Alternating Oscillations and Chaos in a Model of Two Coupled Biochemical Oscillators Driving Successive Phases of the Cell Cycle

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INTRODUCTION

The cell division cycle is certainly one of the most important cellular processes in which nonlinear dynamics plays a major role. During the last decade, experimental evidence has accumulated to show that the onset of the M (mitosis) and S (DNA replication) phases of the embryonic and somatic cell cycles are controlled by the periodic activation of cyclin-dependent kinases (cdks) known as cdk1 and cdk2, respectively.[1–9] Various theoretical models have been proposed to account for the generation of sustained oscillations in the activity of these kinases, particularly the kinase cdc2 (cdk1) which controls the G2/M transition. The early theoretical models were either based on the positive feedback exerted by cdc2 on its own activation[10–12] (such positive feedback is now known to operate via cdc2 dephosphorylation by the cdc25 phosphatase which itself is activated by cdc2) or on the negative feedback involving the cdc2-induced degradation of cyclin which leads to cdc2 inactivation.[13–16]. More detailed models have since been proposed for both the fission yeast[17–20] and embryonic cell cycles.[19–23] The latter models take into account the various checkpoints that ensure the orderly progression through the successive phases of the cell cycle.

If the M and S phases are to follow each other during the cycle, rather than occurring concomitantly, and if each of the two phases is controlled by a biochemical oscillator involving, respectively, cdk1 and cdk2 with their associated cyclins, then it is necessary that the two oscillators be coupled through mutual control. Such control processes are part of the checkpoints mentioned above. There is evidence that this mutual control is of a negative nature. DNA replication is known to inhibit the triggering of mitosis until replication is completed.[3–5,24,25] Moreover, the kinase cdk1 that controls the initiation of mitosis inhibits the transition of the cell to a G1 replication-competent stage as long as the cell is in the S, G2, or M phases.[8,26]

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Coupling two oscillators may profoundly affect their dynamic behavior. We wish to explore here the consequences of a coupling through mutual inhibition of the biochemical oscillators controlling the onset of the M and S phases of the cell cycle. To keep the theoretical study relatively simple, we use for each of the two oscillators the model based on negative feedback previously proposed for sustained oscillations in cdk activity driven by cyclin synthesis and degradation.[13,15] In the present model each of the two oscillators contains three variables, that is, cdk1 or cdk2 with their associated cyclin and enzyme governing cyclin degradation. The coupling is assumed to follow from the direct inhibition of the synthesis of the cyclin of one oscillator by the cdk of the other oscillator. Similar results are obtained when assuming that inhibition occurs through mutual activation of cyclin degradation.

The present work does not aim at proposing a detailed model for the somatic cell cycle. Rather, we wish to investigate in a skeleton model how the mutual inhibition of two biochemical oscillators controlling different phases of the cell cycle may influence the oscillatory dynamics of such a coupled system. When studying the dynamic behavior of the coupled oscillators as a function of the strength of mutual inhibition, we recover, as most common behavior, alternating oscillations that likely correspond to the sequential activation of cdk1 and cdk2 and thus to the observed alternation between mitosis and the S phase. Such periodic alternation between the two kinases is, however, not the only mode of dynamic behavior predicted by the model. Thus, we also uncover the possibility, in slightly different conditions, of autonomous chaotic behavior.

In the next section we present the skeleton model of the double cdk1–cdk2 oscillator, as well as the kinetic equations that describe its time evolution. In the section ALTERNATING AND CHAOTIC OSCILLATIONS, we determine the various modes of periodic or chaotic oscillatory behavior as a function of the strength of the inhibitory coupling. The results are discussed in the DISCUSSION in regard to their physiological significance and to other examples of biological oscillators coupled through mutual inhibition.

SKELETON MODEL OF A DOUBLE OSCILLATOR CONTROLLING SUCCESSIVE PHASES OF THE CELL CYCLE

The model considered is schematized in FIGURE 1. It consists of two coupled minimal cascade models generating oscillations on the basis of cdk-induced cyclin degradation. The first oscillator, controlling the initiation of mitosis at the G2/M transition, involves the activation of cdk1 (M_1) by cyclin B (C_1), and the cdk1-induced degradation of cyclin B by an ubiquitin ligase (X_1) which is part of the ubiquitin-mediated proteolysis system. The second oscillator, controlling the initiation of DNA replication at the G1/S transition, is based on the activation of cdk2 (M_2) by cyclin E (C_2), and on the cdk2-induced degradation of cyclin E by another ubiquitin ligase (X_2). Note that, for simplicity, we do not consider the formation of a cyclin-cdk complex, but rather an activation of cdk by cyclin. Because the precise nature of the coupling is not yet fully characterized, we assume that the mutual inhibition of the two oscillators occurs as follows (see FIG. 1): cdk1 (M_1) inhibits the synthesis of cyclin E (C_2), while cdk2 (M_2) inhibits the synthesis of cyclin B (C_1).

