - V_{5,f} \quad (6)$$

$$\frac{dR_E}{dt} = V_{5,f} - V_{5,r} - V_3 \quad (7)$$

$$\frac{dE}{dt} = V_3 - V_{5,r} + V_{5,f} \quad (8)$$

$$\frac{dI}{dt} = V_{6,r} - V_{6,f} \quad (9)$$

where  $C$  is the cyclin E concentration;  $K$  is the cdk2 concentration;  $K_P$  is the phosphorylated cyclin E-cdk2 complex

concentration;  $K_{p,1}$  is the concentration of cyclin E-cdk2 phosphorylated complex bound to inhibitor,  $R$  is the concentration of the hypophosphorylated form of pRb;  $R_p$  is the concentration of the hyperphosphorylated form of pRb;  $R_E$  is the concentration of the hypophosphorylated form of pRb that binds to E2F;  $E$  is the E2F concentration; and  $I$  is the concentration of the of cyclin E-cdk2 complex inhibitor. The subscripts “f” and “r” denote the forward and the reverse step, respectively, of the reversible reactions.

We can further assume that the reversible reactions ( $V_5$  and  $V_6$ ) are operating near equilibrium and that the total amounts of cdk2, pRb, E2F, and inhibitor remain invariant throughout the cell cycle. Under these assumptions, the mathematical model can be further simplified into the following set of ordinary differential and algebraic equations given in a dimensionless form:

$$\frac{d\tilde{C}}{d\tau} = \tilde{V}_s - \gamma\tilde{V}_1 + \gamma\tilde{V}_2 - \tilde{V}_d, \quad (10)$$

$$\frac{d\tilde{K}}{d\tau} = \tilde{V}_2 - \tilde{V}_1, \quad (11)$$

$$\frac{d\tilde{R}_p}{d\tau} = \tilde{V}_3 - \tilde{V}_4, \quad (12)$$

$$\begin{aligned} \tilde{K} + \tilde{K}_p + \tilde{K}_{p,I} &= 1; \quad \tilde{R} + \tilde{R}_p + \tilde{R}_E = 1; \quad \tilde{E} + \sigma\tilde{R}_E = 1; \\ \tilde{I} + \lambda\tilde{K}_{p,I} &= 1, \end{aligned} \quad (13)$$

$$\theta_E = \frac{\tilde{R}_E}{\tilde{R} \cdot \tilde{E}}, \quad \theta_I = \frac{\tilde{K}_{p,I}}{\tilde{K}_p \cdot \tilde{I}}, \quad (14)$$

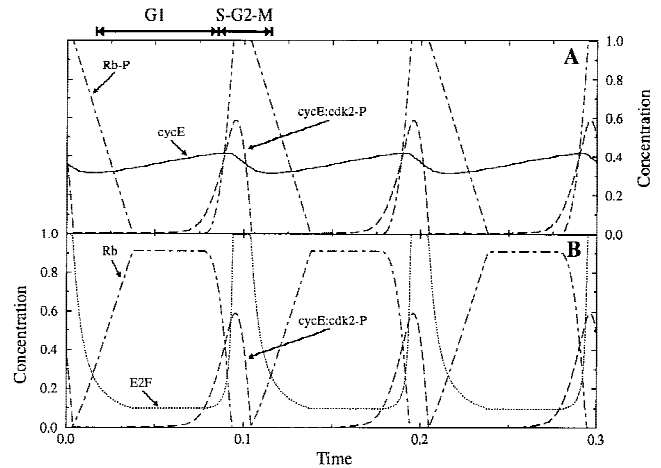
where tilde denotes dimensionless variables and rate expressions,  $\tau$  denotes dimensionless time, and the dimensionless parameters  $\gamma$ ,  $\sigma$ ,  $\lambda$ ,  $\theta_E$ , and  $\theta_I$  are defined in the Appendix.

