Entrainability of Cell Cycle Oscillator Models with Exponential Growth of Cell Mass

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Abstract— **Among various aspects of cell cycle, understanding synchronization mechanism of cell cycle is important because of the following reasons. (1)Cycles of cell assembly should synchronize to form an organ. (2) Synchronizing cell cycles are required to experimental analysis of regulatory mechanisms of cell cycles. (3) Cell cycle has a distinct phase relationship with the other biological rhythms such as circadian rhythm. However, forced as well as mutual entrainment mechanisms are not clearly known. In this study, we investigated entrainability of cell cycle models of yeast cell under the periodic forcing to both of the cell mass and molecular dynamics. Dynamics of models under study involve the cell mass growing exponentially. In our result, they are shown to allow only a limited frequency range for being entrained by the periodic forcing. In contrast, models with linear growth are shown to be entrained in a wider frequency range. It is concluded that if the cell mass is included in the cell cycle regulation, its entrainability is sensitive to a shape of growth curve assumed in the model.**

I. Introduction

The cell division cycle is a fundamental process of cell biology, and a detailed understanding of its function and regulation is critical to many applications in biotechnology and medicine. Molecular mechanisms underlying cell cycle regulation have been clarified [1]. The master regulatory molecules are enzymes called cyclin-dependent protein kinases (Cdks). When associated with appropriate cyclins, Cdks trigger major events of the chromosome cycle such as DNA replication, chromosome condensation, and spindle assembly, by phosphorylating target proteins. Deactivation of mitotic Cdk allows cells to divide and enter the interphase. Regulatory mechanism of cell cycle has been studied for variety of organisms including budding yeast, fission yeast, and mammalian cells. Although there are significant differences in machinery from one specie to another, the underlying cell cycle engine is conserved [2]. Among various aspects of cell cycle, understanding entrainment mechanism of cell cycle is important because of the reasons shown below. First of all, forming an organ likely to need the synchronization between cell cycles [3]. Second, entraining cell cycles is required to experimental analysis of regulatory mechanisms of cell cycles [4]. Third, cell cycle keeps a distinct phase relationship with a circadian rhythm, which have been attracting attention from the viewpoints of oncogeny and chronotherapy of cancer [5,6]. However, forced as well as mutual entrainment mechanisms are not clearly known.

A number of mathematical models have been developed to describe the dynamics of cell cycle. One of major trends of modeling is that the cell cycle engine is regarded as a limit cycle or a cascade of bifurcations including a limit cycle driven by the growth of cell mass [2,7,8,9]. In this study, owing to these models we investigate entrainmenability of cell cycle under the periodic forcing to the cell mass and molecular regulatory dynamics. Simulation and analysis show significance of growth curve of cell mass in entrainability and limitation of entrainment under the exponential growth.

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II. Periodic Forcing of Cell Cycle Models with Exponential Growth of Cell Mass

A. Cell Cycle Model

Since, as shown above, the cell cycle engine is structurally similar to each other over the eukaryote species, the model of budding yeast cell is subject to investigation here [10]. Our investigation is to get general insight into the constituent mechanism shared commonly. Therefore, the model used here is only a sample for study. The model equations are given as follows.

$$\begin{aligned}
&\frac{dX}{dt} = m(k_1 + k_2 W^*) - (k_3 + k_4 Y^* + k_5 Z)X, \\
&\frac{dZ}{dt} = (k_{10} + k_{11} X) - k_{12} Z, \\
&\frac{dm}{dt} = \mu m, \\
&W^* = G(X, P, J_2, J_2), \\
&Y^* = G(k_6 + k_7 Z, k_8 m + k_9 X, J_1, J_1), \\
&G(a,b,c,d) = \frac{2ad}{\beta + \sqrt{\beta^2 - 4ab(b-a)}}, \beta = b - a + bc + ad,
\end{aligned} \tag{1}$$

where, X denotes the concentration of heterodimer of cyclin and Cdk, and Z that for regulatory agent of mitosis. For more details, please refer to Battogtokh and Tyson [10]. Used parameter values are those of the original, i.e., k_1=0.002, k_2=0.053, 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}=0.005, k_{11}=0.07, k_{12}=0.08, μ=0.005776, P=0.15, J_1=0.005, and J_2=0.01. The cell is set to divide into "daughter cells" when X gets across the threshold, 0.05, downward. The cell mass of "daughter cell" is a half of the "mother cell" at division (the proportional division rule). Key

