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Linking Cell Division to Cell Growth in a Spatiotemporal Model of The Cell Cycle

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Abstract

Cell division must be tightly coupled to cell growth in order to maintain cell size, yet the mechanisms linking these two processes are unclear. It is known that almost all proteins involved in cell division shuttle between cytoplasm and nucleus during the cell cycle; however, the implications of this process for cell cycle dynamics and its coupling to cell growth remains to be elucidated. We developed mathematical models of the cell cycle, which incorporate protein translocation between cytoplasm and nucleus. We show that protein translocation between cytoplasm and nucleus not only modulates temporal cell cycle dynamics, but also provides a natural mechanism coupling cell division to cell growth. This coupling is mediated by the effect of cytoplasmic-to-nuclear size ratio on the activation threshold of critical cell cycle proteins, leading to the size-sensing checkpoint (*sizer*) and the size-independent clock (*timer*) observed in many cell cycle experiments.

Keywords

Protein translocation; cell cycle; cell size; nuclear size; mathematical model

INTRODUCTION

Biologically, it is critical for dividing cells to time their mitosis appropriately with respect to cell size. If the cell divides before doubling its mass, the size of daughter cells will progressively shrink, whereas if the cell divides after size doubling, cell mass will progressively increase. To maintain proper cell size, a tight coupling between cell division and cell growth is essential. Mathematical modeling studies (1–11) have shown that the nonlinear dynamics of bistability and limit cycles underlie temporal regulation of cell cycle checkpoints and progression. However, the mechanisms linking these cell cycle events to cell growth are unclear (12–14). Experimental studies (15–18) in yeast have shown that a cell has to grow to a critical size to begin DNA replication and cell division. It has also been shown that when a cell's birth size is smaller than the critical size, the cycle time consists of two components: a *sizer* phase, which is the time for the cell to reach the critical size, followed by a *timer* phase, which is a constant period nearly independent of cell size. Similar size control of the cell cycle also exists in higher eukaryotes such as in *Xenopus laevis* (19,20), *Drosophila* (21), animal cells (22), and HeLa cells (23). Other experimental studies (24–29) have demonstrated the cytoplasmic-to-nuclear volume ratio to be an important factor coordinating cell size and cell division. In previous

modeling studies, cell size or cell mass has been *phenomenologically* set as a parameter modulating other rate constants without experimental justification. Although the sizer and timer phases (7), the cycle time versus cell size in yeast (4,9), or cytoplasm-to-nuclear volume ratio (5,30), can be readily simulated in this type of modeling, the biological mechanisms remain unclear.

To date, cell cycle models have only taken into account the *temporal* regulation of the cell cycle proteins, i.e., how different cyclins, cyclin-dependent kinases (CDKs), and other cell cycle proteins are activated and inactivated in an ordered manner to control the transitions between the different cell cycle phases (G1, S, G2, and M). However, these proteins are also *spatially* regulated during the cell cycle by shuttling between the cytoplasm and nucleus to carry their biological functions (31–34). How spatial protein translocation interacts with temporal regulation to control the cell cycle dynamics has not been elucidated. Accordingly, in this study, we developed mathematical models that include both temporal and spatial protein regulation in the transition from G2 to M phases. We show that protein translocation between cytoplasm and nucleus not only modulates the temporal dynamics of the cell cycle, but also provides a natural mechanism coupling cell growth to cell division.

MATERIALS AND METHODS

Mathematical modeling

The left panel of Fig.1 shows a schematic diagram of the protein interaction (the *temporal* regulation) for the G2 to M transition. The major proteins or protein complexes are cyclin B1, CDK1, CDC25C, wee1, APC-CDC20, cyclin B1-CDK1, and their various phosphorylated forms. CDK1 is activated (35,36) by binding with its partner cyclin B1, which opens its T-loop for phosphorylation. This phosphorylation is carried out by CDK-activating kinase. CDK1 is inactivated by phosphorylation at Thr 14 and Tyr 15 by wee1/Myt1. CDC25C dephosphorylates these two sites and thus activates CDK1. CDC25C itself is activated after being phosphorylated by active CDK1 while wee1 is inactivated after being phosphorylated by active CDK1, forming positive feedback loops. Active CDK1 also activates protein complex, such as APC-CDC20, for cyclin ubiquitination and degradation, forming negative feedback loops.

The right panel of Fig.1 illustrates the protein translocation between cytoplasm and nucleus (the *spatial* regulation) for the G2 to M transition. For example, cyclin B1 and CDC25C are located primarily in the cytoplasm during interphase, but reside in the nucleus during prophase. CDC25C is sequestered in the cytoplasm by 14-3-3 binding due to phosphorylation at Ser216/278 by chk1 and chk2. Phosphorylation of cyclin B1 and CDC25C causes the rapid import of cyclin B1 and CDC25C into the nucleus through the nuclear pore complex. Other proteins, such as wee1 and APC-CDC20, also shuttle between cytoplasm and nucleus (31, 32,37). Although wee1 was found to be mainly nuclear (38), but it was also found in the cytoplasm (39,40) during interphase. Detailed description of the mathematical model and numerical simulation methods are presented in the Appendix. Here we briefly summarize the key modeling aspects.

Spatially extended model—We developed a spatially extended model in which these proteins and their phosphorylated forms are temporally regulated as shown in the left panel of Fig.1, and spatially regulated by passive diffusion in the cytoplasm and nucleus, and active import into and export from the nucleus. The governing partial differential equations for the proteins in the cytoplasm and nucleus have the following forms:

$$\begin{aligned}\frac{\partial p_q^c}{\partial t} &= k_{s,q} + f(p_q^c, \dots) - k_{d,q}^c p_q^c + D_q^c \nabla^2 p_q^c, \\ \frac{\partial p_q^n}{\partial t} &= f(p_q^n, \dots) - k_{d,q}^n p_q^n + D_q^n \nabla^2 p_q^n,\end{aligned}\quad (1)$$

where p_q^c is the q^{th} -type or form of protein or protein complex in the cytoplasm, and p_q^n is that in the nucleus. $k_{s,q}$ is the protein synthesis rate of the q^{th} protein. We assume that the proteins are synthesized only in the cytoplasm (41), and selectively imported into the nucleus through nuclear pore complexes (Fig.1). $f(p_q, \dots)$ is a function describing protein interactions, which we assume to be the same whether occurring in the cytoplasm and nucleus. $k_{d,q}^c$ and $k_{d,q}^n$ are the protein degradation rates in the cytoplasm and the nucleus, respectively. D_q^c and D_q^n are the diffusion constants in the cytoplasm and nucleus, respectively.