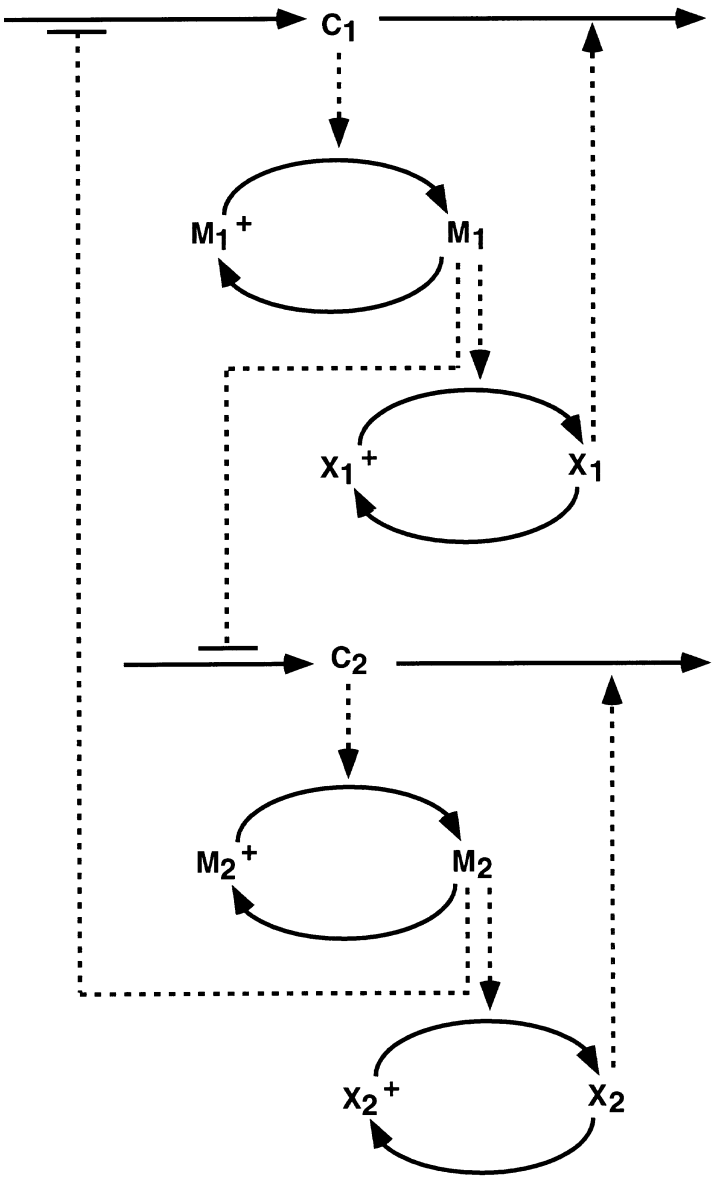

FIGURE 1. Skeleton model of two coupled biochemical oscillators controlling the M and S phases of the cell cycle. Each oscillator consists of a three-variable cascade involving a cyclin (C_1 or C_2), a cyclin-dependent kinase (cdk) (M_1 or M_2), and a cdk-activated ubiquitin ligase (X_1 or X_2) that controls cyclin degradation. The + sign indicates the inactive form of the enzymes. The dashed lines ending with a horizontal bar represent the inhibition exerted by M_1 and M_2 on the synthesis of C_1 and C_2, respectively.

The inhibitory action of cdk1 and cdk2 is described phenomenologically by treating the two kinases as inhibitors that directly modulate the rate of cyclin synthesis. Similar results are obtained if we assume that the coupling occurs through activation by M_1 (M_2) of cyclin C_2 (C_1) degradation (see below).

The time evolution of the system of two coupled biochemical oscillators is governed by the following system of kinetic equations[13,15]:

$$\frac{dC_1}{dt} = v_{i1}\frac{K_{im1}}{K_{im1} + M_2} - v_{d1}X_1\frac{C_1}{K_{d1} + C_1} - k_{d1}C_1 \quad \textbf{(1a)}$$

$$\frac{dM_1}{dt} = V_1\frac{(1 - M_1)}{K_1 + (1 - M_1)} - V_2\frac{M_1}{K_2 + M_1} \quad \textbf{(1b)}$$

$$\frac{dX_1}{dt} = V_3\frac{(1 - X_1)}{K_3 + (1 - X_1)} - V_4\frac{X_1}{K_4 + X_1} \quad \textbf{(1c)}$$