Eqs. (10)–(12) describe the rate of change of cyclin E, inactive cdk, and hyperphosphorylated Rb, respectively. The equations in (13) give the conservation of mass of cdk, Rb, and inhibitor and the equations in (14) derive from the near-equilibrium of the fast reactions. We use elementary enzyme rate expressions for the irreversible steps (Goldbeter, 1991) to simplify the assumptions needed for the description of the system (Eqs. (10)–(14)) because little quantitative information is available on these processes and interactions. We further describe activation and inhibition of interactions using standard expressions from enzyme kinetics. In this model the sum of all forms of cdk2 is assumed to be invariant through the cell cycle (Pagano et al., 1992). This is also assumed for all forms of pRb and E2F. The simultaneous solution of Eq. (10)–(14) yields a qualitative description of the dynamic behavior of the cell cycle molecular model. Limit-cycle solutions represent conditions that lead to cell cycling; whereas, asymptotically stable solutions correspond to cell-cycle arrest (cell death or quiescence).

## RESULTS AND DISCUSSION

For the parameter values chosen (see Appendix) the biochemical mechanism depicted in Fig. 1 yields oscillations. The parameters were chosen to obtain a reasonable distribution of cell cycle phases in time (i.e., S phase shorter than G1 phase) where the G1-phase is defined as the time for which the amount of hypophosphorylated pRb exceeds the amount of hyperphosphorylated pRb (Hatzimanikatis et al., 1995). As shown in Fig. 2, the time sequence and trajectory of the various components and their interrelationships are consistent with experimentally observed phenomena. In particular, the level of cyclin E varies periodically in time, building to a maximum level before it is degraded relatively quickly in a manner consistent with a late G1-phase maximum (Dulic et al., 1992). Note that the total amount of cyclin E is given by the amount free cyclin E plus the amounts complexed with cdk2 and with cdk2 and inhibitor. The concentration of cyclin E-cdk2 phosphorylated complex also peaks simultaneously with cyclin E, as observed experimentally (Koff et al., 1992). Similarly the level of active pRb peaks at the G1-S transition. The transition from high levels of hypophosphorylated pRb to increasing hyperphosphorylated pRb occurs as the amount of free E2F peaks, consistent with a G1-S phase transition or checkpoint and consistent with prior experiment (Peeper et al., 1994; Nevins, 1992; Hiebert et al., 1992; Helin and Harlow, 1993).

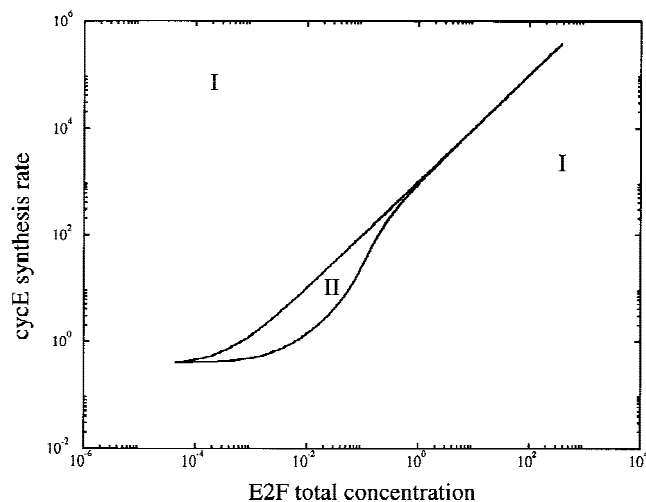
Genetic changes in a cell which affect expression levels or properties of regulatory proteins, or the addition of drugs which interact with one or more of these regulatory proteins,



**Figure 2.** Periodic oscillations of the biochemical mechanism for very low level of total inhibitor concentration. (A) Free cyclin E concentration (solid line), cyclin E-cdk2 active complex concentration (dashed line), and hyperphosphorylated pRb concentration (dashed-dotted line) as functions of time. (B) Level of cyclin E-cdk2 active complex concentration (thick lines), free E2F concentration (dotted line), and hypophosphorylated pRb concentration (dashed-dotted line) as functions of time. Parameters used as in the Appendix except that  $\lambda = 10^7$  (very low level of total inhibitor concentration). G1-phase is defined here as the time for which the amount of hypophosphorylated pRb exceeds the amount of hyperphosphorylated pRb.