We assume that no protein flux occurs through the cell membrane, i.e., no-flux boundary was

used at the cell membrane: $\frac{\partial p_q^c}{\partial \vec{n}} = 0$, where $\vec{n}$ is the unit vector of the normal direction of the cell membrane. At the interface between cytoplasm and nucleus, flux boundary was used to account for the protein import to and export from the nucleus, i.e.,

$$\begin{aligned}D_q^c \frac{\partial p_q^c}{\partial \vec{n}} &= J_{n \rightarrow c} - J_{c \rightarrow n} = \beta_q p_q^n - \alpha_q p_q^c, \\ D_q^n \frac{\partial p_q^n}{\partial \vec{n}} &= J_{c \rightarrow n} - J_{n \rightarrow c} = \alpha_q p_q^c - \beta_q p_q^n,\end{aligned}\quad (2)$$

where $J_{a \rightarrow c}$ is the protein flux from nucleus to cytoplasm, $J_{c \rightarrow n}$ is the flux from cytoplasm to nucleus, and α_q and β_q are the corresponding transport rates. The specific rates depend on the phosphorylation status of the protein or protein complex, and their localization during the cell cycle.

Simplified two-compartment model—We assume that diffusion of proteins within either the cytoplasmic or nuclear compartments are fast, so that the spatial model can be simplified into a two-compartment model. We can then change the partial differential equations (Eq.1), combining Eq.2, into the following ordinary differential equations:

$$\begin{aligned}\frac{dp_q^c}{dt} &= k_{s,q} + f(p_q^c, \dots) - k_{d,q}^c p_q^c + \frac{(\beta_q p_q^n - \alpha_q p_q^c) S_n}{V_c}, \\ \frac{dp_q^n}{dt} &= f(p_q^n, \dots) - k_{d,q}^n p_q^n + \frac{(\alpha_q p_q^c - \beta_q p_q^n) S_n}{V_n},\end{aligned}\quad (3)$$

where S_n is the surface area of the nuclear envelope, and V_c and V_n are the cytoplasmic and nuclear volumes, respectively.

Computer simulation

The differential equations were numerically solved using programs coded in Fortran 77. For the partial differential equation of the *spatially extended model*, we used Forward Euler method. For the ordinary differential equation of the *two-compartment model*, we used fourth-order Runge-Kutta method. Variable time step was used for both cases.

RESULTS

Effects of protein translocation between cytoplasm and nucleus on cell cycle dynamics

Figure 2 compares experimental data to the results of computer simulations using the spatially extended cell cycle model of Eq.1. Fig.2A shows the average cytoplasmic and nuclear concentrations of cyclin B1 and active cyclin B1-CDK1 (MPF) versus time, illustrating that cyclin B1 and its kinase activity oscillated to drive cell cycle progression. Fig.2B shows simulated snapshots of cyclin B1 localization in the cell at different phases around mitosis, in comparison to actual photomicrographs from experimental observations in Fig.2C. Cyclin B1 concentrations progressively increased and plateaued at a high level in the cytoplasm, but not in the nucleus, prior to mitosis. At the beginning of mitosis, the cytoplasmic MPF activity was low but gradually increased until sufficient to trigger rapid cyclin B1 translocation into the nucleus, resulting in high nuclear MPF and an abrupt decrease of cytoplasmic cyclin B1. At the end of mitosis, cyclin B1 was rapidly degraded in the nucleus, reducing MPF activity to a very low level. The normalized whole cell concentrations of cyclin B1 and the nuclear cyclin B1 in our simulation agree well with previous experimental results (Fig.2D) of Clute and Pines (42). Our model also simulated the translocation of CDC25C, wee1, and APC (Fig.3), observed experimentally (31,32,37).

To investigate how protein translocation into the nucleus affects cell cycle dynamics, we simplified the spatially extended model (Eq.1) into a two-compartment model (Eq.3) to reduce computations. The simplified two-compartment model agreed well with the spatially extended model when diffusion was assumed to be fast in both nuclear and cytoplasmic compartments (see Appendix for more details). We then compared the dynamics of the cell cycle signaling network diagrammed in Fig. 1 for a single compartment model, versus a two-compartment model with protein translocation. In the single compartment model, the dynamics were similar to the temporal models previously developed by us and others (1,7,9,11,30). Positive feedback caused bistability and negative feedback caused the system to oscillate following a hysteretic loop. Hysteresis in CDK activity has been experimentally confirmed in *Xenopus laevis* when cyclin B was mutated to be non-degradable (43,44).

In the two-compartment model, the dynamics were qualitatively similar, but quantitatively altered. Bistability was still the key feature of the system, as seen when cyclin B1 was “mutated” so that it could not be degraded through the APC-CDC20 signaling pathway, thereby eliminating the negative feedback loop in the model. By varying the cyclin B1 synthesis rate (to control the total cyclin B1), we obtained the bistable steady state relationship between cytoplasmic MPF, nuclear MPF, and total cyclin B1, as shown in Figs. 4A and B. When total cyclin concentration was low, MPF in both cytoplasm and nucleus were very low. But when the total cyclin reached a critical value (around 35 nM), the cytoplasmic and nuclear MPF jumped to high levels. It is worth noting that MPF levels in cytoplasm (1.5 nM) were much lower than in the nucleus (>60 nM). This is because, once cytoplasmic MPF reached a critical value, it triggered the rapid phosphorylation of cyclin B1 and other proteins, facilitating their import into the smaller volume of the nucleus, producing a concentrating effect. This leads to a rapid rise in nuclear MPF activity, while suppressing cytoplasmic MPF activity (if protein translocation between cytoplasm and nucleus was blocked, the cytoplasmic MPF could also reach a much higher level >20 nM).

