Synchronization by Irregular Inactivation

Taunia L. L. Closson and Marc R. Roussel*

Department of Chemistry and Biochemistry, University of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4
(Received 25 February 2000)

Many natural and technological systems have on/off switches. For instance, mitosis can be halted by biochemical switches which act through the phosphorylation state of a complex called mitosis promoting factor. If switching between the on and off states is periodic, chaos is observed over a substantial portion of the on/off time parameter plane. However, we have discovered that the chaotic state is fragile with respect to random fluctuations in the on time. In the presence of such fluctuations, two uncoupled copies of the system (e.g., two cells) controlled by the same switch rapidly synchronize.

Development of multicellular organisms often seems to require the synchronization of cellular activity [1], whether that activity is replication [2,3], morphological changes [4], or physiological activity [5]. How is that synchrony achieved? Living organisms no doubt use a variety of methods depending on the nature and interactions of the components to be synchronized. Here we focus on division synchrony which, in at least some cases, seems to be obtained by halting cell cycling, then restarting it [2].

There are now a number of models of mitotic control [6–10]. One of the smaller, simpler ones is the two-variable model of Novak and Tyson [8]:

$$\frac{du}{dt} = k_1' g - u(k_2' + k_2'' u^2 + k_{wee})$$
$$+ (k_{25}' + k_{25}'' u^2)(g v - u),$$
$$\frac{dv}{dt} = k_1' - v(k_2' + k_2'' u^2).$$

In these equations, u is the concentration of active MPF (mitosis promoting factor [11], a complex of cdc2 and cyclin B), while v is the cyclin B concentration, both scaled by the total amount of cdc2. The other symbols are parameters [8,12]. An above-threshold concentration of active MPF (i.e., a high value of u) is required for mitotic progression.

Progress to mitosis is often controlled by turning MPF on or off through adjustments in the phosphorylation state of cdc2 [13]. The mechanisms which control progress through the cell cycle are called checkpoints [14]. Phosphorylation cascades [15] or other mechanisms [16] ensure that these processes are reasonably switchlike, i.e., that the MPF activation pathway can be rapidly down-regulated or the inactivation pathway rapidly up-regulated [17]. There are two biologically plausible points of action for checkpoints in this model: Cell cycling can be halted by switching the parameter g to a low value, this parameter determining equilibrium for the phosphorylation state of the activating residue threonine-167 [12]. Alternatively, k_{wee}, the effective rate constant for the activity of the wee1 kinase which phosphorylates the inhibitory residue tyrosine-15, can be switched to a high value. The latter checkpoint mechanism is the more biologically significant [8]. We have studied the first scenario somewhat more thoroughly, but our results are virtually identical for both checkpoint mechanisms.

Since the activation and deactivation events are fast relative to cell cycling, we treat these events as instantaneous switching between "on" and "off" states, each characterized by a particular value of g or of k_{wee}, depending on the checkpoint mechanism considered. We consider two different temporal programs for on-off switching. In the first, switching between the on and off states occurs periodically, i.e., $g(t)$ [or $k_{wee}(t)$] is a square wave with active and inactive periods of fixed durations τ_{on} and τ_{off}. This on-off switching protocol may describe the dynamics in certain experimental settings [18,19] but it may also model a two-oscillator system in which one oscillator is slaved to the other. Both recent molecular biological studies [20] and classical cell biological observations [21] suggest that coupled oscillators may be involved in controlling the cell cycle. In the second case, $g(t)$ [or $k_{wee}(t)$] is a random function representing the effect of naturally occurring or externally imposed [19] environmental fluctuations on cell cycling. Here we chose a two-state continuous time Markov process with exponentially distributed switching times [22] and mean active and inactive periods $\bar{\tau}_{on}$ and $\bar{\tau}_{off}$, the inverses of which are the switching probabilities per unit time.

We used parameters of the Novak-Tyson model for which the on state ($g = g_{max} = 0.9$, thr-167 actively phosphorylated [8]) corresponds to a limit-cycle regime [23]. In the off state ($g = 0$ or some other small value such that thr-167 dephosphorylation dominates), the system has a stable equilibrium point on the $u = 0$ axis. On-off parametric switching through g therefore results in a selection between two different flows in the $u \times v$ phase plane. Dichotomous selection between flows has previously been studied, with very different objectives than those pursued here [24].