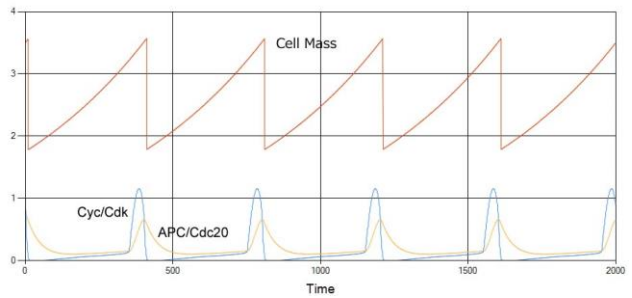

Figure1. Dynamics of the cell cycle model

features under special concern here are the exponential growth of cell mass, m, and its involvement in production of X in a monotonically increasing fashion. Such m-dependency of the cell cycle dynamics is a central idea that m irreversibly induces a cascade of dynamics bifurcations [2]. Model's dynamics are shown in Fig.1, where the period of cycle is given by $T_0=ln2/\mu$ from the division rule and exponential growth of cell mass [10]. That is, μ alone determines T_0 regardless of the machinery of cell cycle control.

B. *Periodic Forcing of Cell Cycle Model*

Experimentally, synchronizing cell cycles is realized by periodic exchange between galactose and glucose media or acute activation of cyclin transcription [4]. The media exchange possibly affects both of cell growth and molecular machinery. Biologically, the circadian clock is expected to affect the progress of cell cycle through the molecular agents *Per1/2* and *Wee1* [5]. In order to mimic this situation, according to Battogtokh and Tyson [10], the periodic forcing to the cell mass growth and molecular machinery of cell cycle are implemented by

$$\frac{dm}{dt} = [\mu + A\mu(1+\sin(2\pi ft))]m, \qquad (2)$$
$$k_{10} \leftarrow k_{10} + Ak_{10}[1+\sin(2\pi ft)],$$

where f is a frequency of periodic forcing. As shown in eq.(1), k_{10} controls production of Cdc20.

Naturally, f around f_0 ($=1/T_0$) or f roughly satisfying $pf:qf_0$ (p, q: natural number) could allow synchronization by the periodic forcing (entrainment), and the entrainable range of f depends on the coupling strength, A. Entrainability of limit cycle oscillator is characterized by the entrainable frequency range as a function of the coupling strength, i.e., "Arnold's tongue" [11]. Battogtokh calculated the Arnorld's tongue for the model (1) [12]. Here, we re-compute the Arnold's tongues under periodic forcing to both of the cell mass and k_{10}, and

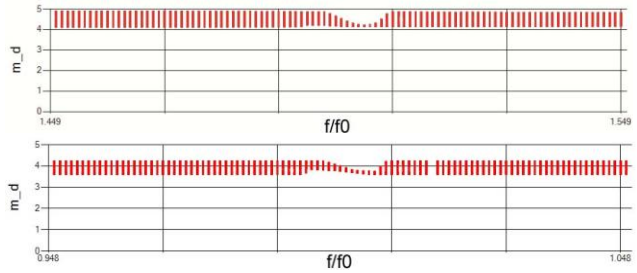

Figure 2. Variations of cell mass at division for perturbations to m and k_{10} (top) and only to k_{10} (bottom). A=0.45. Resolution of f/f_0=0.001.

