Computational Analysis of Mammalian Cell Division Gated by a Circadian Clock: Quantized Cell Cycles and Cell Size Control
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A fundamental attribute of a cell is its ability to divide and multiply. The cell cycle executes a precise control mechanism with multiple checkpoints for proper cell division. Its oscillatory dynamics are extensively studied from yeasts to mammals (Nurse, 2000). Although not as essential as cell cycle for viability, the...
existence of a circadian clock can be observed from cyanobacteria to humans (Dunlap, 1999; Matsuo et al., 2003; Vanselow et al., 2006). In most cases, conserved transcription–translation negative feedback loop (TTFL) is a foundation of robust oscillations in clock mechanisms (Dunlap, 1999). Both the cell cycle and circadian clock are robust oscillatory systems (Chen et al., 2004; Forger and Peskin, 2005; Gonzé et al., 2002; Hong et al., 2007; Morohashi et al., 2002). Their properties, however, are significantly different. The most distinct differences are temperature and nutrient compensations. The period of the circadian clock is relatively invariant over a physiologically relevant range in temperature, whereas the cell cycle or mass doubling time is greatly influenced by temperature and/or nutrient conditions (i.e., cell cycle time decreases as a function of temperature, leading to a Q_{10} [rate change with increase of 10 °C of temperature] of about 3, whereas Q_{10} of a circadian period is close to 1; Tsuchiya et al., 2003). On the other hand, all eukaryotic cell cycles have multiple checkpoints that ensure the proper progress of the cell cycle, but it is still unknown whether checkpoints exist for the biological clock. In any case, the harmonious progress of the cell cycle and circadian rhythms is necessary for the well-being of organisms as malfunctions in the cell cycle and/or clock can lead to tumorigenesis (Fu et al., 2002; Kastan and Bartek, 2004).

The molecular regulatory mechanisms of the cell division cycle are fundamentally identical in all eukaryotes (Nurse, 1990). Although multicellular organisms proliferate only when permitted by specific growth factors, the key enzymes of the cell cycle are functionally conserved across different eukaryotes (Csikasz-Nagy et al., 2006). The key transitions of the cell cycle are regulated by Cyclin-dependent kinases (Cdks) bound to their regulatory Cyclin (Cyc) partners. Four crucial Cdk/Cyc complexes (Cdc2/CycB, Cdk2/CycA, Cdk2/CycE, and Cdk4/CycD) and their regulated sequential functions are necessary for proper mammalian cell cycle progress. Their orders of appearance are meticulously controlled by inhibitors (Rb, p27Kip1), transcription factors (E2F, Mcm), and degradation factors (p55CdC/APC, Cdh1/APC; Sherr, 1996).

We would also like to emphasize the fact that in HeLa cells, the inhibitory kinase Wee1 plays a crucial role in regulating Cdc2 activity and the entry into mitosis, as it does in fission yeast (Chow et al., 2003). Most of this regulatory network of the cell cycle has been mathematically analyzed by Novak and Tyson (2004).

Yeast cells have to reach a critical size for proper cell division. This active size control mechanism prevents yeasts from delayed or premature cell division, resulting in imbalanced cell mass population (Rupes, 2002; Sveiczer et al., 1996). The existence of cell size control is controversial in mammalian cells (Conlon and Raff, 2003; Grebien et al., 2005; Sveiczer et al., 2004; Wells, 2002). In cultured mouse fibroblasts, smaller newborn cells take longer to enter the S-phase compared to larger cells at birth, which indicates a possible cell size checkpoint as in Saccharomyces cerevisiae (Johnston et al., 1979; Killander and Zetterberg, 1965). On the other hand, recent findings from Rat Schwann cells suggest absence of size control (i.e., small cells took several cell divisions to reach their typical size; Conlon et al., 2001). This discrepancy is suggested partly because of differences in growth rates: linear vs. exponential. Recently, however, this hypothesis was challenged with results of different cell types readjusting their size in the next cycle, even when the “linear mode” was observed (Dolznig et al., 2004). With our computational modeling, we propose that periodic influences of the circadian clock on cell cycle contribute to the cell size control mechanism regardless of growth type differences.