In the periodically switched case, chaos as well as a variety of phase-locked modes were found in extended regions of the $\tau_{on} \times \tau_{off}$ parameter plane. Chaos was confirmed by computing Lyapunov exponents [25]. The chaotic

region consists of a series of islands in the $\tau_{on} \times \tau_{off}$ plane, some of which are quite large (Fig. 1). Chaos, when it occurs, implies an inability of similarly prepared systems (e.g., two sister cells) to synchronize, even if driven by the same switch [26].

We next turned our attention to the Markov-switched version of the model. We had expected (perhaps naively) that the statistically more complex switching signal provided by the Markov process would more readily produce chaos than periodic switching. However, in an extensive numerical study, we found that the Lyapunov exponents of the Markov-switched model were *always* negative, implying that similarly prepared systems will always synchronize under this switching protocol. Numerical integration confirms that synchronization normally occurs rather rapidly, even when the initial conditions are very different (Fig. 2). We emphasize that the cells are receiving a common external switching signal (modulating g) but are not coupled to each other in any way.

The $g = 0$ flow allows only trivial dynamics. To test the robustness of Markov switching as a synchronizer, we next studied the biologically more significant variant in which k_{wee}, rather than g, is a function of time. The wee1 kinase is inhibitory so the oscillator is on when k_{wee} has a low value ($1.5\,min^{-1}$) and off when k_{wee} has a high value ($3.5\,min^{-1}$). In the off state, the fixed point is excitable [8]. The results were identical to those obtained for our first model: For periodic switching, chaos is observed in similarly shaped and located regions of the $\tau_{on} \times \tau_{off}$ parameter plane as in Fig. 1. In the Markov-switched case, the Lyapunov exponents are always negative.

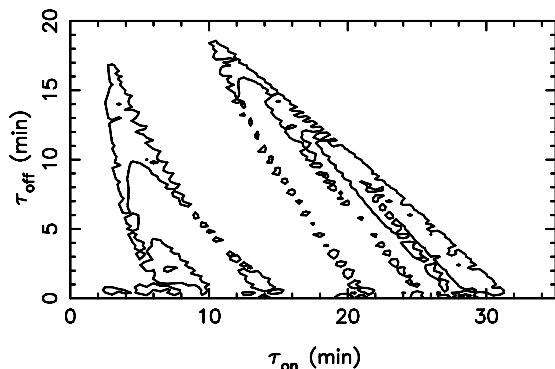We then wanted to convince ourselves that our results were not specific to models involving switching between planar flows so we briefly studied Goldbeter's three-variable mitotic control model [7]. We assumed that Goldbeter's V_2, the maximum activity of the wee1 kinase (roughly equivalent to k_{wee} in the Novak-Tyson model), is the kinetic parameter through which checkpoints halt the cell cycle. All other parameters were set as in [7], Fig. 3. Again, periodic switching between active ($V_2 = 1.5\,min^{-1}$, limit-cycle) and inactive ($V_2 = 3\,min^{-1}$, stable node) states commonly results in chaotic behavior while Markov switching is always an effective synchronization protocol.

The difference in behavior of the periodically and Markov-switched systems can be understood by a relatively simple argument. The leading Lyapunov exponent of a system with a limit cycle is exactly zero: While there are regions of local expansion and regions of local contraction [27], after one full period two points on the limit cycle must be exactly where they started so the expansion and contraction must average out to zero along the limit cycle [26]. In the periodically switched version of the model, it is possible to choose the on and off times in such a way that the contracting region is undersampled in the on flow. For the off flows studied here, the behavior is purely contractive in the relevant parts of the phase space. Expansion during the on phase must be sufficient to overcome the off flow's contractive contribution. This occurs over significant regions of
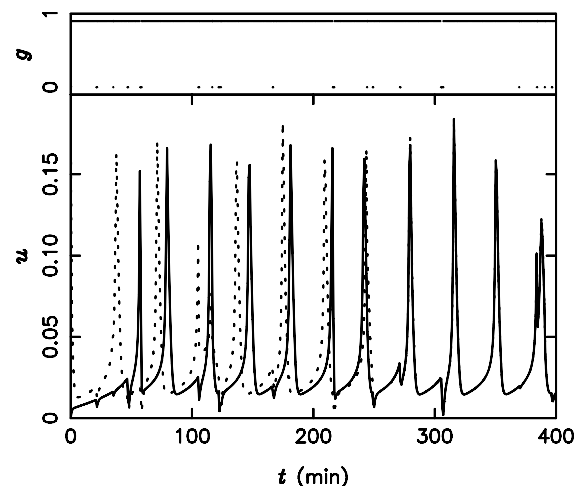