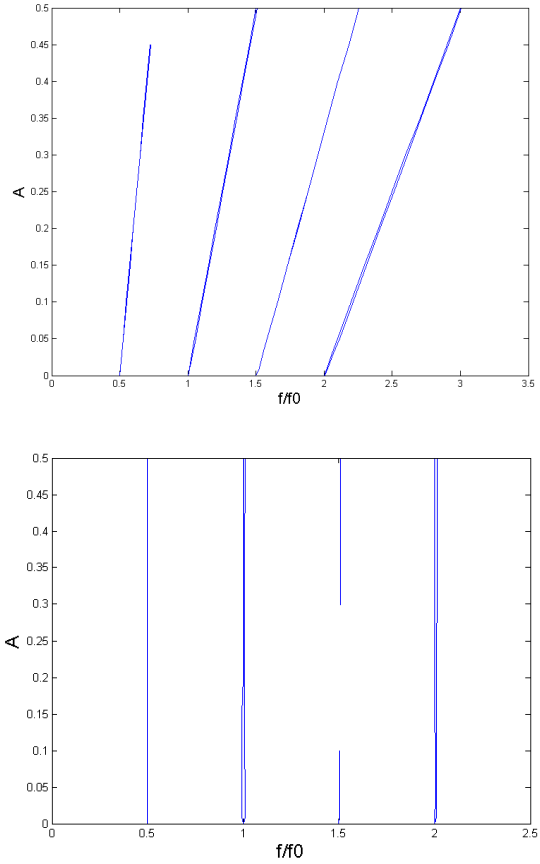

Figure 3. Arnold's tongues for perturbations to m and k_{10} (top) and only to k_{10} (bottom). Resolution of f/f_0=0.001. Missing parts are only due to numerical instability within the resolution used here.

under forcing only to k_{10}. Figure 2 shows the variations of the cell mass at division (m_d), where a large variation indicates that one-to-one entrainment does not take place. The range of entrainable frequency is estimated as an interval between frequencies at which the variance of m_d dips to half its depth from the reference level obtained by a linear regression as a function of f/f_0. Estimated Arnold's tongues are shown in Fig.3. The results for different forcing conditions share the property that only a limited entrainable frequency range is allowed independent of A, although the frequency range for each

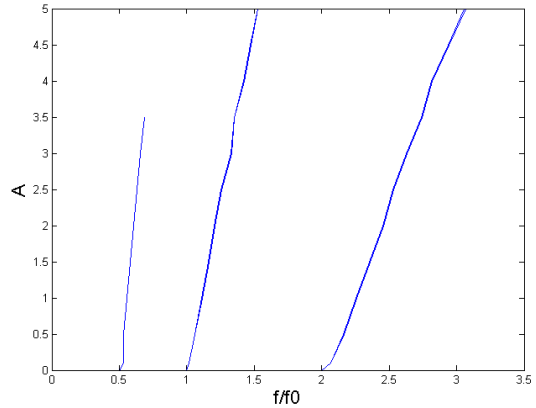

Figure 4. Arnold's tongue for the perturbations to m and k_{a20}. Resolution of f/f_0=0.001. A missing part is only due to numerical instability within the resolution used here.

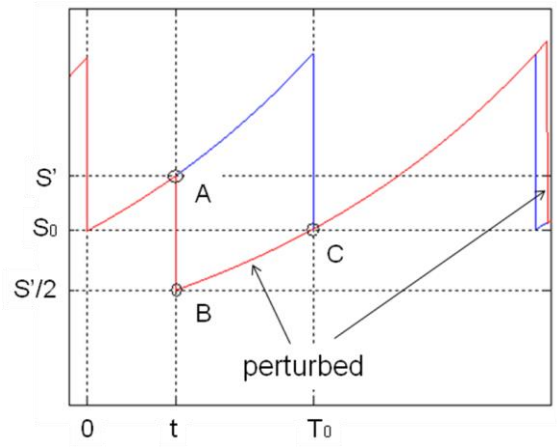

Figure 5. Trajectories of exponential growth of cell mass with and without perturbation.

entrainment ratio moves higher as A increases for the case of forcing to m and k_{10}. Such a limited entrainable frequency range was also shown in [12].