In mammalian systems, the central clock is located in the suprachiasmatic nucleus (SCN) situated in the hypothalamus. Neurons in the SCN display synchronized endogenous clocks (Yamaguchi et al., 2003), receive input information (i.e., light, temperature, etc.), and transmit output signals. The clock is also present in peripheral tissues (i.e., fibroblast, liver, bone marrow, etc.). Peripheral clocks in both mouse and rat 1 fibroblast cells in culture, however, do not communicate with each other, resulting in desynchronization of the clock as a population (Welsh et al., 2004). Nevertheless, identical components are present in both peripheral tissues and in the SCN neurons. The details of the mammalian clock are complex, with an autoregulatory network of TTFLs. Mammalian mPer1 and mPer2 genes are activated by heterodimeric bHLH-PAS transcription factors Bmal1:Clk. The mPers are translated and form complexes with mCry1 and mCry2 proteins. The complexes are translocated into the nucleus and inhibit the activity of the Bmal1:Clk heterodimeric transcription factors. This is a nutshell of the time-delayed negative feedback mechanism that generates a robust oscillation of about 24 h. Posttranscriptional and translational regulations of mPers, mCrys, and Bmal1:Clk add multiple layers of complexity in the system (Hardin, 2004).

Earlier studies from the late 1950s to the 1980s indicate that cell divisions in Euglena, Tetrahymena, and Gonyaulax occur only at particular times of the circadian cycle (Edmunds, 1974a, 1974b; Sweeney
and Hastings, 1958). Gated cell division cycle is also observed in some cyanobacteria, with average doubling times less than 24 h (Mori et al., 1996). These data indicate gating of the cell cycle by the clock. Although there has been physiological evidence suggesting circadian-gated cell cycle for more than 4 decades, the molecular link between cell cycle and the clock remained in a black box until recently. Matsuo and his colleagues showed that a cell cycle regulator, wee1, is directly regulated by clock components via wee1’s E-box elements in mammalian cells (Matsuo et al., 2003). Wee1 phosphorylates Cdc2/CyclinB (Cdk1/CycB) complex and inhibits the entry into mitosis from G2. This regulation is reflected in partial hepatectomy (PH) experiments showing that PH performed at different zeitgeber times (ZT0 vs. ZT8) resulted in similar timing of entry into the S-phases but showed an 8-h delay in the entry of M-phase from the ZT0 PH liver (Matsuo et al., 2003). Wee1 and its kinase activity peaked during the dark phase (~ZT 16–20) after the PH, and wee1 mRNA peaked at ZT 8. A high level of Wee1 activity determines the duration of the G2-phase, and it has to drop before cells enter into the M-phase. Intrigued by these results, we present the first coupled mathematical model of mammalian cell cycle and circadian clock with Wee1 as a coupling factor.

Our model results in (1) quantized cell cycles and (2) cell size control when the mass doubling time (MDT) deviates from 24 h in our stochastic simulations. Quantized cell cycles in mammalian cell lines were first reported by Robert R. Klevecz in 1976 (Klevecz, 1976). In the 1980s, David Lloyd and his colleagues identified quantized cell cycles in lower eukaryotes and demonstrated with mathematical modeling that ultradian pulses created quantized cell cycles (Lloyd and Kippert, 1987; Lloyd and Volkov, 1990). Although quantized cell cycles were shown both in yeast and mammals (Klevecz, 1976; Sveiczer et al., 1999), a clock-regulated quantized mammalian cell cycle with a known molecular link has never been addressed. More interestingly, our simulations show that the clock-influenced cell cycle via Wee1 triggers cell size control. The cell size control becomes apparent when the clock enforces circadian regulation on Wee1 when the MDT differs greatly from 24 h.