When negative feedback due to degradation of cyclin B1 by APC-CDC20 was restored into the model, a Hopf bifurcation leading to limit cycle oscillation occurs in the model as the synthesis rate of cyclin B1 increases (Figs.4 C and D). Fig.4E shows a trajectory of the limit cycle in a three-dimensional phase space. At the beginning of the cycle, the total cyclin was below the critical value due to cyclin degradation in the previous cycle, and MPF in both cytoplasm and nucleus remained low. As total cyclin increased as time (arrow 1) to the critical

value (~40 nM, close to that in Figs.4A and B), MPF in the cytoplasm increased abruptly (to ~5 nM), while nuclear MPF was still low (arrow 2). After cytoplasmic MPF reached a critical value triggering protein import into the nucleus, nuclear MPF (arrow 3) increased rapidly. Since APC-CDC20 was also activated and imported into the nucleus at the same time, cyclin B1 degradation occurred in the nucleus, bringing the total cyclin and MPF down to a low level (arrow 4). This process automatically repeated itself to give rise to the MPF oscillations driving the cell cycle.

MPF activation and its oscillation were also affected by the nuclear import rate. Fig.4F is a phase diagram illustrating how protein phosphorylation and nuclear import rate alter the oscillatory dynamics. When the protein import rate was very high (large α_H) or the phosphorylation threshold was high (large b_p), MPF oscillation stopped. In the MPF oscillatory regime, when the import rate was very low (small α_H), little or no MPF oscillation occurred in the nucleus, while large amplitudes oscillations occurred in the cytoplasm.

Size control of the cell cycle

A novel dynamics arising from our model is the natural coupling of cell growth to cell division. In Eq.3, the cytoplasm volume (V_c), nuclear surface (S_n), and volume (V_n) are parameters which change as the cell grows. Before the onset of mitosis, the cyclin B1 and CDK1 levels are very low in the nucleus, so we can ignore the nuclear export and approximate the differential equations for these proteins (p_q^c) in Eq.3 as:

$$\frac{dp_q^c}{dt} = k_{s,q} + f(p_q^c, \dots) - \left(k_{d,q}^c + \frac{\alpha_q S_n}{V_c}\right) p_q^c \quad (4)$$

Eq.4 shows that the nuclear import is equivalent to an additional protein removal term for the cytoplasm (degradation + import to the nucleus), which increases the protein synthesis threshold required for CDK1 activation, as shown in previous modeling studies (7,8). As the cell grows, however, V_c increases faster than S_n , so that the effect of nuclear import on protein removal from the cytoplasm becomes weaker. When the cell reaches a critical size, the protein synthesis threshold required for CDK1 activation is reduced below the protein synthesis rate of the system, allowing the activation of MPF.

To demonstrate how the ratio of cytoplasmic to nuclear size affects the activation of MPF, we first examined the case when cyclin B1 was made non-degradable (as in Fig. 4A–B). Fig.5A shows the steady states of cytoplasmic MPF versus cyclin synthesis rate for two different cytoplasmic volumes, with a fixed nuclear radius of 10 μm . The critical cyclin synthesis rate required for MPF activation (indicated by the arrows in Fig.5A) was larger for the smaller cytoplasmic volume. In other words, when cytoplasmic volume was small, the same size nucleus was more effective as a sink for cyclin B1.

Fig.5B shows the case when cyclin B1 was degradable through the negative feedback due to APC-CDC20. The threshold for cyclin B1 synthesis rate at which MPF activation occurred is plotted against the ratio of the cytoplasmic volume to nuclear surface area (V_c/S_n), calculated for two different fixed sizes of the nucleus, $r_n=10 \mu\text{m}$ (filled circles) and $r_n=20 \mu\text{m}$ (open circles). The results show that for a given cyclin B1 synthesis rate, a transition occurred at a critical value of the V_c/S_n ratio which caused MPF to oscillate. The critical values of V_c/S_n ratio were very close for the two nucleus sizes, suggesting that it may be a robust parameter regulating the transition from the sizer phase to the timer phase. Thus, the results shown in Fig. 5B indicate that if a cell is born bigger than the critical size (i.e. V_c/S_n ratio), it is ready for MPF activation right after cell division and can undergo a new round of mitosis without cell growth. However, if the cell is born smaller than the critical size, it needs to grow until it passes

the critical V_c/S_n ratio (see arrows in Figs.5B) for MPF activation. In other words, a size checkpoint (*sizer*) is naturally built in the system due to protein translocation between the cytoplasm and nucleus. After the cell passes the critical size, it enters the limit cycle regime of MPF oscillation (*timer*).

We then tested the ability of the model to reproduce experimental observations by letting the virtual cell grow. In a previous study (45), we showed that cell growth is proportional to its surface area at birth, and for a spherical cell the growth equation is:

$$\frac{dV}{dt} = \mu r_B^2, \quad (5)$$

where V is the cell volume and r_B is the cell radius at birth. For a spherical cell, the solution of Eq.5 is $r = \sqrt[3]{r_B^3 + \lambda r_B^2 t}$. We used $\lambda = 0.28 \mu\text{m}/\text{min}$ for the simulation shown in Fig.6. Using the concepts of *sizer* and *timer* (17,18), we have derived the cell cycle time versus birth size as (45):

$$T = \begin{cases} T_0 + \alpha \frac{r_c^3 - r_B^3}{r_B^2}, & r_B < r_c, \\ T_0, & r_B \geq r_c, \end{cases} \quad (6)$$

where T_0 is the timer period and r_c is the critical cell radius. If a cell is born smaller than r_c , its cycle time is size-dependent, i.e., cycle time is the timer period T_0 plus the time that takes for the cell to grow from r_B to r_c . If the cell is born larger than r_c , then its cycle time is size-independent and simply constant T_0 . We have shown in our previous modeling study {Qu, 2003 #187} that T_0 is the period of the limit cycle oscillation.