$$\frac{dC_2}{dt} = v_{i2}\frac{K_{im2}}{K_{im2} + M_1} - v_{d2}X_2\frac{C_2}{K_{d2} + C_2} - k_{d2}C_2 \quad \textbf{(1d)}$$

$$\frac{dM_2}{dt} = U_1\frac{(1 - M_2)}{H_1 + (1 - M_2)} - U_2\frac{M_2}{H_2 + M_2} \quad \textbf{(1e)}$$

$$\frac{dX_2}{dt} = U_3\frac{(1 - X_2)}{H_3 + (1 - X_2)} - U_4\frac{X_2}{H_4 + X_2} \quad \textbf{(1f)}$$

where

$$V_1 = \frac{C_1}{K_{c1} + C_1}V_{M1},\ V_3 = M_1 \cdot V_{M3} \quad \textbf{(2a,b)}$$

$$U_1 = \frac{C_2}{K_{c2} + C_2}U_{M1},\ U_3 = M_2 \cdot U_{M3} \quad \textbf{(2c,d)}$$

In the above equations, C_1 and C_2 denote the concentrations of cyclins B and E, while M_1, M_2 and X_1, X_2 refer to the fractions of activated cdk1 and cdk2 or of enzymes X_1 and X_2. Moreover, v_{ij} and v_{dj} ($j = 1, 2$) denote the constant rate of cyclin synthesis and the maximum rate of cyclin degradation by enzyme X_j reached for $X_j = 1$ for the first ($j = 1$) and second ($j = 2$) oscillator, respectively; K_{d1} and K_{c1} (K_{d2} and K_{c2}) denote the Michaelis constants for cyclin degradation and for cyclin activation of the phosphatase acting on the phosphorylated (inactive) form of the kinase cdk1 (cdk2); k_{d1} (k_{d2}) represents an apparent first-order rate constant related to nonspecific degradation of cyclin. Moreover, V_i (U_i) and K_i (H_i) ($i = 1, \ldots, 4$) denote the effective maximum rate and the Michaelis constant for each of the four enzymes in-

volved in the two cycles of the cascade for each oscillator, namely, on one hand, the phosphatase and the kinase acting on cdk1 (cdk2), and on the other hand, the kinase cdk1 (cdk2) and phosphatase acting on the enzyme governing cyclin B (cyclin E) proteolysis (see FIG. 1). For each converter enzyme, the two parameters V_i (U_i) and K_i (H_i) are divided by the total amount of relevant target protein, that is, the total amount M_{1T} (M_{2T}) of cdk1 (cdk2) or the total amount X_{1T} (X_{2T}) of ubiquitin-conjugating enzyme acting on cyclin B (cyclin E); M_{1T}, M_{2T}, X_{1T}, and X_{2T} are considered as constant throughout the cell cycle.

For each oscillator the coupling between the two cycles of the cascade arises from the expressions for the effective, maximum rates V_1 (U_1) and V_3 (U_3) given by Equations (2a–d). Expression (2a) reflects the assumption that cyclin B activates in a Michaelian manner the phosphatase that acts on cdk1; V_{M1} denotes the maximum rate of that enzyme reached at saturating cyclin levels. On the other hand, Equation (2b) expresses the proportionality of the effective maximum rate of cdk1 to the fraction M_1 of active enzyme; V_{M3} denotes the maximum velocity of the kinase reached for $M_1 = 1$. Whereas Equations (2a) and (2b) pertain to the cdk1 oscillator controlling the G2/M transition, Equations (2c) and (2d) yield the expressions for the maximum rates U_1 and U_3 of the corresponding enzymes in the second oscillator, which controls the G1/S transition through the periodic activation of cdk2.

The coupling between the two oscillators is introduced via the first term in the kinetic equations for C_1 and C_2. This term reflects the Michaelian inhibition of C_1 synthesis by M_2 and of C_2 synthesis by M_1. Parameter K_{im1} (K_{im2}) denotes the inhibition constant divided by the total amount of cdk2 (cdk1). The smaller K_{im1} and K_{im2}, the stronger the inhibition.

We shall restrict the present analysis to the symmetric case in which corresponding parameter values are identical for each of the two oscillators. In particular, the maximum rate of cyclin synthesis ($v_{i1} = v_{i2} = v_i$) and the inhibition constant ($K_{im1} = K_{im2} = K_{im}$) have the same values for both oscillators. In the following we shall determine the dynamic behavior of the system of Equations (1a–f) as a function of the strength of the mutual inhibition measured by parameter K_{im}. We shall briefly discuss at the end of this paper the effect of introducing asymmetries in parameter values between the two oscillators. Focusing on the symmetrical case provides a convenient reference situation, since asymmetries in parameter values may be treated, in a second stage, as perturbations from such a reference state.