**MODELING METHODS**

Our purpose is not to address a comprehensive mammalian circadian rhythm model. For simplicity’s sake, we want to have a minimal but robust oscillator that generates an endogenous cycle enforcing a periodic influence on the cell cycle. Hence, we built a simplified version of a 4-variable mammalian circadian clock model (Fig. 1) that consists of transcription factors (TF: Bmal1 and Clk), clock message (M: mPer or mCry mRNA), clock protein (CP: mPer or mCry), and a dimer complex of clock proteins (CP₂; see Appendix A). For the simplicity of the model, we assume that mPer and mCry are the same species. Therefore, CP₂ represents combinations of mPer/mPer, mPer/mCry, and mCry/mCry dimers. This assumption will be relaxed in our future work when we study a more comprehensive model of circadian clock. We also assume that the CP₂ are more stable than the CP, which introduces an autocatalytic positive feedback in the system (Tyson et al., 1999). The CP is activated by the TF, and the TF is inhibited by the
CP, which closes the negative feedback loop. Our simplified clock model shows robust endogenous oscillations with a period of 24 h (top panel of Fig. 2).

For our cell cycle model, we adapted Novak and Tyson’s mammalian model (2004), which focuses on restriction point control. They simulated “transient inhibition of growth” in mammalian cells upon cycloheximide treatment and its removal (Zetterberg and Larsson, 1995), with in-depth descriptions of cell growth and the Cdk regulatory system. This model, however, did not focus on Wee1 and G2/M transition because of an already complicated molecular network with 4 different Cdk/Cyclin complexes. We introduce a Wee1 and Cdc25 regulatory module emphasizing the G2/M transition into the Novak and Tyson (2004) mammalian model. The Wee1 and Cdc25 module regulates the activity of Cdc2/CycB for proper progress of the cell cycle into mitosis. In addition to the basal transcriptional activity of Wee1, we introduce another level of transcriptional activity of Wee1 that is directly regulated by clock components, Bmal1:Clk (Fig. 1). This connection creates a link between the cell cycle and circadian clock in which periodic regulation of Wee1 is modulated by the clock (Appendix B). The cell cycle model shows robust oscillations with an MDT determined by different growth rates in the absence of a connection with the clock module (i.e., coupling factor $[k_{w5}] = 0$).

Multiple runs of stochastic simulations with different combinations of coupling strength (Appendix C) at different mass doubling times of the cell cycle are executed. For stochastic simulations, we introduce noise into the cell cycle regulatory equations by rewriting the cell cycle model as Langevin-type equations with multiplicative noise (Steuer, 2004; van Kampen, 1981):

$$\frac{d}{dt} x_i = f_i + w(t) \sqrt{2D_i} x_i$$

where $f_i$ means the original deterministic equation, $w(t)$ is Gaussian white noise with 0 mean and unit variance, and $D_i$ is the noise amplitude. For simplicity, we kept the noise amplitude constant (0.005) for all variables. This number was set by matching the coefficient of variation (CV) of simulated uncoupled cell cycle length (at MDT = 24 h) to experimentally observed CV = 10% (Tyson, 1985). We do not introduce stochasticity in the circadian clock module because its sensitivity to noise may not reflect a truly robust clock mechanism, being an overly simplified version of a clock model. In this article, we only concentrate on the unidirectional effect of the clock on cell cycle. We discuss the possibility of cross-talk between the cell cycle...
and circadian rhythms below. We also keep the cell growth equation deterministic because we cannot take into account the fluctuations in the complex process of cell growth in the current model. Differential equations are solved and analyzed with the software tool XPP-AUT (Ermentrout, 2002). Readers can find our XPPAUT readable ODE files and the description of rate constants of our model on our Web site (http://www.cellcycle.bme.hu/).

We run multiple stochastic simulations for the cell cycle time distribution histograms and related figures (Fig. 4–7). For each simulation, we calculate 50 consecutive cell cycles. We assume that interactions between individual cells are weak and single cells behave independently. In such a case, the investigation of multiple cycles of an individual cell is equivalent to the analysis of a cell population at a given time. This is supported in cell culture systems (i.e., NIH3T3) in which cells do not communicate with each other in terms of the clock.

**RESULTS**

*Circadian regulation of Wee1 results in quantized cell cycles.* For initial simulations, it seems natural to start with the MDT of 24 h. The cell cycle synchronizes with the circadian clock regardless of its initial conditions, with an MDT at 24 h (Fig. 2C). A stronger coupling (large $k_{mCry}$) ensures tighter G2 regulation by inducing high levels of Wee1 (Fig. 2A). As a result, cell division locks into a particular phase of the circadian rhythm (Fig. 2C). Our result is in agreement with the findings that cell divisions frequently occur right after the circadian night (in which mPer and mCry are still high; Hardin, 2004) in different mammalian cell types (Bjarnason et al., 2001).

