Synchronization of Eukaryotic Cells by Periodic Forcing

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We study a cell population described by a minimal mathematical model of the eukaryotic cell cycle subject to periodic forcing that simultaneously perturbs the dynamics of the cell cycle engine and cell growth, and we show that the population can be synchronized in a mode-locked regime. By simplifying the model to two variables, for the phase of cell cycle progression and the mass of the cell, we calculate the Lyapunov exponents to obtain the parameter window for synchronization. We also discuss the effects of intrinsic mitotic fluctuations, asymmetric division, and weak mutual coupling on the pace of synchronization.

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Synchronization of rhythmic processes is a fundamental problem in science with applications ranging from engineering to medicine [1]. A major rhythmic process in the physiology of a cell is the cell cycle, by which a cell replicates all its components and divides into two daughter cells. In modern cell cycle research, it is often required to generate cultures that are growing synchronously. However, it is frequently debated whether some common experimental techniques truly synchronize all culture [2,3]. Because comprehensive mathematical models consistent with available experimental data on the eukaryotic cell cycle have been developed only recently [4,5], there are no theoretical studies to shed light on these debates. These models, describing progression through DNA synthesis-mitosis-cell division and cell growth, are well understood qualitatively by bifurcation analysis [6,7]. Thus, an interesting and important theoretical problem is synchronization of cells whose individual dynamics are governed by a reasonable model of the eukaryotic cell cycle.

A population of deterministic oscillators can be synchronized either by external forcing or by mutual coupling. The former method is often used in cell cycle research, by perturbing a cell culture's environmental parameters, such as heat [8], light [9], hypertonic stress [10], and nutrition [11]. Little is known about synchronization of a cell population by mutual coupling between the cells. The molecular mechanisms of downstream processes—cell to cell signaling, growth factor sensing, signal transduction, etc.—are not known well enough to integrate all the events from cell communication to cell division in a realistic mathematical model. In diploid yeast cultures, mutual coupling between cells is negligible and if nutrition of the culture remains sufficient, they proliferate independently for many generations. A population of such cells can be synchronized by periodic forcing, and the pace of synchronization can be studied by means of mathematical modeling.

Let us consider the wiring diagram shown in Fig. 1, which describes the core of the cell cycle engine regulating the eukaryotic cell cycle. The following minimal model can be derived from Fig. 1,

$$\frac{dX}{dt} = m(k_1 + k_2 W_0) - (k_3 + k_4 Y_0 + k_5 Z)X, \quad (1)$$

$$\frac{dZ}{dt} = (k_{10} + k_{11} X) - k_{12} Z, \quad (2)$$

$$\frac{dm}{dt} = \mu m, \quad (3)$$

where m is the cell's mass. Equation (3) must be supplemented by the cell division rule, $m \longrightarrow \tau m$, where $0 < \tau < 1$, which divides the cell whenever the activity of the cyclin dependent kinase, X, drops below a threshold X_{thr}. The activities of components Y and W are derived from quasi-stationary assumptions [12]: $W_0 = G(X, P, J, J)$ and $Y_0 = G(k_6 + k_7 Z, k_8 m + k_9 X, J, J)$. The Goldbeter-Koshland function [13] is $G(a, b, c, d) = \frac{2ad}{e + \sqrt{e^2 - 4ad(b-a)}}$, $e = b - a + bc + ad$.

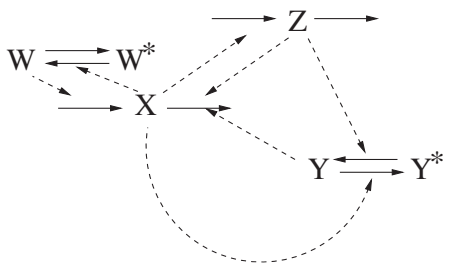

FIG. 1. Core module of cell cycle regulation in budding yeast [4]. X stands for the cyclin dependent kinase Cdc28/Clb2, Y stands for the active form of enzyme Cdh1, Z stands for enzyme Cdc20, and W stands for the active form of the transcription factor, Mcm1. "*" marks inactive forms. By dotted line we indicate regulations involving enzymatic reactions.