We used the two-compartment model to study the effects of cell growth on cell division. The cytoplasm volume $V_c(t) = V(t) - V_n$, where $V(t)$ is the solution of Eq.5. We kept the nuclear volume V_n fixed during the cell cycle. We assumed that cell division occurred when nuclear MPF decreased close to its minimum. At mitosis, a cell divided asymmetrically into two daughter cells with each cell having half the volume of the mother cell with a random 20% variability in cell radius. The nuclear radius of the daughter cell at division was also randomly chosen, varying between 8 μm and 10 μm in Fig.6A and from 8 μm and 12 μm in Fig.6B. Figure 6A shows the cell radius versus time for wild type (solid) and *wee1* mutant (dashed) cell, showing the variation in cell size and duration of the cycle time. The cycle time was longer for a cell born smaller, and *wee1* mutation reduced the overall cell size. Fig.6B plots the cycle time versus cell birth size from our computer simulation for wild type cell. The simulation from our model agrees well with the analytically derived cycle time and birth size relationship of Eq.6 and the experimental observation in *Xenopus laevis* by Wang et al (19,20). Note that the parameters in Fig.6 were rescaled from the simulations of Hela cell shown in Fig.2–Fig.5 to quantitatively fit the *Xenopus* data (see Appendix for detailed parameter settings).

DISCUSSION

In this study, we used mathematical modeling to investigate the effects of protein translocation on spatiotemporal dynamics of cell cycle control. We show that while cell cycle dynamics is driven primarily by temporal protein regulation, it is significantly modulated by protein translocation between the cytoplasm and nucleus. The most important finding, however, is that protein translocation between the cytoplasm and nucleus provides a mechanism which naturally couples cell growth to cell division, including the size-sensing checkpoint (*sizer*) and

the size-independent clock (timer) observed in various cell cycle experiments. Thus, it was not necessary to incorporate phenomenological cell growth-dependence into any of the rate constants in the model; instead, changes in volumes of the cytoplasm and nucleus as the cell grew were all that were required to account for cell growth-dependent features of cell cycle dynamics.

Effects of protein translocation on cell cycle dynamics

Bistability has been experimentally detected in both *Xenopus* oocytes (43,44) and budding yeast (46), and proposed to be the fundamental basis of cell cycle dynamics. Our present study shows that when spatial protein translocation is taken into account, temporal protein regulation is still the primary factor in the genesis of bistable and limit cycle dynamics, but spatial protein translocation acts as a modulator of these dynamics. Previous experiments (47–49) showed that in *Xenopus* egg extracts without nuclei, MPF oscillations still occurred, demonstrating that temporal protein regulation in cytoplasm is sufficient to generate cell cycle dynamics. However, our modeling suggests that the nucleus plays an important modulatory role by acting as an amplifier to increase MPF concentrations to higher levels, due to the differences in cytoplasmic and nuclear volumes. For example, without a nucleus, or if nuclear import and export are blocked, the peak MPF in our model is roughly 10–20 nM, whereas with the nucleus, peak MPF reached 60 nM and above.

Coupling cell division to cell growth

Protein translocation also provides a natural mechanism coupling cell division to cell growth, since a cell has to grow to reach a critical cytoplasmic volume to nuclear surface area ratio in order for MPF to reach its threshold of self-oscillation. Experimental data indicate that cytoplasmic-to-nuclear size ratio is an important factor regulating mitosis (see the review by Masui and Wang (19)), since: 1) Haploid embryos underwent one more synchronous cycle than diploid ones (24,26,28), moreover, *Xenopus* diploid blastomeres ceased division at 17th cycle while haploid stopped at 18th cycle (20); 2) Reducing the cytoplasm volume reduced the number of synchronous cycles (25,26,29); 3) When sperm nuclei were incubated in *Xenopus* egg extracts at 100/ μ L, nuclei underwent cell cycle divisions at intervals equal to the normal cell cycle duration, but cycle time lengthened as nuclei to cytoplasm content increased, and were arrested at S phase at nuclei-to-cytoplasm ratio of 1500/ μ L (27).

Our study shows that the cytoplasmic volume to nuclear surface area ratio (V_c/S_n) may be a critical parameter controlling the onset of nuclear events. However, nuclear volume (V_n) is also a parameter in our modeling equation, which was the reason why the two data sets shown in Fig.5B did not exactly overlap. Nuclear size has also been shown experimentally to affect the initiation of DNA replication (50). An experimental protocol that accurately measures the cell size and the nuclear size simultaneously is necessary to test our theoretical predictions.

In Fig.5, we showed that the threshold for MPF activation depended on cytoplasmic volume-to-nuclear surface area size ratio. However, the specific value of the ratio triggering MPF activation is also set by the synthesis rates of cyclin B1 and other proteins, their degradation rates, and their reaction rates, as shown in many other studies by Novak and Tyson {Novak, 1993 #115; Novak, 1995 #306; Novak, 2004 #298} and by us {Qu, 2003 #187; Qu, 2003 #168}. In other words, altering the rate constants of protein regulation will also alter the critical cytoplasm to nuclear size ratio required to trigger mitosis. This may account for the biological variability in cytoplasm-to-nuclear size ratios in different cell types, and also for the effects of interventions which alter the relationship between cell division and cell growth. For example, overexpressing cyclins D1 and E (51,52) or CDC25A (53) accelerated the transition from G1 to S, while enhanced degradation of CDC25A due to DNA damage delayed this transition (54). This is also true for the G2 to M transition (52). It is conceivable that such *acceleration*

or *delay* in cell cycle phases might be mediated by changes in the critical cytoplasm to nuclear size ratio.

In conclusion, our study is the first attempt to model spatial as well as temporal regulation of the cell cycle, and provides an experimentally testable hypothesis for linking cell growth to cell division.

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Appendix

Appendix

The following text describes in detail the mathematical modeling and computer simulation used in the present study.