FIG. 1.   Chaotic "archipelago" in the $\tau_{on} \times \tau_{off}$ parameter plane for the Novak-Tyson system with $k_1' = 0.01$, $k_2' = 0.01$, $k_2'' = 10$, $k_{wee} = 1.5$, $k_{25}' = 0.04$, $k_{25}'' = 100\,min^{-1}$, and g periodically switched between 0 and $g_{max} = 0.9$. The outline of the chaotic region was obtained by computing Lyapunov exponents on a grid and drawing the contour of zero leading Lyapunov exponent. No chaotic states are found for $\tau_{off} > 20\,min$. The picture is roughly periodic along the τ_{on} axis with a period equal to the natural period of the oscillator (34.35 min) since adding integer multiples of the period to τ_{on} only adds additional transits around the limit cycle (with a zero contribution to the Lyapunov exponent) and does not affect the balance of expansion and contraction.

FIG. 2.   Two trajectories (bottom) of the Novak-Tyson system driven by the same realization of a Markov process (g, top). The parameters are as in Fig. 1, except $\bar{\tau}_{on} = 14\,min$ and $\bar{\tau}_{off} = 0.8\,min$. The first trajectory (solid) was started from $(u, v) = (0, 0)$ while the second (dotted) was started from $(0.2, 0.2)$. Because of the stochastic nature of the process, the time to synchronization is highly variable, but this realization is not untypical. The leading Lyapunov exponent for these values of the parameters is $-0.012 \pm 0.002\,min^{-1}$. In the periodically switched case ($\tau_{on} = 14\,min$ and $\tau_{off} = 0.8\,min$ exactly), chaos is observed with a leading Lyapunov exponent of $0.024\,97 \pm 0.000\,02\,min^{-1}$.
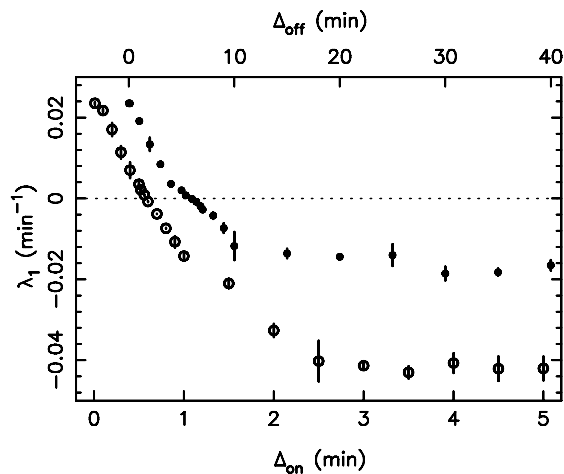
FIG. 3. Leading Lyapunov exponents (λ_1) for the nearly periodic switching protocol described in the text. The parameters are as in Fig. 1, except $\bar{\tau}_{on} = 4\,min$ and $\bar{\tau}_{off} = 11\,min$. The open symbols were obtained for $\Delta_{off} = 0.01\,min$ and variable Δ_{on} (bottom scale). The filled symbols correspond to a fixed $\Delta_{on} = 0.01\,min$ and variable Δ_{off} (top scale). Note that the chaotic state is much more fragile with respect to variability in the on time (transition to negative Lyapunov exponents near $\Delta_{on} = 0.58\,min$) than with respect to variability in the off time (crossover near $\Delta_{off} = 5.9\,min$. For comparison, in the periodic switching limit, the leading Lyapunov exponent is $0.023\,553 \pm 0.000\,007\,min^{-1}$, while in the Markov switching limit, $\lambda_1 = -0.0427 \pm 0.0002\,min^{-1}$. The Lyapunov exponent in the hybrid process approaches the Markov limit at large values of Δ_{on}, even in the absence of significant variability in the off time, underlining the central role of the on time in the dynamics.

the $\tau_{on} \times \tau_{off}$ parameter plane so that chaotic states are readily observed. Although the off time is clearly important, it follows from these observations that the on time is much more critical to the attainment of a chaotic state. In the Markov-switched versions, the time spent in the on state is variable so the system explores the limit cycle uniformly. Contraction during the off flow then ensures that the Lyapunov exponents will be negative and that synchronization will occur.