More detailed studies of Eqs. (1)–(3), including phase portraits and bifurcation diagrams are presented elsewhere [12]. In Fig. 2 we show a typical simulation of Eqs. (1)–(3). At small values of mass, m, the ratio, $\frac{X}{Z}$, is small because X is small in the G_1 phase of the cell cycle. As the cell mass grows, there are transitions through cell cycle phases $S/G_2/M$. When the level of X drops below the threshold, X_{thr}, mass is divided ($m = \tau m$) and the system reenters into the G_1 phase.

Different methods have been used for achieving synchronized cell populations, including pulse treatment with toxic drugs, physical separation of cells by size, perturbation of temperature and nutrient conditions, and genetic restriction [14,15]. Some of these methods simultaneously perturb both the cell cycle engine and mass growth [12]. For example, suppose the cyclin protein Clb2 (X in our model) is transgenically expressed from a *GAL-CLB2* gene by shifting the culture from glucose medium to galactose medium and back to glucose medium. Mathematically, this treatment perturbs both Eqs. (1) and (2) and Eq. (3). With such experimental protocols in mind, here we consider periodic forcing that modulates the synthesis rate of a single protein ($k_{10} \rightarrow k_{10} + \delta k_{10}$, where $\delta k_{10} = k_{10}A[1 + \sin(ft)]$), and the mass growth rate ($\mu m \rightarrow \mu m + \mu B[1 + \sin(ft)]$).

Experimentally, as an asynchronous population of cells has a broad distribution of cell mass, it is difficult to narrow the mass distribution by treating all cells equally. This is one of the arguments of Cooper, who thinks that whole-culture methods cannot truly synchronize cell populations [2]. Although cells may be aligned at a certain phase in the cell cycle, their masses are still widely distributed and so the cells are in very different physiological states, according to Cooper. This argument also can be seen from Eqs. (1)–(3) where the growth process is decoupled from the dynamics of cell cycle proteins; therefore, even if cell cycle phases of different cells are synchronized by whole-culture treatments, the mass distribution remains broad and unsynchronized. However, in the mode-locked regime of the forced cell cycle, the mass distribution narrows, as cells divide only at certain mass values [12]. Mode locking also constrains the dynamics of proteins involved in cell cycle regulation, which attain only certain fixed values at divisions, regardless of their initial levels. As a result, forcing leads to synchronization of noninteracting cells described by the eukaryotic cell cycle model. Figure 3 shows cell generations from 128 cells described by Eqs. (1)–(3) with the forcing terms as described above, and with random initial values for X, Z, and m. In Fig. 3(a), the increase of N (the number of cells) is irregular; therefore, the first generations of cells are not synchronized. But, in later generations, N doubles within short time intervals, indicating an onset of synchronization, Fig. 3(b).

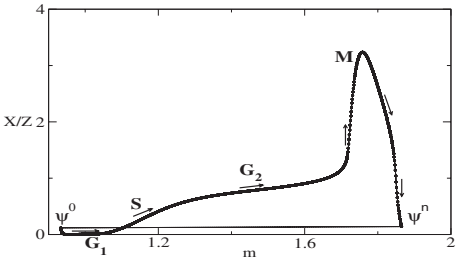

FIG. 2. Cell cycle dynamics in Eqs. (1)–(3). Arrows mark the pace of cell cycle progression. G_1, S, G_2, M refer to the four standard phases of the eukaryotic cell cycle. Cell birth and division are denoted by ψ^0 and ψ^n. Parameters in units of min^{-1}: $k_1 = 0.002$, $k_2 = 0.0795$, $k_3 = 0.01$, $k_4 = 2$, $k_5 = 0.05$, $k_6 = 0.04$, $k_7 = 1.5$, $k_8 = 0.19$, $k_9 = 0.64$, $k_{10} = 0.0025$, $k_{11} = 0.07$, $k_{12} = 0.08$, $\mu = 0.005\,776$. Other parameters: $P = 0.15$, $J = 0.05$, $X_{\text{thr}} = 0.05$, and $\tau = 0.5$.