ALTERNATING AND CHAOTIC OSCILLATIONS

The most natural parameter for studying the effect of a coupling between the two enzymatic cascades controlling the periodic activation of cdk1 and cdk2 is K_{im}, which measures the strength of mutual inhibition of the two oscillators. In FIGURE 2 we present a series of phase portraits obtained by projecting the trajectory of the full, six-variable system, on the C_1–C_2 plane, for decreasing values of the inhibition constant K_{im}. The data show the existence of a rich spectrum of dynamic behavior. The actual sequence of oscillatory behavioral modes depends on the value of parameter v_i. The influence of this and other parameters will be examined in a subsequent publication. Here, we select the value of v_i so as to show the variety of oscillatory phenomena that the coupling of the two oscillators may bring about.

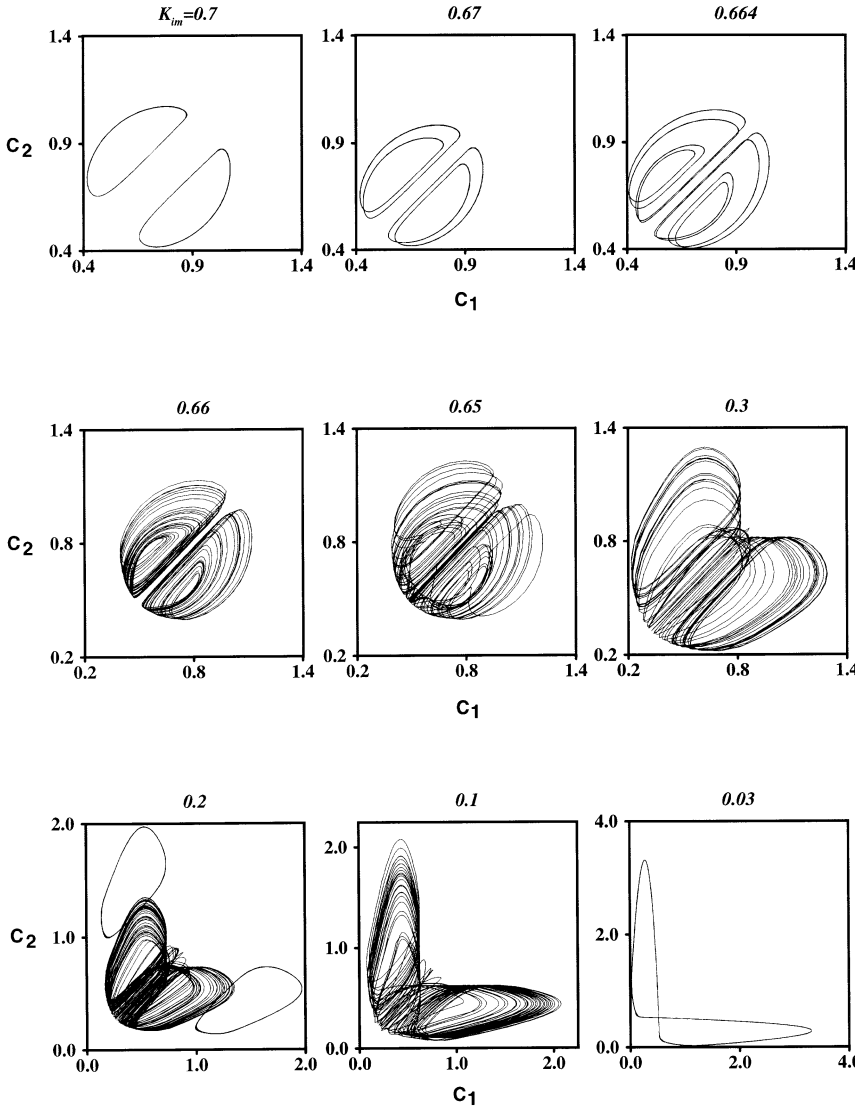

FIGURE 2. Dynamic behavior of the double cdk1-cdk2 oscillator model as a function of the inhibition constant K_{im}. Shown is the trajectory followed by the six-variable system (1a–1f) projected onto the C_1–C_2 plane (concentrations C_1 and C_2 are both expressed in μM). The sequence of diagrams is obtained at a constant value of the rate of cyclin synthesis ($v_i = 0.05$ μM min^{-1}). When $K_{im} = 0.7$ a pair of nonsymmetric limit cycles arise. In the range $0.7 < K_{im} < 0.664$ a period doubling cascade is observed. For $K_{im} = 0.66$ a pair of nonsymmetric chaotic attractors are found, and when K_{im} reaches the value of 0.65 they merge into a single antisymmetric, chaotic attractor. In the range $0.3 < K_{im} < 0.2$ a coexistence between an antisymmetric chaotic attractor and a pair of nonsymmetric limit cycles is observed. For $K_{im} = 0.1$ the two nonsymmetric limit cycles disappear and only the antisymmetric chaotic

