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Abstract

A typical proliferating human cell divides on average every 24 h. This division timing allows cells to synchronize with other physiological processes and with the environment. The circadian clock, which orchestrates daily rhythms, directly regulates the cell division cycle and is a major synchronizing factor. There is, however, no evidence that the circadian clock is able to entrain the cell cycle to a 24 h period. We show here, using a computational model for the cell cycle, that cells under circadian control that have an interdivision time close to multiples of 24 h proliferate faster. Moreover, growth of cell populations with a markedly different cell cycle time is impaired. We propose that this resonance effect in cell proliferation has a role to play in efficient normal cell proliferation and suppression of tumor growth.

Keywords: cell cycle, circadian clock, programmed cell death, resonance, tumor suppression, delay differential equations

1 Introduction

A fast proliferating human cell divides on average every 24 h. Given that many physiological processes such as activity, psychophysical performance, blood pressure, and liver metabolism show daily variation, it is not surprising that cells cycle in a circadian fashion. The circadian clock, which orchestrates these daily rhythms, consists of a set of genes showing self-sustained rhythmicity in expression with a period of approximately 24 h [21]. On a cell population level, the circadian clock regulates DNA synthesis and mitotic activity [4, 20, 22], and on a genetic level, tumor suppression [11], liver regeneration [18], and DNA damage control [13]. The kinetics of the cell cycle has been studied using cell population-based models [3, 17], and molecular-based models [8]. In this paper, we look at how an external periodic force, the circadian clock, influences the cell cycle kinetics. We show, using a computational model, that cells with an division time close to multiples of 24 h proliferate faster. Circadian variation of the cell cycle kinetics interacts with the cell division clock and the cell death program to create a resonance effect that promotes certain cell division times. This phenomenon contributes to the emergence of cells with specific cell cycle durations. The selectivity of cell cycle times could play a role in the suppression of tumor development by slowing down the growth of cells that have a perturbed cell cycle.

2 Model

We use a population-based model for the human cell cycle [3, 5, 17, 23] (Figure 1). To mimic the effect of the circadian clock, we allow the kinetic parameters to vary with a circadian period. We

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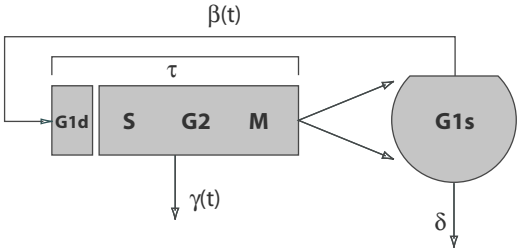

Figure 1: Model for the human cell cycle.

first describe the model as a stochastic system, where cell fate—death, arrest or division—are random events. We then formulate the model as a deterministic system, valid when the cell population is large.

Cells go successively through four phases: G1, S, G2, and M, at the end of which cells divide and reenter G1 phase. Most of the variability in duration of the cell cycle comes from differences in length of G1 phase. Durations of S phase (8–12 h), G2 (2–4 h), and M (1 h) are relatively constant under normal conditions. The G1 phase has a minimal duration, during which cells are in G1d phase, with an average duration of 9–11 h. The G1d, S, G2, and M phases have a fixed total duration τ, between 12 and 25 h in human cells [1], and constitute the proliferative phase of the cell cycle. The variable part of the G1 phase (called G1s) has a duration exponentially distributed with parameter $\delta + \beta$. G1s phase cells go in proliferative phase with probability $\beta/(\delta + \beta)$, or leave the cell cycle by programmed cell death or cell cycle arrest with probability $\delta/(\delta + \beta)$. Cell progressing in the proliferative phase have constant probability $1 - \exp(-\gamma\tau)$ to undergo programmed cell death, or apoptosis. The time a cells spend in proliferative phase is exponentially distributed with parameter γ when $a < \tau$ and τ otherwise. At time τ after entering G1s phase, surviving cells divide and go back in the G1s cell pool.