The two principal processes in our model are cell cycle progression Eqs. (1) and (2), and cell growth Eq. (3). We propose to reduce Eqs. (1) and (2) to a single variable, ϕ, for cell cycle phase, $\frac{d\phi}{dt} = \tilde{\mu} m$, with cell division at $\phi = \phi_{\text{thr}}$ and cell birth at $\phi = m_{\text{birth}}$. In the presence of periodic forcing of both variables, our model is

$$\frac{dm}{dt} = \mu m + \mu B[1 + \sin(ft)], \tag{4}$$

$$\frac{d\phi}{dt} = \tilde{\mu} m + \mu A[1 + \sin(ft + \chi)]. \tag{5}$$

Equations (4) and (5) are subject to the following resets: $m \rightarrow \tau m$ and $\phi \rightarrow m$ if $\phi = \phi_{\text{thr}}$. Parameter χ characterizes a phase shift in forcing. We numerically confirmed that Eqs. (1)–(5) display qualitatively similar dynamics.

Because they involve reset dynamics associated with cell division [5,16,17], cell cycle models are quite complicated for theoretical and numerical analyses. The advantage of the toy model, Eqs. (4) and (5), is that it allows explicit calculations of the Lyapunov exponents [18] which are given by $\lambda^{1,2} = \lim_{n\rightarrow 0} \frac{1}{2n} \ln(\omega_n{}^{1,2})$, where n is number of cell divisions, and $\omega^{1,2}$ are the eigenvalues of the symmetrical matrix $M_n^T M_n$. The superscript T denotes matrix transposition and M_1 is set to the identity matrix. For $n > 1$

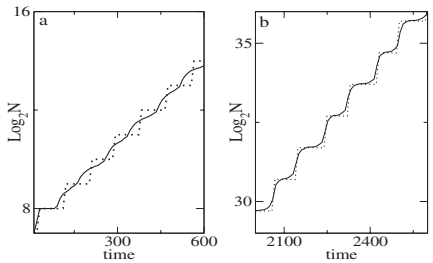

FIG. 3. Onset of synchronization in a periodically forced population with cells described by Eqs. (1)–(3). We monitor number of cells, N, generating initially from 128 cells. The dotted lines show perfect cell synchrony. (a) First generations are not synchronized. (b) Onset of synchronization. $A = B = 0.52$ and other parameters are the same as in Fig. 2.

the M matrix can be computed as $M_{n+1} = m_n M_n$, where $m_n = D_{t_{n+1}-t_n} C_{t_n}$. The D_t matrix characterizes the dynamics of small perturbations, $\delta m(t)$ and $\delta\phi(t)$, during continuous time evolution between cell birth and division. For Eqs. (4) and (5), D_t is a vector given by $D_t^T = \{\exp(\mu t), \exp(\tilde{\mu} t)\}$. C_t matrix characterizes dynamics of the small perturbations at cell division and is given by $C_t = \tau\{\{1, \frac{(\tau^{-1}-1)\tilde{A}}{\tilde{\mu} m + \tilde{A}}\}, \{1, \frac{(\tilde{\mu}-\mu)m + (\tau^{-1}-1)\tilde{A}}{\tilde{\mu} m + \tilde{A}}\}\}$, where $\tilde{A} = \mu A[1 + \sin(ft)]$. For simplicity we assume $A = B$ and $\chi = 0$ in Eqs. (4) and (5).