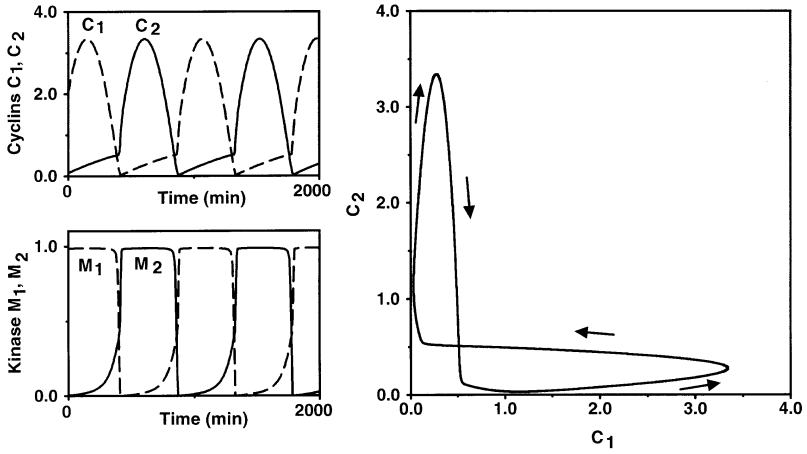

FIGURE 3. Alternating oscillations of cyclins C_1 and C_2 (*left upper panel*) and of kinases M_1 and M_2 (*left lower panel*) in the double cdk1-cdk2 oscillator model. These oscillations correspond to the antisymmetric limit cycle (*right panel*) obtained in FIGURE 2 for $K_{im} = 0.03$. The time evolution is obtained by numerical integration of Equations (1a)–(1f) using a routine for solving stiff differential equations.

Starting with weak inhibition corresponding to a relatively large value of the inhibition constant ($K_{im} = 0.7$), we observe the presence of two limit cycles that are symmetric with respect to each other. In each cycle, however, the variations of C_1 and C_2 are not symmetrical, so that the two coexisting trajectories can be described as nonsymmetric limit cycles. Upon decreasing K_{im} down to 0.67 and 0.664, we observe for each cycle a sequence of period doubling, which eventually leads to the coexistence of two strange attractors ($K_{im} = 0.66$). Further decrease of K_{im} to 0.65 causes the fusion of the two chaotic attractors. The resulting, unique strange attractor remains present at smaller values of K_{im} (e.g., $K_{im} = 0.3$), and later is seen to coexist with two nonsymmetric limit cycles ($K_{im} = 0.2$). For $K_{im} = 0.1$, the trajectory is again unique and takes the form of an antisymmetric chaotic attractor. Finally, for very low values of the inhibition constant ($K_{im} = 0.03$), that is, when the mutual inhibition is strong, we observe an antisymmetric limit cycle.

In the present paper we wish to focus on two of the behaviors shown in the sequence of FIGURE 2 that appear to be of particular biological significance. The first is the case of antisymmetric oscillations which correspond to the trajectory obtained for $K_{im} = 0.03$. This trajectory in the C_1–C_2 plane is again shown in FIGURE 3, together with the corresponding oscillations in C_1, C_2, M_1, and M_2. We see that in the presence of strong mutual inhibition, the two oscillators operate out of phase, so that

attractor remains (see also FIG. 4). For very low values ($K_{im} = 0.03$) of the inhibition constant an antisymmetric limit cycle is obtained (see also FIG. 3). The other parameters for the first and second oscillators are: H_i ($i = 1, \ldots, 4$) = K_i ($i = 1, \ldots, 4$) = 0.01; $V_{m1} = U_{m1} = 0.3\ \text{min}^{-1}$; $V_2 = U_2 = 0.15\ \text{min}^{-1}$; $V_{m3} = U_{m3} = 0.1\ \text{min}^{-1}$; $V_4 = U_4 = 0.05\ \text{min}^{-1}$; $K_{c1} = K_{c2} = 0.5\ \mu\text{M}$; $v_{d1} = v_{d2} = 0.025\ \mu\text{M min}^{-1}$; $K_{d1} = K_{d2} = 0.02\ \mu\text{M}$; $k_{d1} = k_{d2} = 0.001\ \text{min}^{-1}$.