When the cell population is large, stochastic fluctuations can be neglected and only the average population needs to be considered. This leads to the deterministic version of the model [3, 17]. The population in G1s phase at time t, $N(t)$, is sufficient to describe the dynamics of cell proliferation. Cells in G1s phase leave the cell cycle at a rate δ and enter the proliferative phase at a rate β. Cells in proliferative phase die at a rate γ, and surviving cells divide a time τ after entering G1d phase. The G1s population is described by a delay differential equation with periodic coefficients,

$$\frac{\mathrm{d}N(t)}{\mathrm{d}t} = -\left[\delta + \beta(t)\right]N(t) + 2\sigma(t)\beta(t-\tau)N(t-\tau). \tag{1}$$

The fraction of cells surviving the active phase is

$$\sigma(t) = \exp\left(-\int_{t-\tau}^{t} \gamma(u)\mathrm{d}u\right), \quad 0 \le \sigma(t) \le 1. \tag{2}$$

Two cell cycle kinetic parameters, the G1s→G1d transition rate β and the apoptosis rate γ, are subject to circadian fluctuation. A variation in the G1s→G1d transition rate is motivated by the periodic expression of proliferation promoting genes such as *c-Myc* and *cyclin D1* [11]. The variation of the apoptosis rate is relevant in the context of periodic, or chronomodulated cancer therapy [19]. Detailed molecular models of the mammalian circadian clock [16] and the mammalian cell cycle [8] exist, but a link between them has not been made. We therefore consider the circadian clock input as a sine wave. The G1s→G1d transition rate has the form

$$\beta(t) = \beta_0(1 + A\sin(2\pi t/T)), 0 \le A \le 1. \tag{3}$$

For the apoptosis in the proliferative phase, we allow either the apoptosis rate γ or the survival fraction σ to vary sinusoidally,

$$\gamma(t) = \gamma_0(1 + B\sin(2\pi t/T)), \ 0 \le B \le 1, \tag{4}$$

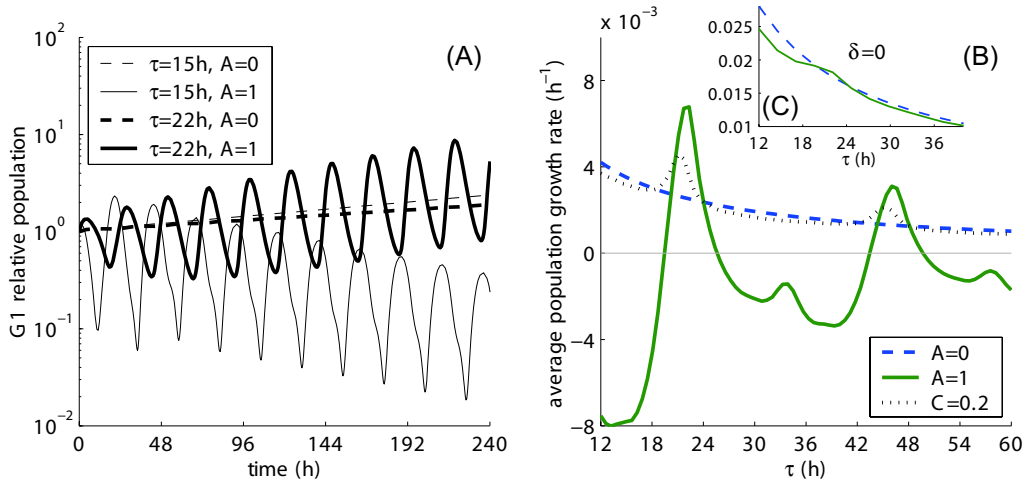

Figure 2: Resonance effect for periodic G1s→G1d transition rate $\beta(t)$ and proliferative phase apoptosis $\gamma(t)$. (A) Exponential growth of populations $N(t)$. For $A = 0$ (dashed lines), cells are rapidly desynchronized and the population grows exponentially. For $A = 1$ (solid lines), cells are synchronized and the population oscillates with a 24 h period in addition to the exponential growth. The initial population $N(t) = 1$ for $-\tau \leq t \leq 0$. (B) Average population growth rate λ_0 as a function of the proliferative phase duration τ. For $A = 0$ (dashed line), the growth rate decreases monotonically with respect to τ. For $A = 1$ (solid line), the growth rate λ_0 peaks approximately every 12 h. For $C = 0.2$ (dotted line), the resonance is also present, albeit in a weaker form. The growth rate is not affected by a periodic γ ($B > 0$, same as $A = 0$). In every case, the circadian variation of parameters does not affect the growth rate for $\tau = nT$. Parameters are $\delta = 0.1$ h^{-1}, $\beta_0 = 0.2$ h^{-1}, $\sigma_0 = 0.8$. The coupling coefficients A, B, and C are zero unless specified. (C) Growth rate for $\delta = 0$ and $A = 1$.