We confirmed our reasoning by undertaking a series of Lyapunov exponent calculations with a switching protocol [applied to $g(t)$ in the Novak-Tyson model] in which we could control the variability of the on and off times. Specifically, we introduced "bandwidths" Δ_{on} and Δ_{off} such that transitions from the on to the off state are forbidden if the time since activation is less than $\bar{\tau}_{on} - \Delta_{on}$ and forced when the time since activation reaches $\bar{\tau}_{on} + \Delta_{on}$. Between these two limits, there is a constant switching probability per unit time of $p_{on\rightarrow off} = 1/\bar{\tau}_{on}$. Transitions from off to on are analogously treated. While this stochastic process is somewhat unusually defined [28], it has the advantage of reducing, in suitable limiting cases, either to a purely periodic signal (Δ_{on} and Δ_{off} both small) or to an ordinary Markov process (Δ_{on} and Δ_{off} both large), which makes the results of these and our previous computations

directly comparable. It turns out that it is always necessary to strongly constrain the variability of the on time to observe a chaotic state. It is often necessary to limit the variability of the off time as well, but the chaotic state is clearly less sensitive to Δ_{off}. Typical results are shown in Fig. 3, where we can see that the chaotic regime can tolerate a much larger value of Δ_{off} than of Δ_{on}. The decrease in the leading Lyapunov exponent with increasing bandwidth is not always monotonic, but we have yet to observe a case in which the Lyapunov exponent crosses the zero axis more than once. In some cases, chaos is observed even when the off time bandwidth is unlimited. These results lead us to conclude that the good synchronization properties of the Markov switch are due mainly to irregularity in the duration of the active phase preventing expansion in the limit cycle regime from dominating the dynamics.

The general conclusion to be drawn from this study is that on-off parametric switching with variable on times facilitates synchronization by suppressing chaos. Even a modest amount of variability in the on time is generally sufficient to suppress chaos.

Two recent modeling studies have demonstrated synchronization of identical uncoupled systems by a common additive stochastic input [29,30]. The phenomena treated in these studies and in ours may be of particular significance in neurobiology [30] where "chattering cells" may play a role in synchronizing cortical activities [31], in developmental biology where synchronization of cellular activities is often required [1], and in cell physiology where cell division synchrony is frequently desirable [32].

*Corresponding author.
Email address: roussel@uleth.ca
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[12] Our notation is identical to Novak and Tyson's except that our g is the inverse of their G: $g = K_{167}/(1 + K_{167}) \in [0, 1)$, where K_{167} is the effective equilibrium constant for phosphorylation of the activating residue thr-167 of MPF.

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[23] The general physiological significance of the oscillatory regime has been questioned by Thron [9], who argues that bistability and excitability are more typical dynamical behaviors for the cell cycle than limit cycles. However, even if the pieces of the cell cycle control system display only stable steady states, it may be that the overall system displays limit cycle behavior, as argued by Goldbeter and co-workers [10]. Since the conclusions of our study depend very little on the detailed kinetics used, our results can reasonably be expected to extend to the more complex situation of a limit cycle built up from bistable or excitable circuit elements or, for that matter, to completely unrelated natural or technological systems in which on-off switching operates.

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[25] G. Rangarajan, S. Habib, and R. D. Ryne, Phys. Rev. Lett. **80**, 3747 (1998). Our implementation uses a fourth-order Runge-Kutta integrator and seeks consistency of the exponents when both the length of the trajectory is extended and the step size is reduced. A pure relative error criterion was used, resulting in tight error control in the critical region where the leading Lyapunov exponent is near zero. The results were spot-checked using an independently written integrator and a different numerical method by running pairs of trajectories to verify qualitative agreement with the exponent calculations.

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[27] The regions of expansion and contraction are bounded by the curve in the $u \times v$ plane on which the real part of the leading eigenvalue of the Jacobian is zero. Equivalently, this is the locus of points at which the divergence of the flow is zero.

[28] If $\Delta_{on} > \bar{\tau}_{on}$, the system is subject to Markov-process switching to the off state as soon as it is activated. Δ_{on} then controls only the length of the tail of the distribution. Additionally, note that this stochastic process is normalized only in the trivial sense that if the system is still in the on state when $\bar{\tau}_{on} + \Delta_{on}$ time units have elapsed, a transition from the on to the off state is forced. Similar comments can obviously be made for switching from the off to the on state. Calculations reported elsewhere [M. R. Roussel and J. Wang (to be published)] indicate that the results are not very sensitive to the details of the stochastic process which generates variability.

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