The MDT of mammalian cell culture varies greatly depending on cell types and growth conditions (i.e., temperature, nutrients, etc.). Hence, we changed the MDT from 16 to 28 h in our simulations and observed the cell cycle time profile over multiple runs of cell division cycles with different coupling strengths. A strong coupling ($k_{mCry} = 2 h^{-1}$) results in uneven distribution of cell cycle time (Fig. 3). A periodic influence on wee1 transcription imposes a delay in G2, depending on the timing of Bmal1:Clk and Wee1 (Fig. 3B). This pattern repeats itself every 6 cell cycles at MDT = 20 h or 28 h and every third at MDT = 16 h (Fig. 3A–C). Similar repetitions cannot be observed with weak coupling in our stochastic simulations ($k_{mCry} = 0.25 h^{-1}$; Fig. 3D-F). In the absence of any coupling factor ($k_{mCry} = 0 h^{-1}$), the two oscillators run with their endogenous periods independently of each other (not shown). The observed pattern with strong coupling is dictated by the least common multiple of the 24-h period and the MDT (Fig. 5C, 5D). This “mode-locking” behavior of two oscillators results in quantized cell cycle times at different MDTs with strong coupling. Figure 4A–C represents histograms with multiple peaks of cell cycle time at MDT = 16, 20, and 28 h, with strong coupling. These multimodal cell cycle distributions show a resemblance to previous experimental results (Klevecz, 1976; Nagoshi et al., 2004). Quantitative comparisons, however, cannot be achieved, because of lack of experimental details. We wish to pursue this in our future work. Weak coupling results in normal distributions of cell cycle times (Fig. 4D–F). Further stochastic simulations are performed with randomly chosen MDTs to investigate cell cycle time across MDTs. This simulation allows us to visualize the distribution patterns of cell cycle time with both strong and weak couplings across a large range of MDTs. Similarly, as shown in Figure 4, the strong coupling results in quantized cycles, whereas the weak coupling reflects normal distribution cycle times from the stochastic modeling (Fig. 5A, 5B). As the MDT deviates from 24 h, the clock-enforced cell cycle goes through repeated cycles of “mode-locking,” which create large deviations in cell cycle time. Analysis of the variations in cell cycle time and cell mass agree with experimental data.

The quantized cell cycles with compensatory shorter or longer cell cycle times create smaller or larger cell mass influenced by the circadian clock. Periodic influence of the clock reduces the effect of noise and synchronizes the cell cycle when the MDT is close to 24 h. As the MDT deviates from 24 h, the clock-enforced cell cycle goes through repeated cycles of “mode-locking,” which create large deviations of cell cycle time to compensate for differences in cell mass. To measure these deviations, the coefficients of variation (CV = [standard deviation/mean] × 100 [%]) of cell cycle time and cell mass are calculated from 50 cell cycle times.
cycle simulations each, with randomly generated MDTs (Fig. 6). With strong coupling, our simulations show that populations of cells reflect a unique relationship between 2 CVs: the CV of cycle time is roughly twice the CV of cell mass at division, which is in agreement with experimental results (Tyson, 1985; Fig. 6).

The circadian clock contributes to the regulation of cell size control. Cell size control is apparent when smaller or larger cells at birth undergo different durations of growth to reach the critical size for proper cell cycle progression. In other words, it would take less time for large cells at birth to reach the critical cell mass than smaller cells. Experimentally, this phenomenon is reflected by negative correlation (slope of about –1) of net growth throughout the cycle (mass ∆ = mass at division–birth mass) and birth mass (mass 0; Sveiczer et al., 1996). To investigate the existence of size control in our model, we studied the relationship between mass ∆ as a function of mass 0 from our stochastic simulations of 50 cell cycles each at different MDTs (Fig. 7A, 7B).

We acknowledge that in mammalian system, it is difficult to measure mass ∆ as a function of mass 0 because of technical limitations, as it was done in fission yeast. With computational simulations, however, this can be easily measured. This is one of the advantages of computational modeling.
critical cell mass of a cell depends on growth conditions leading to larger cells with rich nutrients [Johnston et al., 1979]). Hence, we categorize our results according to the MDTs. To our surprise, we observe negative correlations with a strong coupling factor in distinct populations of cells when the data are sorted according to the MDTs (Fig. 7A).