or

$$\sigma(t) = \sigma_0(1 + C\sin(2\pi t/T)),\ 0 \leq C \leq 1/\sigma_0 - 1. \tag{5}$$

The circadian coupling coefficients, A, B, and C, control the strength of the circadian clock on the cell cycle parameters. The parameter C is limited by the constraint that the survival fraction $\sigma(t)$ must be between 0 and 1. The period T is fixed to 24 h. We simulated the stochastic model with a Gillespie algorithm adapted for delay equations and the deterministic model with the delay equation solver dde23 (Matlab).

3 Results

Because equation 1 is linear, one would expect an exponential growth for the G1s population, $N = K(t)\exp(\lambda_0 t)$. With constant coefficients, $N(t)$ indeed grows exponentially, with $K(t)$ rapidly converging to a constant (Figure 2 (A), $A = 0$, dashed lines). The growth rate is slightly larger for $\tau = 15$ h than for $\tau = 22$ h (thin and thick dashed lines, respectively). For periodic coefficients, the average growth is also exponential, but the population oscillates periodically, with $K(t)$ converging to a T-periodic function. In contrast to the constant case, the growth rate increases as τ varies from 15 to 22 h when the G1s→G1d transition rate β is periodic (Figure 2 (A), $A = 1$, solid lines). For $\tau = 15$ (solid thin line), cell deaths outnumber cell births and the population decays. When τ is increased to 22 h (solid thick line), the population grows faster than any other, despite the longer cell cycle time. This suggests that a rhythmic β interacts with the successive waves of cell divisions. The proliferation phase duration τ is the key parameter that determines the population growth under circadian control.

The population dynamics with respect to τ behave differently depending on the values taken by the circadian coupling strengths A, B, and C (Figure 2 (B)). For $A = 0$ (dashed line), the growth rate decreases monotonically with respect to τ. For $A = 1$ (solid line), the growth rate λ_0 peaks every

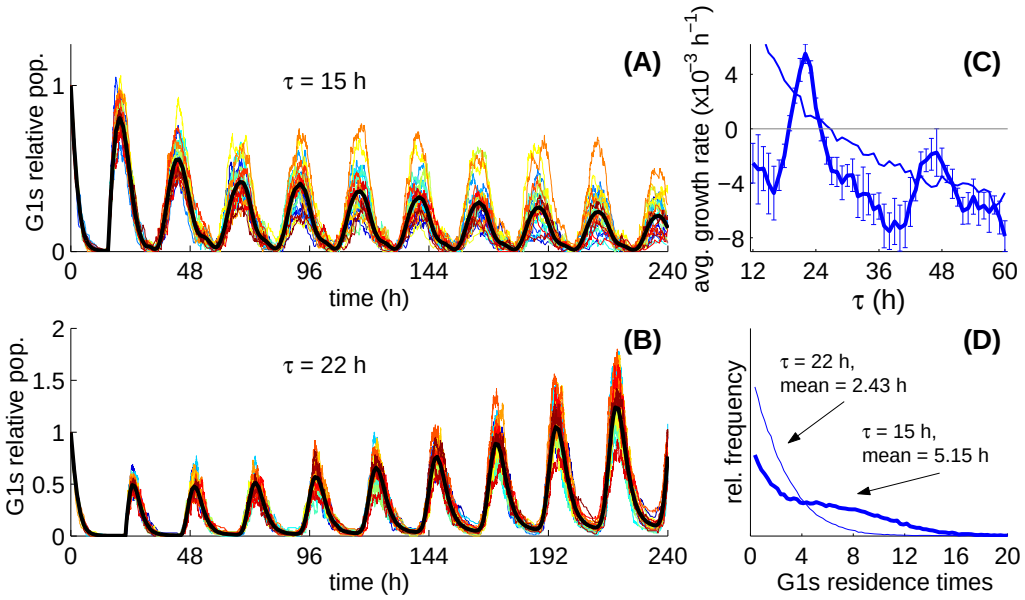

Figure 3: Stochastic simulations for periodic G1s→G1d transition rate β. (A–B) Ten stochastic time series for $\tau = 15$ and 22 h are shown with their respective deterministic simulations (thick black line). Parameters are $\delta = 0.1\text{h}^{-1}$, $\beta_0 = 0.2\text{h}^{-1}$, $\gamma_0 = 0.01\text{h}^{-1}$, initial number of G1s cells for each run $n_0 = 100$, duration of G1d phase 6 h, $A = 1$, $B = C = 0$. (C) Resonance effect in the stochastic model. The mean growth rate was estimated from 20 stochastic runs for each τ, for $A = 0$ *(thin line)* or $A = 1$ *(thick line)*, the error bars show the standard deviation for different runs. (D) Average residence time in the stochastic part of G1 (G1s) of cells entering the S-phase for $A = 1$.