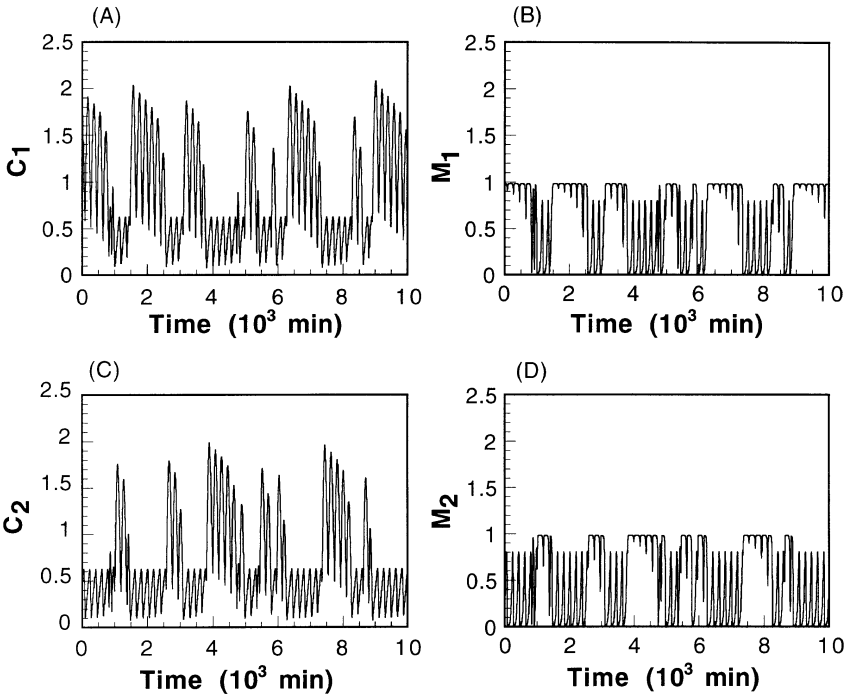

FIGURE 4. Autonomous chaos in the double cdk1-cdk2 oscillator model. The aperiodic oscillations correspond to the strange attractor shown for $K_{im} = 0.1$ in FIGURE 2. Panels (A) and (C) show the aperiodic evolution of the cyclins C_1 and C_2 (in μM). Panels (B) and (D) show the aperiodic evolution of the kinases M_1 and M_2. The curves are obtained as described in FIGURE 3.

cdk1 (M_1) reaches its maximum when cdk2 (M_2) is close to its minimum, and vice versa. This situation of alternating oscillations in cdk1 and cdk2 likely corresponds to the physiological case (see DISCUSSION). Because this study is of a qualitative rather than quantitative nature, the values of the parameters in FIGURE 3 have been selected arbitrarily so as to yield a period of mitotic oscillations of the order of somatic cell cycle lengths.

A second case of particular interest is that in which aperiodic oscillations result from the coupling between the cdk1 and cdk2 oscillators. Shown in FIGURE 4 are the chaotic oscillations corresponding to the strange attractor obtained in FIGURE 2 for $K_{im} = 0.1$. The variations in M_1 and M_2 are again out of phase, but have lost their periodic nature.

The question arises as to whether the results depend on the precise form of the coupling through mutual inhibition. To address this point we have studied another version of the model in which mutual inhibition is achieved through activation of C_1 (C_2) degradation by M_2 (M_1). For simplicity, we treat this putative regulation as a direct activation process. Then, Equations (1a) and (1d) for C_1 and C_2 are replaced by Equations (3a) and (3b):