I. Mathematical Modeling

Two mathematical models were developed in this study to simulate protein translocation between the cytoplasm and nucleus and its effects on cell cycle dynamics. The first model is a spatially extended model including protein diffusion in the 3-dimensional space. The second one is a two-compartment model which assumes protein diffusion in the cytoplasm and nucleus to be very fast so that one can treat the protein distribution in each compartment to be uniform.

Modeling the protein interaction

The key factors of protein interaction and translocation are illustrated in Fig 1 in the paper. Fig. A1 shows the detailed protein interaction network used in our model, which is similar to our previous model (7). The major proteins or protein complexes are: cyclin B1 (marked as symbol y in Fig.A1), inactive cyclin-CDK (xI), active cyclin-CDK (x), CDC25 (z), *wee1* (w), Myt1 (m), APC-CDC20 (u), and their different phosphorylated forms. The variables used in the differential equations for these proteins and their different phosphorylated forms are indicated in Fig.A1.

We assumed that all proteins were synthesized only in cytoplasm (41) at constant rate, $k_{s,q}$, where q represents y , z , w , m , and u . Each protein has a degradation rate proportional to its concentration, i.e., $k_{d,q}q$, where q represents all the proteins and their different phosphorylated forms. An additional degradation term for free cyclin B1 and its phosphorylated form was used, i.e., $k_{apc}y$. $k_{apc} = C_u u^3$, where u^3 is the three-site phosphorylated and activated APC-CDC20 and C_u is constant. For computational purpose, we simplified multi-step cyclin B1 phosphorylation catalyzed by active CDK1 to a one-step one with the rate constant to be $k_p = a_p(x+x_p)^3$. The phosphorylations of CDC25, *wee1*, Myt1, and APC-CDC20 are all catalyzed by active CDK1 with the rate constant for each phosphorylation step to be $k_q^+ = a_q + b_q(x+x_p)$, where $q=z$, w , m , and u . All dephosphorylation rates were set to be constant, k_q^- . We assumed only three-site phosphorylated CDC25 ($z3$) was active, which dephosphorylates CDK1 at a rate of $k_{cdc25} = c_z Z3$, in which c_z is constant. Similarly, only the unphosphorylated *wee1* or Myt1 is active, which phosphorylates CDK1 at a rate $k_{wee1} = c_w w0$ or $k_{myt1} = c_m m0$, in which c_w and c_m are constants. Unlike *wee1*, Myt1 was assumed to remain only in cytoplasm (32).

The differential equations for the proteins in the cytoplasm are:

$$\frac{\partial y}{\partial t} = k_{s,y} + k_{dp}y_p - k_p y + k_2 x_1 - k_1 y - (k_{d,y} + k_{apc})y + D_y \nabla^2 y$$

$$\frac{\partial x_1}{\partial t} = k_{dp}x_{1p} - k_p x_1 + k_1 y - k_2 x_1 + (k_{wee1} + k_{myt1})x - k_{cdc25}x_1 - (k_{d,x1} + k_{apc})x_1 + D_{x1} \nabla^2 x_1 \quad (A1a)$$

$$\frac{\partial x}{\partial t} = k_{dp}x_p - k_p x + k_{cdc25}x_1 - (k_{wee1} + k_{myt1})x - (k_{d,x} + k_{apc})x + D_x \nabla^2 x$$

$$\frac{\partial y_p}{\partial t} = k_p y - k_{dp}y_p + k_2 x_{1p} - k_1 y_p - (k_{d,y} + k_{apc})y_p + D_{y_p} \nabla^2 y_p$$

$$\frac{\partial x_{1p}}{\partial t} = k_p x_1 - k_{dp}x_{1p} + k_1 y_p - k_2 x_{1p} + (k_{wee1} + k_{myt1})x_p - k_{cdc25}x_{1p} - (k_{d,x1} + k_{apc})x_{1p} + D_{x_{1p}} \nabla^2 x_{1p}$$

$$\frac{\partial x_p}{\partial t} = k_p x - k_{dp}x_p + k_{cdc25}x_{1p} - (k_{wee1} + k_{myt1})x_p - (k_{d,x} + k_{apc})x_p + D_{x_p} \nabla^2 x_p \quad (A1b)$$

$$\frac{\partial zb}{\partial t} = k_{s,z} + k_{2,z}z_0 - k_{1,z}zb - k_{d,z}zb + D_{zb} \nabla^2 zb$$

$$\frac{\partial z_0}{\partial t} = k_{1,z}zb - k_{2,z}z_0 + k_z^- z_1 - k_z^+ z_0 - k_{d,z}z_0 + D_{z_0} \nabla^2 z_0$$

$$\frac{\partial z_1}{\partial t} = k_z^+ z_0 - k_z^- z_1 + k_z^- z_2 - k_z^+ z_1 - k_{d,z}z_1 + D_{z_1} \nabla^2 z_1 \quad (A1c)$$

$$\frac{\partial z_2}{\partial t} = k_z^+ z_1 - k_z^- z_2 + k_z^- z_3 - k_z^+ z_2 - k_{d,z}z_2 + D_{z_2} \nabla^2 z_2$$

$$\frac{\partial z_3}{\partial t} = k_z^+ z_2 - k_z^- z_3 - k_{d,z}z_3 + D_{z_3} \nabla^2 z_3$$

$$\frac{\partial w_0}{\partial t} = k_{s,w} + k_w^- w_1 - k_w^+ w_0 - k_{d,w} w_0 + D_{w0} \nabla^2 w_0$$

$$\begin{aligned} \frac{\partial w_1}{\partial t} &= k_w^+ w_0 - k_w^- w_1 + k_w^- w_2 - k_w^+ w_1 - k_{d,w} w_1 + D_{w1} \nabla^2 w_1 \\ \frac{\partial w_2}{\partial t} &= k_w^+ w_1 - k_w^- w_2 + k_w^- w_3 - k_w^+ w_2 - k_{d,w} w_2 + D_{w2} \nabla^2 w_2 \end{aligned} \quad (\text{A1d})$$