12 h approximately, with main peaks at 22.1 and 46.2 h, and secondary peaks at 33.6 and 57.6 h. The maximal growth rate, at $\tau = 22$ h, is twice as large as for $A = 0$. When the fraction of cells surviving the proliferative phase σ is periodic, the growth rate peaks every 24 h, without showing secondary peaks ($C = 0.2$, dotted line). The situation is different for periodic apoptosis. A periodic parameter γ ($B > 0$) does not affect the growth rate [7]. That is, the growth rate has the same values as in the constant case. This is because a periodic rate γ pushes up the average survival fraction, $\langle \sigma_{per} \rangle \geq \sigma_{const}$, in a τ-dependent manner, preventing direct analogy between the effects of $B > 0$ and $C > 0$. When $\tau = nT$, the growth rate is independent of A, B, and C (see the intersections of the three lines at $\tau = 24$ and 48 h in Figure 2 (B)). If δ is negligible, the resonance effect becomes weaker (Figure 2 (C)). All these results show that cell populations under periodic conditions grow faster for specific proliferation phase durations. The resonance effect relies on the cell death or arrest rate δ.

For arbitrary ratio between τ and T, the growth rate is best determined by numerical methods. Some analytical treatment, however, is possible for particular cases. The population growth rate λ_0 of N is the largest (real) root determined by the eigenvalue problem

$$\frac{\mathrm{d}K(t)}{\mathrm{d}t} = -[\lambda + \delta + \beta(t)]K(t) + 2\sigma(t)\beta(t-\tau)e^{-\lambda\tau}K(t-\tau), \tag{6}$$

where $K(t)$ must be a T-periodic solution. When all the coefficients are constant, the eigenproblem reduces to the equation $\lambda + \delta + \beta = 2\sigma\beta e^{-\lambda\tau}$. For periodic coefficients, the eigenproblem can be approached by spectral methods [15] or by trying a reduction to a system of ordinary differential equations. If the proliferative phase duration τ is an integer multiple of the period T, the latter method leads to the case of constant coefficients.

The sensitivity of the growth rate on τ for periodic coefficients is reminiscent of resonance effects in physical systems. In a periodically forced damped oscillator, the amplitude is maximal when the intrinsic and the forcing frequencies coincide. For constant coefficients, the fraction of G1s cells,

$X = N/(N + P)$ (where P represents the proliferative population) is a nonlinear damped oscillator. Its intrinsic frequency can be related to the average interdivision time, with the variance in G1s duration slowly desynchronizing the cells and damping out X. If the interdivision time were such that cells minimized their residence times in G1s, a smaller fraction would die or arrest due to the term δ. Consequently, a larger fraction would reenter the proliferative phase. This is enough to compensate the longer τ needed to achieve this effect. Together, the probability of a G1s cell to enter the proliferative phase eventually, the rate of entry, and the length of the proliferative phase determine the growth rate. The stochastic formulation of the cell cycle model is best suited to determine the G1 residence times and the probability to enter the proliferative phase.

With the stochastic version of the cell cycle model, cells can be tracked individually. Time courses of the model for $\tau = 15$ and 22 h have a dynamics similar to the deterministic simulations (Figure 3 (A), (B)). The average population growth rate shows a resonance effect as in the deterministic version of the model (Figure 3 (C)). To understand this, we looked at the average time cells spent in G1 phase before entering S phase (Figure 3 (D)). The mean G1s residence time is 5.0 h for $\tau = 15$ h and 2.4 h for $\tau = 22$ h. Shorter residence time for $\tau = 22$ h means that fewer cells go to apoptosis. The probability of G1s cells to transit to proliferative phase is 0.54 for $\tau = 15$ h and 0.74 for $\tau = 22$ h. The difference is due to the presence of a "shoulder" in the residence time distribution for $\tau = 15$ h. G1s cell death or arrest (δ) plays an important role and is the major factor shaping of the growth rate resonance. For vanishing δ, the resonance effect is still present but becomes much weaker (Figure 2 (C)).