correspond to changes in values of the model parameters. In turn, this implies changes in the functions of the cell and corresponding changes in behavior of the model. Particularly important for cell-cycle regulation are changes which result in a transition from cycling to quiescence or vice-versa—which correspond to bifurcations. A bifurcation analysis of two critical model parameters—the cyclin E synthesis rate and the total level of E2F in the cell, which is depicted in Fig. 3, recapitulate prior experiment. The operating diagram suggests that, for certain fixed concentrations of total E2F, increase in cyclin E synthesis rate can cause quiescent cells to begin cell cycling (proliferation). Likewise, overexpression of E2F, for certain synthesis rates of cyclin E, causes a bifurcation from quiescence to proliferation. Experimentally obtained constitutive overexpression of either cloned cyclin E or cloned E2F-1 can result in a transition to proliferation in serum-free medium for Chinese hamster ovary cells (Lee et al., 1995; Renner et al., 1995) and in low serum medium for rat embryo fibroblasts (Singh et al., 1994). Furthermore, inducible expression of cyclin E in Rat-1 cells results in early-entry into S-phase (Resnitzky et al., 1994). The exact shape of region II is based on the chosen parameters and is therefore tissue specific, although the qualitative nature of region II is not sensitive to alteration in the kinetic and equilibrium parameters.

The bifurcation map of Fig. 3 suggests that overexpression of cyclin E or E2F can result in uncontrolled cell growth, one of the hallmarks of cancer. Indeed, deregulated expression of these two molecules have been implicated in a wide array of cancers (Hartwell and Kastan, 1994; Keyomarsi and Pardee, 1993). Interestingly, the bifurcation map also predicts that substantial overexpression of cyclin E,

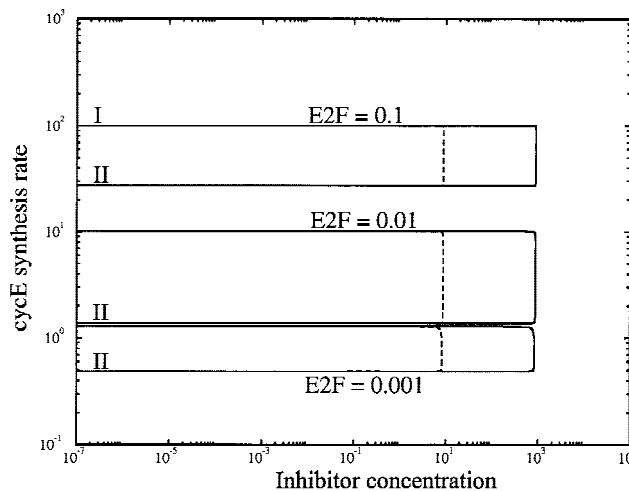


**Figure 3.** Bifurcation analysis of the system of Eqs. (10)–(14). Two regions (I and II) are depicted in the operating diagram. Phase I depicts combinations of constitutive cyclin E synthesis rate and total E2F concentration for which the system does not oscillate (cell cycle arrest) and phase II corresponds to combinations for which the system oscillates (cell cycling). The parameter values used are given in the Appendix. The bifurcation parameters (constitutive cyclin E synthesis rate and E2F total concentration) are presented as relative changes from their reference values.

E2F, or both of these regulatory proteins can result in cell cycle arrest. These results are consistent with observations from E2F-1 overexpression in human gliomas where this genetic manipulation arrests the growth of human gliomas in vivo (Fueyo et al., 1998). This prediction could be tested further by expressing cyclin E or both genes in a construct affording inducible expression of these two genes. Our results further suggest that high level constitutive overexpression of cyclin E or E2F can lead to cell-cycle arrest (and apparent death). This implies a selection *against* cells successfully transfected with an unregulated cyclin E or E2F expression vector at very high levels of cyclin E or E2F expression. Therefore, cells with significant overexpression of these cell cycle regulatory molecules may not be selected for in transfection studies as we predict that these cells will not proliferate in the presence of antibiotic despite having significantly elevated levels of cyclin E or E2F.

Since the model simulates well qualitative characteristics of cell proliferation responses to different levels of cyclin E and E2F-1, we expanded model calculations and analysis to predict how different levels of a cyclin-cdk complex inhibitor affect transitions between proliferation and quiescence. Bifurcation analysis (Fig. 4) reveals that for given values of all other model parameters, an increase in the concentration of inhibitor will not affect cell proliferation (qualitatively) until a critical inhibitor concentration (threshold concentration) is reached. Once this level of inhibitor is reached, cell-cycle arrest occurs.