$$\frac{\partial w_3}{\partial t} = k_w^+ w_2 - k_w^- w_3 - k_{d,w} w_3 + D_{w3} \nabla^2 w_3$$

$$\frac{\partial u_0}{\partial t} = k_{s,u} + k_u^- u_1 - k_u^+ u_0 - k_{d,u} u_0 + D_{u0} \nabla^2 u_0$$

$$\begin{aligned} \frac{\partial u_1}{\partial t} &= k_u^+ u_0 - k_u^- u_1 + k_u^- u_2 - k_u^+ u_1 - k_{d,u} u_1 + D_{u1} \nabla^2 u_1 \\ \frac{\partial u_2}{\partial t} &= k_u^+ u_1 - k_u^- u_2 + k_u^- u_3 - k_u^+ u_2 - k_{d,u} u_2 + D_{u2} \nabla^2 u_2 \end{aligned} \quad (\text{A1e})$$

$$\frac{\partial u_3}{\partial t} = k_u^+ u_2 - k_u^- u_3 - k_{d,u} u_3 + D_{u3} \nabla^2 u_3$$

$$\frac{\partial m_0}{\partial t} = k_{s,m} + k_m^- m_1 - k_m^+ m_0 - k_{d,m} m_0 + D_{m0} \nabla^2 m_0$$

$$\begin{aligned} \frac{\partial m_1}{\partial t} &= k_m^+ m_0 - k_m^- m_1 + k_m^- m_2 - k_m^+ m_1 - k_{d,m} m_1 + D_{m1} \nabla^2 m_1 \\ \frac{\partial m_2}{\partial t} &= k_m^+ m_1 - k_m^- m_2 + k_m^- m_3 - k_m^+ m_2 - k_{d,m} m_2 + D_{m2} \nabla^2 m_2 \end{aligned} \quad (\text{A1f})$$

$$\frac{\partial m_3}{\partial t} = k_m^+ m_2 - k_m^- m_3 - k_{d,m} m_3 + D_{m3} \nabla^2 m_3$$

The different equations for the proteins in the nucleus are the same as for the proteins in the cytoplasm except: no protein synthesis ($k_{s,q}=0$) and no Myt1 ($k_{\text{myt1}}=0$).

The differential equations for the two-compartment model are obtained by dropping the diffusion terms in Eq. A1 and adding the rate change terms due to the protein flux through the nuclear pore complex. For example, for the free cyclin B1 (y), the ordinary different equations for cytoplasm and nucleus are:

$$\begin{aligned}\frac{dy^c}{dt} &= k_{s,y} + k_{dp} y_p^c - k_p^c y^c + k_2 x_1^c - k_1 y^c - (k_{d,y} + k_{apc}^c) y^c + (\beta_y y^n - \alpha_y y^c) S_n / V_c \\ \frac{dy^n}{dt} &= k_{dp} y_p^n - k_p^n y^n + k_2 x_1^n - k_1 y^n - (k_{d,y} + k_{apc}^n) y^n + (-\beta_y y^n + \alpha_y y^c) S_n / V_n\end{aligned}\quad (\text{A2})$$

where the superscripts c and n indicate cytoplasm and nucleus, respectively.

II. Numerical Simulation

Due to the isotropic diffusion and spherical symmetry of the cell, we can reduce the three-dimensional problem into a one-dimensional problem to reduce computational difficulty. In the spherical coordinate (ρ, θ, ϕ) system, the diffusion terms in the partial differential equations can be simplified as

$$\begin{aligned}D\nabla^2 p &= D \left\{ \frac{1}{\rho^2} \left[\frac{\partial}{\partial \rho} (\rho^2 \frac{\partial p}{\partial \rho}) \right] + \frac{1}{\rho \sin \theta} \left[\frac{\partial}{\partial \theta} (\sin \theta \frac{\partial p}{\partial \theta}) \right] + \frac{1}{\rho^2 \sin^2 \theta} \frac{\partial^2 p}{\partial \phi^2} \right\} \\ &= D \frac{1}{\rho^2} \left[\frac{\partial}{\partial \rho} (\rho^2 \frac{\partial p}{\partial \rho}) \right] = D \left(\frac{\partial^2 p}{\partial \rho^2} + \frac{2}{\rho} \frac{\partial p}{\partial \rho} \right),\end{aligned}\quad (\text{A3})$$

since the protein concentration p and its boundary conditions are independent on θ and ϕ . In numerical simulation, we used the following equation in our computer program to calculate the diffusion effect:

$$D \frac{(p_{i+1} - p_i) [(i+1)\Delta\rho]^2 - (p_i - p_{i-1})(i\Delta\rho)^2}{(\Delta\rho)^2 [(i+\frac{1}{2})\Delta\rho]^2}, \quad (\text{A4})$$

where $\Delta\rho = 0.4 \mu\text{m}$ is the space step we used. The cell radius $r = N\Delta\rho$. At the boundary of cell membrane, a virtual point $p_{N+1}^c = p_N^c$ was used to result in no-flux boundary condition. At the boundary of nuclear surface (nuclear radius $r_n = M\Delta\rho$), virtual points were used to handle the boundary conditions Eq. A2. For example, if $(M+1)\Delta\rho$ is in cytoplasm and $M\Delta\rho$ is in nucleus, we set $p_M^c = p_{M+1}^c - (\beta p_M^n - \alpha p_M^c)\Delta\rho/D_q^c$ and $p_{M+1}^n = p_M^n + (\beta p_M^n - \alpha p_M^c)\Delta\rho/D_q^n$. Explicit Euler method was used to integrate the discretized equations with a variable time step Δt between 1/20000 min and 1/200 min.

We numerically solved the differential equations for the two-compartment model using the fourth-order Runge-Kutta method with a variable time step between 1/800000 min and 1/50 min.

III. Parameters estimation

Most of the rate constants for cyclin B1 and CDK regulation in this study were based on the estimation of Marlovits et al (55) for *Xenopus oocyte*. Since the cell cycle time of *Xenopus oocyte* is much shorter than that of Hela cells (42), we rescaled the rate constants to fit to the experiments (42). Other parameters were chosen to better fit the experimental data and the dynamics. Table A1 summarizes the parameters used for the simulations shown in Fig.2–Fig. 5.