The slopes of regression lines (from the previous calculations) as a function of MDT provide relationships between cell size control and different coupling factors (Fig. 7C, 7D). Our stochastic simulations show that weak coupling of the clock with the cell cycle results in no clear correlation between the mass₃ and the mass₀ (Fig. 7D). Our simulations with zero coupling are identical to those of weak coupling (not shown). However, we see a general trend of decrease in the slope of regression lines with increasing MDTs because of the innate properties of the cell cycle module as proposed in previous work (Csikasz-Nagy et al., 2006). On the other hand, the strong coupling results in both positive and negative slopes of regression lines, depending on the MDT (Fig. 7C). With strong coupling, cell size control is apparent (slope about –1) when the MDT is either significantly shorter or longer than 24 h. This is because of compensatory cycles in which very large or very small cells undergo short or long cell cycles, as seen in Figure 3 (therefore resulting in quantized cell cycles). The compensatory cycles (hence, resulting cell size control) become apparent when cells experience significant changes in their cell cycle regulatory dynamics by the clock. In other words, the periodic influence of the clock on Wee1 expression perturbs cell cycle dynamics, resulting in cells that are either too large or too small when the MDTs are significantly different from the clock period length. This, in turn, triggers cell size control. Positive slopes of regression lines are observed when the MDTs are close to 24 h, because of “rare” compensatory cycles resulting in loss or gain of cell mass depending on the MDT. For

Figure 6. Coefficients of variation of cell cycle time and cell mass as a function of mass doubling time (MDT) at different coupling strengths. Coefficients of variation (CV) of cell cycle length (A, B) and cell mass at division (C, D) are calculated. The CV for cell cycle length is small at a MDT close to 24 h but is large at other MDTs because of the strong influence of the circadian clock (A) compared to the weakly coupled (B) case. The CV for division mass is higher in the strong coupling case (C) than the weak coupling case (D). The CV of cell cycle is roughly twice the CV of cell mass at divi-

Figure 5. Mode-locked distribution of cell cycle time as a function of mass doubling time (MDT). (A) A cluster of quantized cell cycle populations is observed with a strong coupling in a large range of MDTs. (B) Weak coupling results in normal distribution of cell cycle time (CT) in various MDTs, with the average CT = MDT. About 250 simulation runs are performed in various MDTs, and each simulation calculated 50 cell cycles. (C, D) Deterministic simulations of both strong (C) and weak (D) coupling cause mode-locking. The pattern of cell cycle time repeats with the least common multiple (noted on panel D) of the circadian-imposed 24 h and the MDT. The degree of separation between different cell cycle lengths, however, is very different from strong vs. weak couplings. The weak coupling results in normal distribution of cell cycle time even with mode-locking behavior. The abscissa of each histogram is vertically shifted to the MDT value that is used for the given simulation. Histograms describe distributions of cell cycle lengths depending on periodic repeat sequences. In the deterministic case with zero coupling, we get a simple peak at each MDT at a cycle time = MDT (not shown).
Figure 7. Analysis of critical mass control. (A, B) Growth from cell birth to division (mass) is plotted as a function of birth mass (mass) for multiple simulations at different mass doubling times (MDTs). Data points are color coded and clustered according to particular MDTs. Cell size control from the right-hand side of dCycB/dt. Unique slope of regression lines of mass growth rate. For these simulations, we change the equation of cell growth by eliminating the mass. Weak coupling shows no apparent mass control (D). (E, F) Similar results are shown with linear short or longer than 24 h, but size control is not observed when the MDT is close to 24 h (C). Strong coupling results in strict size control when cell masses are either large or small but no apparent correlation at intermediate cell masses (A). Weak coupling (B) shows no clear size control. About 250 simulation runs are calculated at different MDTs. For clear representation, not all data points are displayed on panels (A) and (B), and the legends for both panels are inserted on panel (B). (C, D) Slopes of linear regression lines from (A, B) are plotted as a function of the MDT. Strong coupling results in strict mass control (slope about –1) when the MDTs are either much shorter or longer than 24 h, but size control is not observed when the MDT is close to 24 h (C). Weak coupling shows no apparent mass control (D). (E, F) Similar results are shown with linear growth rate. For these simulations, we change the equation of cell growth by eliminating the mass from the right-hand side of dCycB/dt. Unique slope of regression lines of mass vs. mass plots are observed with strong coupling as a function of MDT (E), as seen with exponential growth rate (C). The MDT is calculated from the average cell cycle time of 50 cycles.