These model predictions indicate that the threshold level of inhibitor required to stop cell cycling is independent of



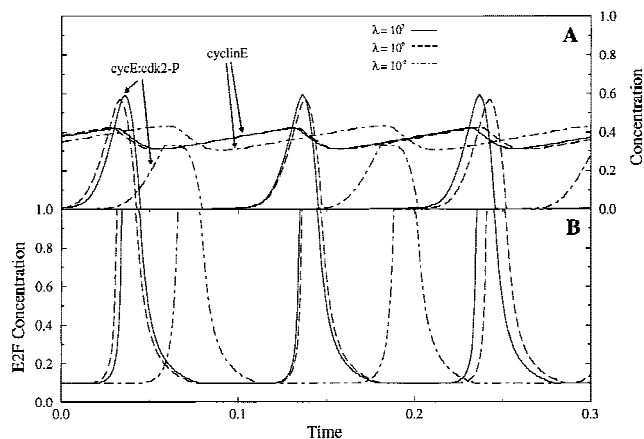
**Figure 4.** Bifurcation analysis of the system of Eqs. (10)–(14) with respect to inhibitor concentration. Phase I corresponds to combinations of constitutive cyclin E synthesis rate and total inhibitor concentration for which the system does not oscillate and phases II correspond to combinations for which the system oscillates for different values of total E2F concentration (values shown on the graph) and of inhibitor-cdk2/cycE association constant ( $\theta_1$ ) (1 (solid lines), 100 (dashed lines)). The parameter values used are given in the Appendix. The bifurcation parameters (constitutive cyclin E synthesis rate, E2F total concentration, and total inhibitor concentration) are presented as relative changes from their reference values.

the total amount of E2F and of the cyclin E synthesis rate. Further analysis reveals that, for a given level of total E2F, the inhibitor arrest threshold is independent of the cyclin E concentration but does depend on the equilibrium ratio,  $\theta_p$ , between the inhibited cyclin-cdk complex and the uninhibited cyclin-cdk complex. This observation suggests that mutations that result in an increase in affinity for an inhibitor will decrease the threshold level of inhibitor required for cell-cycle arrest. Thus, the model predicts that the intracellular level of a cdi affects the proliferation if a particular threshold is crossed, where the threshold is defined by the biochemical properties of the regulatory network. Increasing cdi level without reaching the threshold will not arrest cancer cells. Conversely, cells that are arrested because they are beyond the inhibitor threshold cannot be brought back to cycling by varying the synthesis rate of cyclin E or the expression of E2F.

Figure 4 also suggests that, for a given concentration of inhibitor below the threshold, increasing cyclin E or E2F can act to effect cell cycle arrest. Alternatively, cells that are proliferating because of cyclin E deregulation (to any degree) or because of E2F deregulation (to any degree), can be arrested by adding the same amount of inhibitor in each case. That is, the increase in cdi level needed to effect cell cycle arrest is independent of the levels of cyclin E and E2F expression. Based on this model, the one variable that does appear to have an effect on the amount of inhibitor needed is the value of the previously mentioned equilibrium constant ( $\theta_p$ ) which is tissue specific and/or subject to variation by mutation. An increase in the equilibrium constant of 2 orders of magnitude lowers the amount of inhibitor necessary to effect cell cycle arrest. However, these calculations suggest that the threshold effect of the cdi's is independent of this value.

It has been suggested (Soos et al., 1996) that the appearance of thresholds requires inhibitor binding relationships more complicated than the one employed here in Eq. (14), which follows from the reasonable assumption of equilibrium binding of a single inhibitor molecule to the cycE:cdk2-P complex in dilute solution. This model clearly exhibits thresholds, as frequently observed in experimental studies of cell cycle control (Sherr and Roberts, 1995; Toyoshima and Hunter, 1994; Polak et al., 1994; Zhang et al., 1994; Harper et al., 1995).

The responses of the cell cycle to different amounts of inhibitor below the threshold level were also studied via model simulations (Fig. 5). The oscillating concentration of free cyclin E and E2F are not sensitive to inhibitor concentration, nor are doubling time (the period of the oscillation) or G1-phase duration. The major cell cycle effect of changed cdi level is on the level of the cyclinE-cdk2 active complex. These model predictions suggest that when screening for cyclinE-cdk2 complex inhibitors, the candidate molecules are best evaluated with respect to the level of the cyclinE-cdk2 active complex and not with respect to the effect on the doubling time or levels of any of the other regulatory components.