4 Discussion

Molecular evidence points to an important role of the circadian rhythm for efficient cell proliferation and reduction of tumor growth. This can be demonstrated using knock out mice without essential clock genes such as *Cry* or *Per2*. Free-running rhythmicity is abolished in *Cry*-deficient mice. After partial hepatectomy, liver regeneration in these mice is slower compared to wild type mice [18]. Cells showed a normal entry to S phase but a marked reduction in mitotic fraction compared to wild type. This implies that a functional circadian clock is necessary for efficient cell proliferation.

Following γ-radiation, *mPer2* null mice developed spontaneous salivary gland hyperplasia and teratomas faster than the wild type mice [11]. Moreover, epidemiological studies support the idea that circadian rhythms contribute to slowing down tumor growth. Patients with colorectal cancers exhibiting normal daily rhythms had longer survival times than those with disrupted circadian rhythms [19]. Nevertheless, a direct link between circadian rhythm and development of cancer has not been established. Arrhythmic mice do not systematically display increased sensitivity to cancer, as shown in a $Cry1^{-/-}Cry2^{-/-}$ mouse model [12]. Individual components of the circadian clock might have roles to play that are independent of the circadian rhythm. We suggest here that the presence of a circadian rhythm in cell cycle helps cells that have a normal cell cycle length, in addition to possible effects of individual clock genes.

Circadian regulation of the cell cycle orchestrates normal tissue regeneration and protects cells from oncogenic mutations. We propose that this is done by slowing down the cell cycle. Without circadian regulation, oncogenic mutations would be doubly damaging, because of the proliferative advantage and the possibly reduced anti-tumor mechanisms a short cell cycle length would allow. By shifting the proliferative advantage to slowly dividing cells, the circadian clock hence acts as a tumor suppressor. Slower cells have more time to detect and to repair genetic damages; mutations shortening the cell cycle are thus rendered unfavorable.

Our simulations show that only cells with a proliferative phase τ slightly shorter than 24 h (20 to 24 h) proliferate faster in presence of a circadian clock. This also holds for cells with τ between 44 and 48 h, to a lesser degree. The stochastic simulations allow the calculation of the average G1 residence time. For τ between 20 and 24 h, the G1s phase average residence time ranges from 3.3 to 2.3 h, for a total average cell cycle time between 23.3 and 26.3 h.

In two transplantable mice tumor models, Glasgow osteosarcoma and Pancreatic adenocarcinoma, disruption of circadian rhythmicity accelerated malignant growth [10]. Ablating the suprachiasmatic nuclei, center of the circadian pacemaker, or subjecting the mice to chronic jet lag resulted in the suppression of sleep-wake rhythms and altered body temperature and lymphocyte count rhythms. Our model provides a simple explanation for the accelerated tumor growth: if tumor cells have a deregulated cycle length (i.e. $\tau \ll 20$ h), the mere loss of circadian rhythmicity is sufficient to increase the tumor growth rate, every other quantities being equal (see Figure 2 (B), for $\tau = 12$ h, $A = 1$ and $A = 0$, for instance).

Other computational models have shown similar resonance effects. Clairambault *et al.* [6] recently studied tumor growth in an age-structured model with periodic parameters, and also found that the growth rate is significantly influenced by the ratio between the G1 and the proliferative phase durations. Battogtokh and Tyson [2] used a simplified molecular model of the eukaryotic cell cycle to show that periodic change of parameters is able to synchronize a population initially randomly distributed over the cell cycle. Using their formalism and adding random cell death, we showed a similar resonance effect for different lengths of the cell cycle (Figure 4 in Appendix).

The molecular role of the circadian clock in cell cycle regulation has been clarified in recent studies [11, 12, 13, 18]. Its impact on normal and tumor cell growth, however, can only be assessed when other regulatory mechanisms are taken into account. One of those, programmed cell death, is difficult to quantify because cells undergoing apoptosis are rapidly cleared from the organism, thus the apoptosis rate is generally underestimated [9, 14]. In addition to its role in tissue development and maintenance, it might act together with the circadian clock to coordinate cell proliferation. Programmed cell death, as it is, might finally promote normal cell growth and helps this way to suppress tumor development.