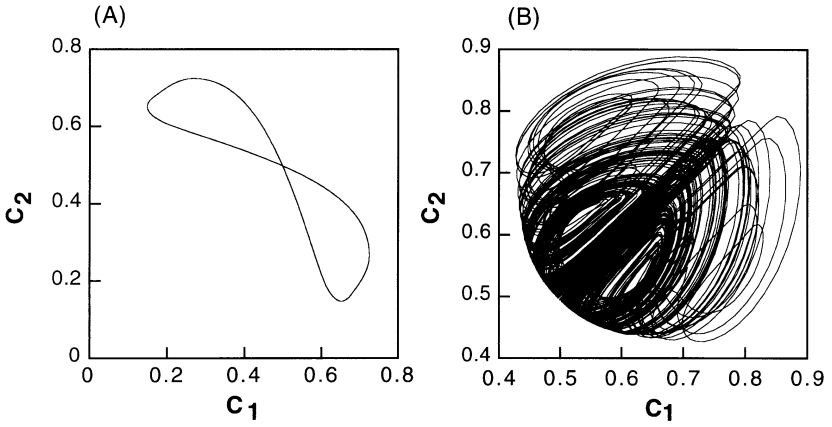

FIGURE 5. Alternating oscillations (A) and chaos (B) obtained in the model when the mutual inhibition of the two oscillators occurs through the putative activation of cyclin degradation by the cyclin-dependent kinases. The evolution of the coupled oscillators is then governed by eqs. (1b), (1c), (1e), (1f), (3a), and (3b). Shown are the projections of the trajectories followed by the system onto the C_1–C_2 plane; C_1 and C_2 are expressed in μM. Parameter values are: H_i (i = 1, …, 4) = K_i (i = 1, ...4) = 0.01; $V_{m1} = U_{m1} = 0.3$ min^{-1}; $V_2 = U_2 = 0.15$ min^{-1}; $V_{m3} = U_{m3} = 0.1$ min^{-1}; $V_4 = U_4 = 0.05$ min^{-1}; $K_{c1} = K_{c2} = 0.5$ μM; $v_{d1} = v_{d2} = 0.025$ μM min^{-1}; $K_{d1} = K_{d2} = 0.02$ μM; $k_{d1} = k_{d2} = 0.001$ min^{-1}; moreover, for (A) $K_{a1} = K_{a2} = 0.1$ μM, $v_{i1} = v_{i2} = 0.005$ μM min^{-1}, and for (B) $K_{a1} = K_{a2} = 1.0$ μM, $v_{i1} = v_{i2} = 0.01$ μM min^{-1}.

$$\frac{dC_1}{dt} = v_{i1} - v_{d1} X_1 \frac{C_1}{K_{d1} + C_1} \frac{M_2}{K_{a1} + M_2} - k_{d1} C_1 \qquad \textbf{(3a)}$$

$$\frac{dC_2}{dt} = v_{i2} - v_{d2} X_2 \frac{C_2}{K_{d2} + C_2} \frac{M_1}{K_{a2} + M_1} - k_{d2} C_2 \qquad \textbf{(3b)}$$

The time evolution of variables M_1, X_1, M_2 and X_2 remains given by Equations (1b), (1c), (1e), and (1f), respectively. The results presented in FIGURE 5 show that alternating oscillations as well as chaos are recovered in that version of the model.

DISCUSSION

Nonlinear phenomena play a key role in the dynamics of the cell cycle. In animal cells, there is evidence for autonomous oscillations in the activity of the cyclin-dependent kinases cdk1 and cdk2, the rise of which initiates mitosis (M phase) and DNA replication (S phase), respectively. The avoidance of concomitancy, that is, the ordering of these events is achieved through checkpoint mechanisms that are partly based on the mutual inhibition of mitosis and DNA replication.[3–5,8,24–26]

Here we have explored theoretically the effects of coupling through mutual inhibition the two biochemical oscillators that control the M and S phases of the cell cycle. To determine the effects of such a coupling, we resorted, for each of the two oscillators, to a simple model of a phosphorylation–dephosphorylation enzymatic cascade regulated by negative feedback. This model was previously shown to provide a simple mechanism for sustained oscillations in the activity of a cyclin-dependent kinase. Moreover, it fits very simply with the fact that cyclin and the active form of cdk, respectively, are slow and fast variables. We coupled the two oscillators by considering that the synthesis of cyclin in each oscillator is inhibited by the cdk of the other oscillator. The strength of this mutual inhibition is measured by the inhibition constant K_{im}. This skeleton model of two coupled oscillators can be viewed as a simple, cdk1–cdk2 double oscillator model for the cell cycle.[13,15] Obviously, it would be desirable to consider a more realistic model, but the number of variables and parameters would rapidly increase. Such a model could, for example, incorporate an inhibitor of both cdk1 and cdk2 that keeps them turned off in G1 phase; release from this inhibition would be connected with cell growth. It appears, indeed, that mitosis is prevented as long as the cell has not reached a critical size.

Our analysis shows that the dynamic behavior of the double oscillator model markedly depends on the strength of mutual inhibition. When this inhibition is strong, antisymmetric (i.e., alternating) oscillations are observed, in which one cdk reaches its maximum while the other is at a minimum. Such an alternation likely corresponds to the physiological situation as it would ensure the ordered progression of the cell through the S and M phases. In FIGURE 3 which illustrates this situation, the fractions of active cdk1 (M_1) and cdk2 (M_2) are seen to reach a plateau during their active phase. This situation with rapid alternations of replication and mitosis without intervening G1 and G2 gaps is most relevant to the embryonic cell cycle. For smaller values of the rate of cyclin synthesis v_i, however, M_1 and M_2 do not reach a prolonged plateau during the active phase, and the latter occupies a shorter portion of the period. The value of v_i selected for FIGURES 2 and 3 was chosen so as to maximize the variety of dynamic phenomena that can be observed in the model.