The first two rows in Fig. 4 show distributions of interdivision time and mass at division upon variations of the amplitude of the periodic forcing in Eqs. (4) and (5). Continuous spectra show quasiperiodic motions, whereas discrete spectra show mode-locked solutions. The third row in Fig. 4 shows the corresponding Lyapunov exponents which are negative when the system is in a mode-locked regime.

Since cells are uncoupled, we need consider only two cells to characterize synchronized motions. Thus, we introduce a quantity, $R_{\text{synch}} = \ln|\frac{\Delta(t)}{\Delta(0)}|$, where $\Delta(t) = \sqrt{[m_1(t) - m_2(t)]^2 + [\phi_1(t) - \phi_2(t)]^2}$. Dotted lines in Fig. 5 show the dynamics of R_{synch} when the forcing amplitude A is outside of the mode locking window. The solid lines in Fig. 5 show the rate of the synchronization when $A = 0.77$. Notable synchrony ($\Delta < 2.5 \times 10^{-3}$) is achieved at $T_{\text{synch}} \sim 22\,000$ min ($\sim$200 generations or $\sim$17 days). For different initial values of Δ_0, we numerically found similar times for $\Delta < 2.5 \times 10^{-3}$.

If we take into account random processes inherent in biomolecular networks, the mode locking window might shift or even disappear as the fluctuations become stronger. Synchronization of the cell cycle in Eqs. (4) and (5) is sensitive to fluctuations of parameters (μ and ϕ_{thr}) defining the resonance condition. Let ϕ_{thr} be a random number with the mean π and the standard deviation σ. Figure 6 shows the order parameter calculated for two descendants of two daughter cells selected in each generation. Synchrony (at $\sigma = 0$) is degraded as σ increases, but some degree of synchrony still can be detected if σ is not too large. On the contrary, stochastic synchronization is also possible; a system desynchronized at $\sigma = 0$ may obtain a certain degree of synchrony when $\sigma_1 < \sigma < \sigma_2$ (see $A = 0.74$ in Fig. 6).

We assume that cells divide symmetrically ($\tau = 0.5$), as fission yeast cells do. However, budding yeast cells divide asymmetrically [19]. For the case of asymmetric division, our simulations show that periodic forcing at fixed A and f fails to synchronize the whole population if the asymmetry of division is too great. However, for appropriate choices of A and f, it is always possible to enforce mode locking for selected cells. For example, in our simulations, after several generations in the mode-locked regime, the daughter cells become synchronized. Also, by alternating forcing parameters, A and f, from the values selectively synchronizing mother or daughter cells, a certain degree of synchrony in the whole culture can be achieved.

We neglect in our models the phenomenon of aging—a physiological feature of budding yeast cells that complicates the production of synchronous cultures. Even if a culture can be synchronized with respect to position in the DNA replication-mitosis-division cycle, the cells within the population will be nonuniform with respect to age and state of senescence. Fortunately, the oldest and most senescent cells are also the rarest, because they are diluted out by the more numerous younger cells in a population. Therefore, their effects on the average physiological state of the culture may be negligible.

Though we ignore mutual coupling between the cells, a cell in a culture senses its environment and exchanges information with other cells, for instance, through growth factors—rapidly diffusing molecules. Growth factors participate indirectly in cell cycle regulation, by interacting with proteins that activate or deactivate key cell cycle proteins [5]. Mathematically, accounting of growth factors in cell cycle dynamics will lead to a reaction diffusion model which under certain conditions can be approximated by a simpler model similar to Eqs. (4) and (5) that can describe qualitative dynamics of realistic models [1,20]. Thus, the simplest way to account for the effects of growth factors in the toy model is by coupling the phase variables, i.e., by introducing a coupling function Γ in the right-hand side of Eq. (5). If coupling is weak, the coupling function