Acknowledgments

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A Appendix

To see if the resonance effect is specific to the population-based model, we tested a minimal molecular model of the eukaryotic cell cycle that can be synchronized by period forcing [2]. The model describes

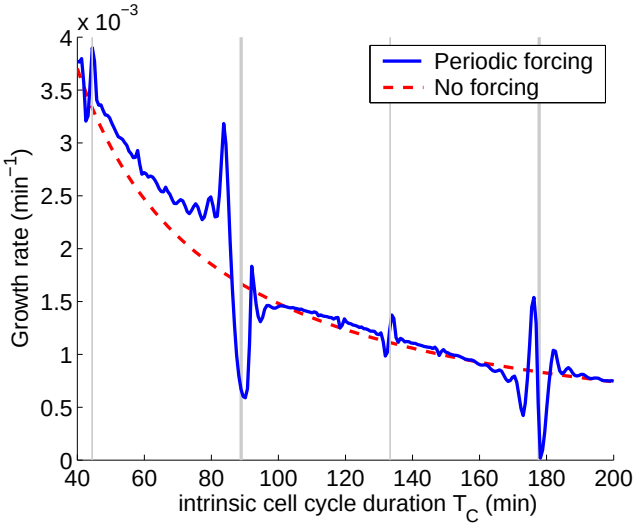

Figure 4: Resonance effect in a molecular-based cell cycle model. Average growth rate of the cell population. Death, or cell cycle arrest, was introduced at the beginning of the cell cycle, corresponding to the G1 phase ($d = 0.04$). In absence of periodic forcing, the growth rate is inversely proportional to the cell cycle length *(dashed line)*. In presence of periodic forcing, the growth rate shows significant resonance effect for cell cycle lengths $T_C = k/2T_0$, $k = 1, ..., 4$ *(solid line)*. The default cell cycle parameters are $A = 0.52$, $B = 0.52$, $\mu = 0.005776$, $k_1 = 0.002$, $k_2 = 0.0795$, $k_3 = 0.01$, $k_4 = 2$, $k_5 = 0.05$, $k_6 = 0.04$, $k_7 = 1.5$, $k_8 = 0.19$, $k_9 = 0.64$, $k_{10} = 0.0025$, $k_{11} = 0.07$, $k_{12} = 0.08$, $P = 0.15$, $J = 0.05$, $f = 2\pi/T_0$, and $T_0 = 88.88$.

the core of the cell cycle engine of the eukaryotic cell cycle.

$$\frac{\mathrm{d}X(t)}{\mathrm{d}t} = m(k_1 + k_2 W_0) - (k_3 + k_4 Y_0 + k_5 Z)X$$
$$\frac{\mathrm{d}Z(t)}{\mathrm{d}t} = k_{10}(1 + A(1 + \sin(ft))) + k_{11}X - k_{12}Z$$
$$\frac{\mathrm{d}m(t)}{\mathrm{d}t} = \mu(m + B(1 + \sin(ft)))$$
$$W_0 = g(X, P, J, J)$$
$$Y_0 = g(k_6 + k_7 Z, k_8 m + k_9 X, J, J)$$
$$g(a, b, c, d) = \frac{2ad}{(e + \sqrt{e^2 - 4ad(b - a)})}$$
$$e = (b - a + bc + ad)$$

A cell division occurs when X crosses downwards the threshold $X_{thresh} = 0.05$, and at this point the cell mass m is divided equally between the two daughter cells (see [2] for more details). We introduced random cell death or cell cycle arrest in the G1 phase by calculating the net increase b in proliferative cell number after each cell division ($0 \leq b \leq 2$). When cells are in G1 phase, cells exit the cell cycle at a rate d, so that the survival fraction after division is $b = 2\exp(-d\,t_{G1})$, where t_{G1} is the time cells spent in G1 (defined as cells having a mass less than 1.2). The external period, $T_0 = 88.88$ min, was set to be equal to the unperturbed cell cycle length, and we varied the cell cycle length from 40 to 200 min. To change the cell cycle length, the time was rescaled between $t \to T_0/200t$ and $T_0/40t$. The growth rates with or without external forcing were similar except for cell cycle lengths $T_C = k/2T_0$, $k = 1, ..., 4$ (Figure 4), where the growth rate under periodic forcing showed a resonance similar to what is seen in the population-based model.