C. Periodic Forcing of Generic Cell Cycle Model

Next, this limitation in entrainment is shown to be general as long as the framework of the model (1) is shared. The generic detailed model of cell cycle is used for this purpose [8], where the cell mass grows exponentially, and the production rates of all cyclins are multiplied by the cell mass. For more detail, please refer directly to [8]. Periodic forcing is introduced in the following way.

$$\frac{dm}{dt} = [\mu + (A/10)\mu(1+\sin(2\pi ft))]m, \qquad (3)$$
$$k_{a20} \leftarrow k_{a20} + A k_{a20}[1+\sin(2\pi ft)],$$

where k_{a20} is modulated because of the model structure that k_{a20} is a production rate of Cdc20 protein [8]. The cell cycle is entrained by the periodic forcing with $f=f_0$ in a one-to-one manner (the results are omitted here), where the parameter values follows the set of budding yeast [8]. The Arnold's tongues are computed based on the variations of m_d under the periodic forcing to both of the cell mass and k_{a20}, and the periodic forcing only to k_{a20}. The result under the former condition is only shown in Fig.4. Similar to the previous model (1), the generic model allows only a limited entrainable frequency range independent of A.

From experimental and biological points of view, the limited ability of entrainment shared by the different models implies that synchronization of cell cycle is difficult to be realized, considering possible diversity in the period of individual cell cycle and difference between intrinsic periods of circadian rhythm and cell cycle. Our concern here is the reason for the limited entrainability. As shown before, the period of the model cell cycle with the exponential growth of cell mass is $T_0=ln2/\mu$. This means not only that μ solely determines the period of cycle independent of the cell cycle machinery, but that the perturbation to the molecular machinery cannot change the period. Because, generally speaking, entrainment needs change in the period of oscillator subject to the periodic forcing, the cell cycle with the exponential growth and proportional division is expected not to allow mutual and forced entrainments for $f \neq f_0$.

D. Exponential Growth Limits Ability of Entrainment

The limitation of ability of entrainment described above can be attributed to the exponential growth of cell mass. Figure 5 illustrates how the exponential growth works under perturbation, where we are confined to a temporal perturbation. Provided that the cell divides at t on the way to the cell cycle completion due to perturbation, the cell mass is reduced from S' (A) to $S'/2$ (B) because of the proportional division. The point B on the perturbed trajectory is always on the extension from the point C on the unperturbed trajectory, which is known from the fact that the following relations are satisfied. For point B, $S'/2= 1/2S_0exp(\mu t)$. On the other hand, the extension curve from the point C at t is expressed by $S(t)=S_0exp(\mu(t-T_0))= S_0exp(\mu t)/exp(\mu T_0)=S'/2$, where please remind the relation $exp(\mu T_0)=2$. Since the moment t at which the cell division takes place under perturbation is arbitrary, it is proven that the perturbed trajectory of the cell mass growth is always on the unperturbed one. Because of stability of cell cycle, the steady relation $T_0=ln2/\mu$ should be reached after the temporal perturbation. Therefore, the perturbed trajectory of the cell mass is completely superposed on the unperturbed one at least asymptotically in the steady state. In other words, even if the molecular machinery delays or advances the division