**Figure 5.** Effects of inhibitor on cell cycle oscillations. (A) Free cyclin E concentration (thin lines) and level of cyclinE-cdk2 active complex concentration (thick lines) as functions of time. (B) E2F concentration as function of time. Parameters used as in Appendix except  $\lambda = 10^7$  (solid lines),  $\lambda = 1$  (dashed lines), and  $\lambda = 10^{-2}$  (dashed-dotted lines).

The main structural features of our model that are responsible for oscillations, for a given set of parameter values, are the activation of cyclin E degradation by free E2F, that is, the reactions characterized by  $V_1$  and  $V_2$ , and the size of the phosphorylation cascade. It can be shown (by nonlinear stability analysis; Hatzimanikatis and Lee, 1999) that if cyclin E and cdk2 were the only species studied and  $V_1$  and  $V_2$  had been omitted from the mass balance equation of cyclin E, then there is no set of parameter values that could lead to oscillations. This further suggests that the length of the phosphorylation cascade and the formation of the cyclin E-cdk2 phosphorylated complex might play a structural/functional role in cell cycle robustness.

On the other hand, the parameter values determine the onset of oscillations, as demonstrated by the bifurcation diagrams. What is not shown by bifurcation diagrams is the role of the  $V_m$ 's on the onset of oscillations. One of the requirements for oscillatory behavior is that the values of  $V_{1,m}$  and  $V_{3,m}$  should generally be greater than the values of  $V_{2,m}$  and  $V_{4,m}$ , respectively, for oscillations to exist for a combination of values for the remaining parameters. Physically, this means that the maximum phosphorylation activity/capacity should be greater than the maximum dephosphorylation activity/capacity.

The current model demonstrates excellent agreement with experimentally observed phenomena and can form the basis for future simulations of a wide variety of experimental observations. For example, overexpression of cyclin D1 results in a shorter G1 phase (Resnitzky et al., 1994) and p53 affects the activity of p21 (MacLachlan et al., 1995). By changing the appropriate model parameters one could use Eqs. (10)–(14) as a minimal model to simulate and to examine the benefit of reformulating the model to incorporate these molecules. Indeed, simulations which accelerate  $V_3$  (overexpression of cyclin D1) result in shorter G1 phase (data not shown). We hope that this model can provide a solid foundation for further mathematical study of cell-cycle

control and related areas as well as help to guide experiment while helping to provide insight into the mechanisms of tumorigenesis and possible interventions.

## APPENDIX

### I. Dimensionless Variables and Parameters

The concentrations of the inactive cdk2, active cyclinE–cdk2 phosphorylated complex, and cdk2/inhibitor species are scaled with respect to the total amount of cdk ( $K_T$ ) as follows:

$$\tilde{K} = \frac{K}{K_T}, \tilde{K}_P = \frac{K_P}{K_T}, \tilde{K}_{P,I} = \frac{K_{P,I}}{K_T}.$$

The total amount of inhibitor concentration ( $I_T$ ) is used for scaling of the inhibitor concentration:  $\tilde{I} = I/I_T$ .

The concentration of the various forms of pRb (hypophosphorylated, hyperphosphorylated, and pRb/E2F complex) are scaled with respect to the total amount of pRb ( $R_T$ ) as follows:

$$\tilde{R} = \frac{R}{R_T}, \tilde{R}_P = \frac{R_P}{R_T}, \tilde{R}_E = \frac{R_E}{R_T}.$$

The concentration of the cyclin E is scaled with respect to an arbitrary reference concentration,  $C'$ . The Greek letters in Eqs. (10)–(14) are defined as ratios of the reference concentrations:

$$\gamma = \frac{K_T}{C'}, \sigma = \frac{R_T}{E_T}, \lambda = \frac{K_T}{I_T}.$$

The dimensionless equilibrium constants are defined as:

$$\theta_E = q_E E_T \text{ and } \theta_I = q_I I_T.$$

Time is scaled with respect to the reference cyclin E concentration and to the kinetic constant of the first order cyclin E degradation (see below),  $V_{d,m}$ , as follows:

$$\tau = tC'V_{d,m}.$$

### II. Rate Expressions

The following expressions were used to describe the kinetics of the irreversible steps depicted in Fig. 1.