The translocation rate of cyclin B1 was estimated from the data shown in the reference(42) (Fig.2b in their paper) and additional data provided to us by Dr. Catherine Lindon in Dr. Pines' group that the maximum fluorescence in the whole cell is almost 1.8 fold of the maximum fluorescence in the nucleus. We subtracted the nuclear fluorescence from whole cell

fluorescence (whole cell data in their Fig.2b multiplied by 1.8), and then normalized it to obtain the cytoplasmic fluorescence curve. Using the data from 26 min to 38 min of the cytoplasmic

fluorescence curve and assuming an exponential decay, i.e., $\frac{dy^c}{dt} = -k_{ly}y^c$, we obtained

$k_{ly} = \frac{\ln(0.94/0.61)}{38-26} = 0.035 \text{ min}^{-1}$. By further assuming that the cell radius $r=20 \mu\text{m}$ and nuclear radius $r_n=10 \mu\text{m}$, we estimated the high import rate constant $\alpha_H=0.82 \mu\text{m}/\text{min}$ by using

$-k_{ly}y^c \approx -\alpha_H \frac{S_n}{V_c} y^c$. The unphosphorylated cyclin B1 import rates are very low and the rate

constant was set as $\alpha_L=0.0001 \frac{V_c}{S_n}=0.0023 \mu\text{m}/\text{min}$. The export rate constants were set as $\beta_L = \alpha_L$ and $\beta_H = \alpha_H$. Because the other protein translocation rates are unknown, we set all high import rate constants as $\alpha_H = 0.82 \mu\text{m}/\text{min}$ and low import rate constants as $\alpha_L = 0.0023 \mu\text{m}/\text{min}$, and all high export rate constants as $\beta_H = \alpha_H$ and low export rate constants as $\beta_L = \alpha_L$, except for the translocation rates of wee1. Since wee1 was found to be mainly nuclear (38), but it was also found in the cytoplasm (39,40) during interphase. Therefore, we still use high import and export rate constants as $\alpha_H = \beta_H = 0.82 \mu\text{m}/\text{min}$, but low import and export rate constants as $\alpha_{L,w} = \beta_{L,w} = 0.082 \mu\text{m}/\text{min}$.

We used the same diffusion constant, $D = 100 \mu\text{m}^2 / \text{min}$, for all proteins based on the study of Elowitz et al (56). Due to the fast diffusion, the gradients in protein concentration in both cytoplasm is very small so that the two-compartment model agrees well with the spatially extended model (Fig.A2)

In the simulation of *Xenopus laevis* in Fig.6, we rescaled the parameters to fit the cycle time in the experiments (19,20). All parameters were 50-fold of those in Table A1, except for the translocation rate constants which were 150-fold of those in Table A1.

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Fig. 1.

Schematic plot of cell cycle protein interaction network (left) and protein translocation between the cytoplasm and the nucleus (right). Phosphorylated cyclin B1, CDC25C and APC-CDC20 have a much larger nuclear import rates (indicated by thick arrows) than export rates (thin arrows), and conversely for their unphosphorylated forms (thin arrows). In contrast, phosphorylated wee1 has a much larger nuclear export rate than the import rate. MPF in our simulation was defined as the active cyclin B1-CDK1 (dashed circle, left panel).

Fig. 2.

Comparison of modeling and experimental results for cyclin B1 translocation. **A.** Concentrations of cytoplasmic cyclin B1, nuclear cyclin B1, cytoplasmic MPF, and nuclear MPF versus time. **B** and **C.** Selected snapshots of cyclin B1 distribution from the computer simulation and from experiments (42). **D.** Normalized whole cell cyclin B1 and nuclear cyclin B1 from model simulations and cell experiments (42). In simulation, the cell radius was fixed at $r=20\ \mu\text{m}$ and the nuclear radius was fixed at $r_n=10\ \mu\text{m}$. The experimental images in **C** and data in **D** were kindly provided by Drs. Catherine Lindon and Jonathon Pines.

Fig. 3. Time course of changes in cytoplasmic and nuclear CDC25C (A), wee1 (B), and APC-CDC20 (C) during mitosis, for the same simulation shown in Fig.2.

Fig. 4.

Dynamics of protein translocation. **A** and **B**. The steady state of cytoplasmic MPF (**A**) and nuclear MPF versus total cyclin B1 (**B**) when APC-CDC20 was removed from the model. Note the different y-axis scales. **C** and **D**. Bifurcation leads to limit cycle oscillation in the parameter $k_{s,y}$ space when APC-CDC20 was added back to the model. Maximum and minimum MPFs for both cytoplasm and nucleus were plotted so that in the limit cycle regime two MPFs were recorded for each $k_{s,y}$, while in the stable regime only one was recorded since they are the same. **E**. A three-dimensional illustration showing the trajectory of the limit cycle oscillation. **F**. Phase diagram showing the effects of protein phosphorylation and import rate on the oscillatory dynamics of the model.

Fig. 5.

Effects of cell and nuclear sizes on cell cycle transitions. **A.** Steady state cytoplasmic MPF versus cyclin B1 synthesis rate when APC-CDC20 is absent, for cytoplasmic-to-nuclear volume ratios of 7:1 (solid) and 2:1 (dashed), respectively. Nuclear volume (V_n) was the same ($r_n = 10 \mu\text{m}$) for both cases. Arrows indicate the onset of MPF activation. The multi-valued complex relation between MPF and cyclin B1 synthesis rate is due to the projection of a high-dimensional system into a two-dimensional plane. **B.** Cyclin B1 synthesis rate versus V_c/S_n for the onset of limit cycle oscillations for two different nuclear sizes when APC-CDC20 is present.

Fig. 6.

Cycle time dependence of cell size. **A**. Cell size versus time for in wild type (solid) and Wee1 mutant (dash) cell. At mitosis, the cell and nuclear sizes of the daughter cell varied randomly by 20%. **B**. Cell cycle time versus birth size from the computer simulation in A (points) and from Eq.5 (line). Nuclear size varied randomly by 40%. **C**. Same as B but from *Xenopus* experiments by Wang et al (20).