**DISCUSSION**

Since the early discoveries of circadian clock–gated cell cycles in lower eukaryotes (Edmunds, 1974a, 1974b; Sweeney and Hastings, 1958), numerous molecular findings that connect the cell cycle and circadian clock are now being addressed (Fu et al., 2002; Matsuo et al., 2003). Preliminary screening has shown that there are multiple cell cycle components that oscillate with a period of about 24 h in mouse liver (i.e., CycB1, p53 ΔC, Cdc2, CycD1, etc.; Fu et al., 2002; Matsuo et al., 2003). Among many candidates, Wee1 stood out as a strong link based on several facts: (1) both wee1 mRNA and Wee1 protein cycle with a period of about 24 h, (2) both Wee1 protein and its relative kinase activity showed about 24-h cycles with a delay of 8 h in their peak levels and kinase activities of the PH samples at ZT0 compared to the PH samples at ZT8, and (3) the wee1 gene contains E-boxes in the 5’ flanking region, where Bmal1:Clk directly regulates wee1 transcription (Matsuo et al., 2003). We acknowledge that there may be other coupling factors in addition to Wee1 at different checkpoints in the progress of the cell cycle. Furthermore, recent research indicates that this connection may be bidirectional rather than unidirectional. The cell cycle kinase Chk2 phosphorylates a core clock component (i.e., FRQ in Neurospora crassa and mPer1 in mice), resulting in DNA damage–dependent reset of the clock (Gery et al., 2006; Pregueiro et al., 2006). The detail of this pathway is still unknown. What we present here, however, is an initiative of computational analysis with a unidirectional link from the circadian clock to the cell cycle via Wee1. In our future computational analysis, we plan to address the following issues: (1) simulations of multiple coupling factors in various checkpoints in cell cycles, (2) use of a comprehensive model of mammalian clock model, (3) effects of cell cycle inhibitors and changes in growth factor levels in the presence of the circadian clock, aimed at better “chronotherapy” (Gardner, 2002; Mormont and Levi, 2003), and (4) cross talk between the cell cycle and circadian clock. At the present moment, we introduce the first coupled mammalian cell cycle and circadian clock model with molecular profiles of both components (Fig. 2–3).
cell cycle model (Cross and Siggia, 2005). The authors found that periodically induced Cln2 or Cln3 transcription led to the “mode-locking” of cell division cycles. Based on recent discoveries of a genomewide ultradian respiratory cycle in yeast (Klevecz et al., 2004), it would be interesting to study possible players in this respiratory cycle that may affect the cell division cycle and whether those components influence the cell cycle via Cln2 or Cln3. This in turn may result in quantized cell cycles in yeast. Our results can be tested in both yeast and mammalian cell culture systems. For example, one can compare cell cycle distributions as a function of MDT (our Fig. 5) in the presence and absence of coupling factors (i.e., knock-down of wee1) in mammalian system or clock (i.e., knock-out of ultradian clock in yeast [Klevecz et al., 2004] or circadian clock in mammals [Okamura, 2004]).