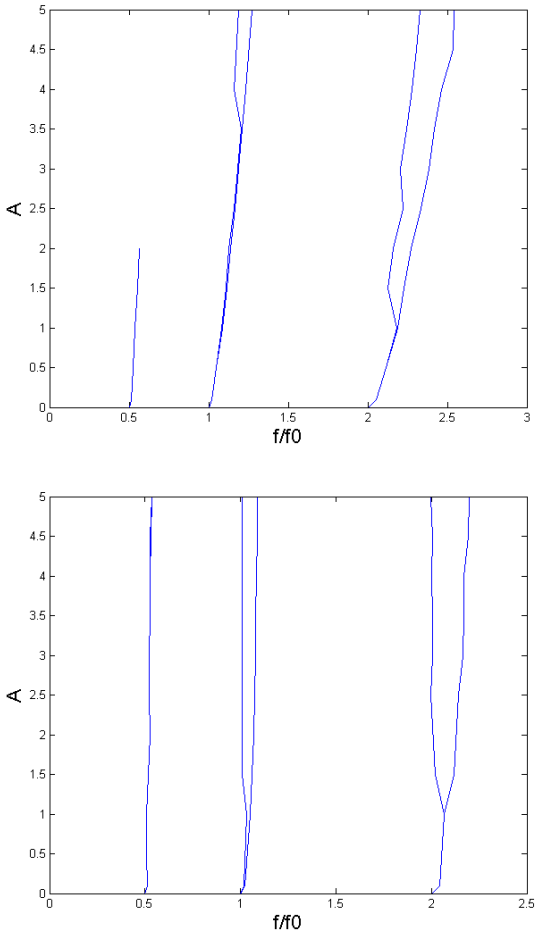

Figure 6. Arnold's tongues for the perturbations to m and k_{a20} (top) and only to k_{a20} (bottom). Resolution of f/f_0=0.005 (top) and 0.003 (bottom). Amissing part is only due to numerical instability within the resolution used here.

due to the perturbation, the phase of cell cycle restores the unperturbed phase of oscillation, i.e., a null phase response. Although further investigation may be necessary for us to understand the entrainment under continuous perturbation, the situation could be regarded as that under the repetition of temporal perturbation.

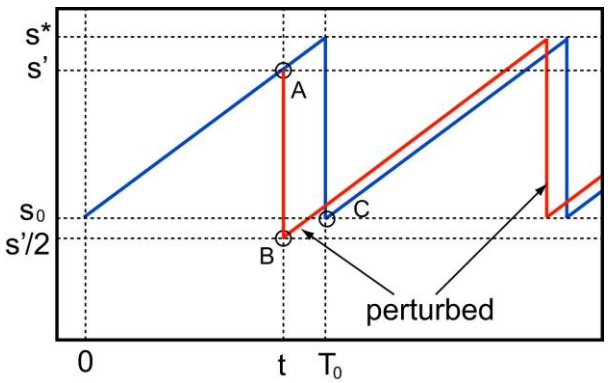

Figure 7. Trajectories of linear growth of cell mass with and without perturbation.

III. Periodic Forcing of Cell Cycle Models with Linear Growth of Cell Mass

Although, as mentioned above, the exponential growth of cell mass is widely shared in the cell cycle models, there are experimental studies showing non-exponential growth [13]. Here, a linear growth mechanism of cell mass is included in the cell cycle models used so far and their dynamics are examined. After confirming steady oscillations in the models with the linear growth, periodic forcing is introduced as follows.

$$\frac{dm}{dt} = \mu + A\mu(1+\sin(2\pi ft)), \qquad (4)$$

where forcing to k_{10} or k_{a20} is applied in the same way as before. Entrainable frequency range as a function of coupling strength A is obtained for the models with the linear growth of cell mass. Since qualitative features are shared by the model (1), only the result for the generic model is shown in Fig.6. Wider entrainable frequency range than in the exponential growth is shown for 1:1 and 1:2 entrainment regardless of the detail model structure. Figure 7 illustrated the unperturbed and perturbed trajectories of cell mass for the linear growth case, where S^* is the cell mass at division in the steady state. For point B, $S'(t)/2 = 1/4S^* + \mu t/2$. The extension from the point C at t is expressed by $S(t)=\mu t$ because of the relation $T_0=S^*/(2\mu)$ in the linear growth case. Both lines meet only at $t=T_0$, i.e., unperturbed division. Therefore, it is proven that the same thing as the exponential growth could not happen in the linear case. If the molecular machinery delays or advances the division due to the perturbation, the phase of cell cycle remains shifted, i.e., a non-null phase response. These results confirm our idea that the exponential growth limits the ability of entrainment due to its own numerical property shown in Fig.5.

IV. Conclusion

Regardless of the detail model structure, it is shown that the exponential growth limits the entrainability of the cell cycle model that includes the cell mass as a regulatory factor for the progress of cycle. This property could not explain the distinct phase relationship between cell cycles and circadian rhythm [14]. Therefore, an alternative growth curve might fit [13], or different regulatory mechanisms from those involving the growth of cell mass might be plausible. Actually, the other trends of modeling are exemplified by a model consists of cascades of bifurcation including limit cycles [15] and modeling within the transport theoretical framework of aged population [16]. Those models appear to accommodate the circadian control mechanisms. Synchronizations with the other cell cycles and biological rhythms are biologically and clinically very important. Our result here provides a criterion for examining the reality of models built in different contexts.

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