Fig. A1.

Schematic plot of cell cycle protein interaction network used in this study. Same network was used for both cytoplasm and nucleus. Rate constants (for each reaction arrow) and variables (for each protein) used in Eq. A5 were labeled.

Fig. A2.

A and B. MPF distributions in space (from the center of the nucleus to the cell membrane) before (A) and during mitosis (B). In both cases, the spatial gradients are very small. **C and D.** Comparison of the *Spatially extended model* (C) and the *Two-compartment model* (D). The parameters used for both models were the same. Note that two models gave rise to virtually the same result.

Table A1

Parameters for the *Spatially extended model* and the *Two-compartment model*. Results of Fig. 2 –Fig.5 were obtained using this parameter set. The parameters in Fig.6 were 50-fold of those in this Table, except for the translocation rate constants which were 150-fold of those in this Table.

$k_{s,y}=0.1 \text{ nM/min}$	$b_w=0.005 \text{ min}^{-1}\text{nM}^{-1}$	$\alpha_{x1} = \alpha_L$	$\beta_{z3} = \beta_L$
$k_I=0.1 \text{ min}^{-1}$	$k_w^-=0.007 \text{ min}^{-1}$	$\alpha_x = \alpha_L$	$\alpha_{w0} = \alpha_H$
$k_2=0.0 \text{ min}^{-1}$	$c_w=0.04/10 \text{ min}^{-1}\text{nM}^{-1}$	$\alpha_{yp} = \alpha_H$	$\alpha_{w1} = \alpha_H$
$k_{d,y}=0.002 \text{ min}^{-1}$	$k_{s,u}=0.1 \text{ nM/min}$	$\alpha_{x1p} = \alpha_H$	$\alpha_{w2} = \alpha_H$
$k_{d,x1}=0.002 \text{ min}^{-1}$	$k_{d,u}=0.001 \text{ min}^{-1}$	$\alpha_{xp} = \alpha_H$	$\alpha_{w3}=0.082 \text{ }\mu\text{m/min}$
$k_{d,x}=0.002 \text{ min}^{-1}$	$a_u=0.0015 \text{ min}^{-1}$	$\beta_y = \beta_H$	$\beta_{w0}=0.082 \text{ }\mu\text{m/min}$
$a_p=0.0001 \text{ min}^{-1}$	$b_u=0.005 \text{ min}^{-1}\text{nM}^{-1}$	$\beta_{x1} = \beta_H$	$\beta_{w1}=0.082 \text{ }\mu\text{m/min}$
$b_p=0.0005 \text{ min}^{-1}\text{nM}^{-3}$	$k_u^-=0.0075 \text{ min}^{-1}$	$\beta_x = \beta_H$	$\beta_{w2}=0.082 \text{ }\mu\text{m/min}$
$k_{dp}=0.01 \text{ min}^{-1}$	$c_u=0.025/100 \text{ min}^{-1}\text{nM}^{-1}$	$\beta_{yp} = \beta_L$	$\beta_{w3} = \beta_H$
$k_{s,z}=1.0 \text{ nM/min}$	$k_{s,m}=0.1 \text{ nM/min}$	$\beta_{x1p} = \beta_L$	$\alpha_{u0} = \alpha_L$
$k_{d,z}=0.01 \text{ min}^{-1}$	$k_{d,m}=0.01 \text{ min}^{-1}$	$\beta_{xp} = \beta_L$	$\alpha_{u1} = \alpha_L$
$k_{I,z}=1. \text{ min}^{-1}$	$a_m=0.0015 \text{ min}^{-1}$	$\alpha_{zb} = \alpha_L$	$\alpha_{u2} = \alpha_L$
$k_{2,z}=0.01 \text{ min}^{-1}$	$b_m=0.005 \text{ min}^{-1}\text{nM}^{-1}$	$\alpha_{z0} = \alpha_L$	$\alpha_{u3} = \alpha_H$
$a_z=0.015 \text{ min}^{-1}$	$k_m^-=0.0075 \text{ min}^{-1}$	$\alpha_{z1} = \alpha_L$	$\beta_{u0} = \beta_H$
$b_z=0.05 \text{ min}^{-1}\text{nM}^{-1}$	$c_m=0.04/10 \text{ min}^{-1}\text{nM}^{-1}$	$\alpha_{z2} = \alpha_L$	$\beta_{u1} = \beta_H$
$k_z^-=0.075 \text{ min}^{-1}$	$\alpha_H=0.82 \text{ }\mu\text{m/min}$	$\alpha_{z3} = \alpha_H$	$\beta_{u2} = \beta_H$
$c_z=0.02/100 \text{ min}^{-1}\text{nM}^{-1}$	$\alpha_L=0.0023 \text{ }\mu\text{m/min}$	$\beta_{zb} = \beta_H$	$\beta_{u3} = \beta_L$
$k_{s,w}=0.1 \text{ nM/min}$	$\beta_H=\alpha_H$	$\beta_{z0} = \beta_H$	$r = 20\mu\text{m}$
$k_{d,w}=0.01 \text{ min}^{-1}$	$\beta_L=\alpha_L$	$\beta_{z1} = \beta_H$	$r_n = 10\mu\text{m}$
$a_w=0.0015 \text{ min}^{-1}$	$\alpha_y = \alpha_L$	$\beta_{z2} = \beta_H$	$D=100 \text{ }\mu\text{m}^2/\text{min}$
$K_z^+ = a_z + b_z(x + x_p)$	$K_w^+ = a_w + b_w(x + x_p)$	$K_u^+ = a_u + b_u(x + x_p)$	$K_m^+ = a_m + b_m(x + x_p)$
$k_{cdc25} = c_z z^3$	$k_{weel} = c_w w^0$	$k_{apc} = c_u u^3$	$k_{myt1} = c_m m^0$
$k_p = a_p + b_p(x + x_p)^3$			