It is important to note that in 2000, Sveiczer and colleagues mathematically modeled quantized cell cycles in fission yeast double mutant (weelΔcdc25Δ) without assuming ultradian influences (Sveiczer et al., 2000). This double mutant’s molecular phenotype (low Wee1 and no Cdc25) abrogates the positive feedback of Cdc2/Cdc13 via Cdc25. In the absence of positive feedback, the system loses bistability and is pushed into a stable oscillatory region with a period much shorter than the MDT (Csikasz-Nagy et al., 2006). In other words, there is a collision of 2 different periods: the MDT and the period set by a stable oscillator. This results in variations in the timing of mitosis entry, which creates quantized cell cycles. This model is significantly different from our model and others (Lloyd and Kippert, 1987) because no external influence (i.e., ultradian cycle) is required to generate quantized cell cycles for the weelΔcdc25Δ double mutant. It will be important to investigate different profiles of quantized cell cycles in both the presence and absence of ultradian cyclic influences in this double mutant. This will enlighten us as to whether an ultradian clock in fission yeast plays a role in cell cycle regulation on wee1 transcription triggers cell size control at different MDTs. Cell size control is observed during specific ranges of MDTs when the circadian clock induces periodic perturbations that force the cell cycle out of homeostasis from its dictated MDTs. There is no evidence of mass control with either weak or zero coupling strengths. Qualitatively similar behaviors are observed with both exponential and linear growth types (Fig. 7). In our model, circadian influences on Wee1 introduce cell size control at the G2/M transition. It is possible that there may be additional cell size control at the G1/S transition in the mammalian system as in budding yeast (Rupes, 2002). Interestingly, mammalian cell types that demonstrate cell size control also feature circadian rhythms (i.e., mouse fibroblast [Nagoshi et al., 2004; Tsuchiya et al., 2003] and bone marrow containing erythroid [Chen et al., 2000]), whereas there is no precedent for a functional circadian clock in Rat Schwann cells (where no critical size control has been reported; Conlon et al., 2001) to our knowledge. We acknowledge that the cell size control mechanism may be a complex network within cell cycle regulation. Our simulations suggest that the clock may play an important role in cell size control via Weel, depending on the MDT. We propose to test quantized cell cycles and cell size control in several ways: (1) observe cell size distribution at different MDTs in mouse fibroblasts that pertain to clock in absence and presence of coupling factors (i.e., Weel) and (2) if it is feasible, create an inducible system in Rat Schwann cells that creates circadian pulsatile induction of Weel and observe the distribution of cell size as a function of the MDT.

**APPENDIX A**

**DIFFERENTIAL EQUATIONS OF THE SIMPLIFIED CIRCADIAN RHYTHM MODULE FOR MAMMALIAN CELLS**

Messenger RNA of the clock proteins and Weel:

\[
\frac{d}{dt} M = k_m \frac{TP^0}{p + TP^0} - k_{we1} M \quad (1)
\]

Monomer clock proteins (mPer or mCry):

\[
\frac{d}{dt} CP = k_p M - k_{pe} CP - 2k_{CP^2} + 2k_{CP_2} \frac{CP}{J_p + CP_{tot}} \quad (2)
\]

Dimer form of clock proteins (mPer/mPer, mPer/mCry, or mCry/mCry):
\[ \frac{d}{dt} CP_2 = k_a CP^2 - k_C CP_2 - k_{\text{prod}} CP_2 + k_{\text{in}} IC - \] 

\[ k_{\text{in}} CP_2 \cdot TP - k_{\text{prod}} CP_2 \] 

(3)

Transcription factor (Bmal1:Clk) of the clock proteins’ mRNA:

\[ \frac{d}{dt} TF = \frac{k_{\text{in}} IC}{J_p + CP_{\text{tot}}} - \] 

\[ k_{\text{prod}} CP_2 \cdot TF - k_{\text{out}} \] 

(4)

Inactive complex of clock dimers and transcription factor:

\[ IC = TF_{\text{tot}} - TF \] 

(5)

Total amount of clock proteins:

\[ CP_{\text{tot}} = CP + 2CP_2 + 2IC \]

Rate constants (h\(^{-1}\)):

\[ k_{\text{in}} = 1, \quad k_{\text{prod}} = 0.1, \quad k_{\text{out}} = 0.5, \quad k_{\text{prod}} = 0.525, \quad k_p = 100, \quad k_{\text{in}} = 0.01, \quad k_{\text{prod}} = 0.0525, \quad k_{\text{out}} = 0.01, \quad k_{\text{in}} = 20, \quad k_p = 10, \quad k_{\text{prod}} = 0.1 \]

Dimensionless constants:

\[ J_p = 0.05, \quad J_p = 0.3, \quad n = 2 \]