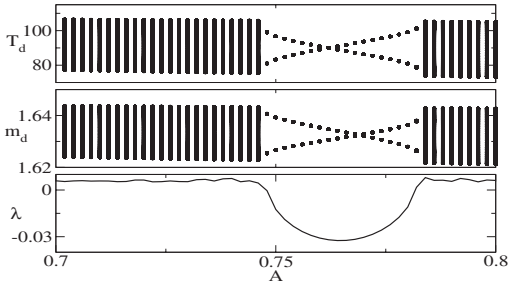

FIG. 4. Mode locking in Eqs. (4) and (5). Parameters are $\tilde{\mu} = 0.9\mu$, $\phi_{\text{thr}} = \pi$, and $f = f_0 : \frac{2}{3}$, where $f_0 = \frac{2\pi}{T_0}$; $\mu = 0.005\,776$ and $T_0 = \frac{Ln2}{\mu}$.

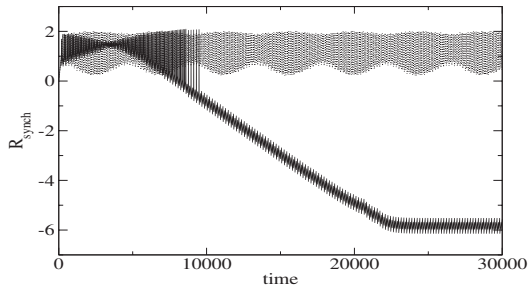

FIG. 5. Time evolution of the order parameter. Solid lines: $A = 0.77$, dotted lines: $A = 0.7$. Parameters are the same as in Fig. 4.

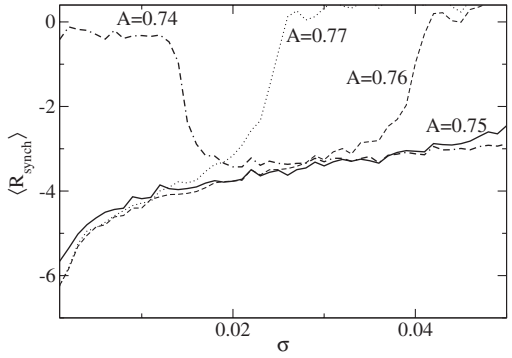

FIG. 6. Synchronization in the presence of fluctuations. R_{synch} is computed by comparing two daughter cells in each generation. We average R_{synch} over time and over different initial conditions. Parameters are the same as in Fig. 4.

for the phase of a cell at the spatial location x can be given by $\Gamma(x) = \int \exp-(\gamma|x-y|)\sin(x-y+\alpha)dy$. Because the time scale of diffusive agents is small, the coupling range of Γ can be global ($\gamma = 0$) or nonlocal ($\gamma \ll 1$) [21,22]. Our simulations show that in the absence of forcing, attractive coupling can synchronize phases, but not the mass distribution as Cooper argued [2]. Thus, forcing of the growth dynamics is essential in synchronization, even when the phases are under global, attractive coupling. When cells are coupled, T_{synch} is only few hours. If the coupling is repulsive and sufficiently strong, the cells are desynchronized even in a mode locking regime. An interesting case for nonlocal coupling is that, in some range of attractive coupling, cells can be in a *chimera* state, where cells split into two domains, synchronized and desynchronized [23].

The dynamics of cell cycle and cell growth can be modulated experimentally. Indeed, Cross and Siggia discussed periodic forcing of the dynamics of genes and proteins involved in cell cycle regulation of budding yeast and suggested ways to enforce mode locking experimentally [24]. As for the perturbations of mass growth, recent experiments indicate that it is possible to modulate cell mass by heat shocks [25] or by perturbations that change the uptake through cell membrane [26]. What can be essential in forced cell synchronization experiments is a coordination of modulations of both growth and cell cycle progression.

In conclusion, we have shown that a cell population described by a model of the eukaryotic cell cycle can be synchronized by periodic forcing that simultaneously modulates the dynamics of the cell cycle engine and of mass growth. The degree of synchrony is higher if the cells divide symmetrically and if fluctuations are not too strong.

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