$$\begin{aligned} \tilde{V}_s &= \tilde{V}_{C,s} + \tilde{V}_{s,m} \frac{\tilde{E}}{\tilde{K}_{s,E} + \tilde{E}}, \tilde{V}_d = \tilde{C} + \tilde{V}_{d,E,m} \tilde{E} \frac{\tilde{C}}{\tilde{K}_{d,C} + \tilde{C}}, \\ \tilde{V}_1 &= \tilde{V}_{1,m} \frac{\tilde{C}}{\tilde{K}_{1,C} + \tilde{C}} \cdot \frac{\tilde{K}}{\tilde{K}_1 + \tilde{K}}, \tilde{V}_2 = \tilde{V}_{2,m} \frac{\tilde{K}_P}{\tilde{K}_2 + \tilde{K}_P}, \\ \tilde{V}_3 &= \tilde{V}_{3,m} \tilde{K}_P \frac{\tilde{R}_E}{\tilde{K}_3 + \tilde{R}_E}, \tilde{V}_4 = \tilde{V}_{4,m} \frac{\tilde{R}_P}{\tilde{K}_4 + \tilde{R}_P}. \end{aligned}$$

The rate of cyclin E synthesis ( $V_s$ ) is described by two terms: one for constitutive expression and one for the E2F-induced expression (Geng et al., 1996). The degradation rate

of cyclin E includes a first order degradation rate and a term that depends on E2F that qualitatively describes the cell-cycle dependent cyclin degradation. The kinetics for the phosphorylation ( $V_1$  and  $V_3$ ) and dephosphorylation ( $V_2$  and  $V_4$ ) steps are assumed to follow Michaelis–Menten kinetics (Goldbeter, 1991.) Moreover, first-order kinetics have been assumed for the activation of the phosphorylation of pRb ( $V_3$ ) by the cyclin E–cdk2 phosphorylated complex ( $K_P$ ).

The dimensionless kinetic parameters are defined as follows:

$$\begin{aligned} \tilde{V}_{C,s} &= \frac{V_{C,s}}{C'V_{d,m}}, \tilde{V}_{s,m} = \frac{V_{s,m}}{C'V_{d,m}}, \tilde{K}_{s,E} = \frac{K_{s,E}}{E_T}, \\ \tilde{V}_{d,E,m} &= \frac{V_{d,E,m}E_T}{C'V_{d,m}}, \tilde{K}_{d,C} = \frac{K_{d,C}}{C'}, \\ \tilde{V}_{1,m} &= \frac{V_{1,m}}{K_TV_{d,m}}, \tilde{V}_{2,m} = \frac{V_{2,m}}{K_TV_{d,m}}, \\ \tilde{K}_{1,C} &= \frac{K_{1,C}}{C'}, \tilde{K}_1 = \frac{K_1}{K_T}, \tilde{K}_2 = \frac{K_2}{K_T}, \\ \tilde{V}_{3,m} &= \frac{V_{3,m}K_T}{R_TV_{d,m}}, \tilde{V}_{4,m} = \frac{V_{4,m}}{R_TV_{d,m}}, \tilde{K}_3 = \frac{K_3}{R_T}, \tilde{K}_4 = \frac{K_4}{R_T}. \end{aligned}$$

### III. Reference Parameter Values

The reference values of the dimensionless kinetic parameters used for the numerical analyses are as follows:

$$\begin{aligned} \tilde{V}_{C,s} &= 1, \tilde{V}_{s,m} = 1, \tilde{K}_{s,E} = 0.1, \tilde{V}_{d,E,m} = 1000, \tilde{K}_{d,C} = 0.005, \\ \tilde{V}_{1,m} &= 50, \tilde{V}_{2,m} = 40, \tilde{K}_{1,C} = 0.1, \tilde{K}_1 = 0.0001, \tilde{K}_2 = 0.0001, \\ \tilde{V}_{3,m} &= 3000, \tilde{V}_{4,m} = 3, \tilde{K}_3 = 0.0001, \tilde{K}_4 = 0.0001. \end{aligned}$$

The reference values of the ratios of the concentrations and the equilibrium constants are as follows:

$$\gamma = 1, \sigma = 10, \lambda = 1, \tilde{q}_E = 0.01, \text{ and } \tilde{q}_I = 1.$$

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