**APPENDIX B**

**DIFFERENTIAL EQUATIONS FOR THE EXTENSION OF THE NOVAK AND TYSON MODEL (2004)**

Extensions to the equation of Cdk1/CycB:

\[ \frac{d}{dt} CycB = \epsilon \left( k_i \cdot \frac{(CycB/J)^2}{1 + (CycB/J)^2} \right) \cdot \text{mass} - \] 

\[ V_2 \cdot CycB + (k_{\text{dec25}} + k_{\text{dec25}}' \cdot Cdc25a) \cdot CycB \] 

(6)

Phosphorylated form of Cdk1/CycB:

\[ \frac{d}{dt} CycBP = (k_{\text{wee1}}' + k_{\text{wee1}}' \cdot \text{Wee1}) \cdot CycB - \] 

\[ k_{\text{dec25}} \cdot Cdc25a \cdot CycBP - \] 

\[ V_2 \cdot CycBP \] 

(7)

Active form of Wee1 kinase:

\[ \frac{d}{dt} \text{Wee1} = \frac{(k_{\text{wee1}}' + k_{\text{wee1}}' \cdot M)}{J_{\text{wee1}} + \text{Wee1}} + \] 

\[ \frac{(k_{\text{wee1}}' + k_{\text{wee1}}' \cdot CycB) \cdot \text{Wee1}}{J_{\text{wee1}} + \text{Wee1}} + \] 

\[ \frac{\text{Wee1P} - k_{\text{wee1}}' \cdot \text{Wee1}}{J_{\text{wee1}} + \text{Wee1P}} \] 

(8)

Inactive form of Wee1:

\[ \frac{d}{dt} \text{Wee1IP} = \frac{(k_{\text{wee1}}' + k_{\text{wee1}}' \cdot CycB) \cdot \text{Wee1}}{J_{\text{wee1}} + \text{Wee1}} - \] 

\[ \frac{\text{Wee1P}}{J_{\text{wee1}} + \text{Wee1P}} - \frac{k_{\text{wee1}}' \cdot \text{Wee1}}{J_{\text{wee1}} + \text{Wee1P}} \] 

(9)

Active form of Cdc25:

\[ \frac{d}{dt} Cdc25a = \frac{(k_{\text{dec25}}' + k_{\text{dec25}}' \cdot CycB) \cdot (1 - Cdc25a)}{J_{\text{dec25}} + (1 - Cdc25a)} - \] 

\[ \frac{\text{Wee1P}}{J_{\text{wee1}} + \text{Wee1P}} - k_{\text{dec25}} \cdot \text{Cdc25a} \] 

(10)

The cell divides (mass is halved) when CycB crosses 0.2 from the above.

Rate constants (h\(^{-1}\)):

\[ k_{\text{dec25}}' = 0.05, \quad k_{\text{dec25}}'' = 10, \quad k_i' = 0.1, \quad k_i'' = 1, \quad k_c = 0.4, \quad k_{\text{wee1}}' = 0.08, \quad k_{\text{wee1}}'' = 10, \quad k_{\text{wee1}}'' = 0.2, \quad k_{\text{wee1}}'' = 2, \quad k_{\text{wee1}}'' = 1, \quad k_{\text{wee1}}'' = 1, \quad (k_{\text{wee1}}' and k_{\text{wee1}}'' \text{ in Appendix C}) \]

Dimensionless constants:

\[ J_{\text{dec25}} = 0.05, \quad J_{\text{wee1}} = 0.05, \quad J_{\text{wee1}} = 0.2, \quad J_{\text{wee1}} = 0.2 \]

The rest of the parameters are same as in the Novak–Tyson model (Novak and Tyson, 2004).

**APPENDIX C**

**THE VALUES OF THE COUPLING PARAMETERS**

<table>
<thead>
<tr>
<th>(k_{wee1}'(h^{−1}))</th>
<th>(k_{wee1}''(h^{−1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zero coupling</td>
<td>1.00</td>
</tr>
<tr>
<td>Weak coupling</td>
<td>1.00</td>
</tr>
<tr>
<td>Strong coupling</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>2.00</td>
</tr>
</tbody>
</table>

a. To keep the average cell size similar, we assume the cell cycle has stronger influence on Wee1 when it is weakly dependent on the circadian clock. Wee1 levels have large influence on cell size, and we want to simulate normal distribution of cell size.

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