When the inhibition is weaker, chaos is found over a relatively large range of parameter values (see FIGURE 2). The question remains as to whether this phenomenon is of physiological significance. Previous theoretical studies have raised the possibility that the variability of the cell cycle duration might be due to the chaotic nature of its underlying dynamics.[27–30] In this context some theoretical models have been proposed in which chaos originates from the periodic forcing of a model biochemical oscillator.[31] Here, in contrast, chaos is autonomous as it occurs in the absence of any periodic forcing. The study of the cdk1–cdk2 double oscillator model shows that aperiodic oscillations can naturally arise from the coupling between the two cdk oscillators when the strength of mutual inhibition is in the appropriate range, that is, neither too strong (in which case periodic, alternating oscillations would occur) nor too weak (in which case two nonsymmetric limit cycles may coexist, as shown in FIGURE 2 for $K_{im} = 0.7$). These results provide a first theoretical indication as to how autonomous chaos may occur in the cell cycle dynamics.

The study of the double oscillator model also shows the possibility of a coexistence between multiple periodic or chaotic attractors (see FIG. 2), as well as other types of oscillations (symmetric or nonsymmetric) not shown there. The very rich repertoire of dynamic behavior will be studied in further detail in a subsequent publication

where a bifurcation diagram in the v_i–K_{im} parameter plane will be presented. Such a diagram shows that the sequence of temporal patterns in FIGURE 2 depends on the value of parameter v_i. For the value of v_i considered in this figure, the system evolves toward a stable steady state in the absence of mutual inhibition, that is, at very large K_{im} values. Thus, in this case, the coupling is necessary for oscillations to occur. Oscillations can occur in the absence of coupling, however, at smaller values of v_i.

Admittedly, the two-oscillator model is a much simplified caricature of the dynamics of the animal cell cycle. More detailed models have been proposed for both the fission yeast and embryonic cell cycles.[17–23] In yeast there is a single cdk that controls the onset of both the M and S phases in conjunction with different cyclins and a cdk inhibitor.[18,19] The question arises whether a detailed exploration of these more realistic models in parameter space could also produce results similar to those presented here, in regard to alternating oscillations and/or chaos in the time evolution of the different cdk–cyclins complexes. A virtue of the present skeleton model is that it brings to light the sorts of dynamic behavior that are expected to occur as a result of the coupling between two oscillators controlling the S and M phases.

We have focused here on the case where the mutual coupling is achieved through inhibition of cyclin synthesis. As shown in FIGURE 5, similar results, including antisymmetric and chaotic oscillations, are also obtained when the coupling is exerted through the activation of cyclin degradation in one oscillator by the cdk belonging to the other oscillator. The results are also recovered, to a large extent, when a limited degree of asymmetry is introduced for the parameter values of the two oscillators.

In contrast to the present approach, some authors have suggested that the cell cycle is not described by a limit cycle oscillator but rather by a bistability phenomenon, each steady state corresponding to a particular phase (M or S) of the cell cycle.[20,32–34] We do not think, however, that the latter view necessarily contradicts the limit cycle description. Indeed, to account for the succession of cell cycle phases, the approach based on bistability corresponds to a sequence of "frozen frames." Including the variation of control parameters, so as to link these frames continuously with one another, should confer a recurrent (i.e., oscillatory) nature on the sequence of events which, each on its own, can be interpreted in terms of bistability.

Our results can be related to those obtained in the study of other biological oscillators coupled through mutual inhibition. Of particular interest in this respect is the analysis of a model of two mutually inhibiting pacemaker neurons carried out by Borisyuk *et al*.[35] These authors also obtained in that different context evidence for both antisymmetric and chaotic oscillations. The present study shows that if the cell cycle is governed by two biochemical oscillators coupled through mutual inhibition, the most prevalent mode of dynamic behavior will be that of periodic, alternating oscillations corresponding to the ordered sequence of the cell cycle phases. Our results indicate, however, that besides this physiological situation, depending on the strength of mutual inhibition, such a coupling may also give rise to more complex dynamic phenomena including chaos.

SUMMARY

The animal cell cycle is controlled by the periodic variation of two cyclin-dependent protein kinases, cdk1 and cdk2, which govern the entry into the M (mitosis) and

S (DNA replication) phases, respectively. The ordered progression between these phases is achieved thanks to the existence of checkpoint mechanisms based on mutual inhibition of these processes. Here we study a simple theoretical model for oscillations in cdk1 and cdk2 activity, involving mutual inhibition of the two oscillators. Each minimal oscillator is described by a three-variable cascade involving a cdk, together with the associated cyclin and cyclin-degrading enzyme. The dynamics of this skeleton model of coupled oscillators is determined as a function of the strength of their mutual inhibition. The most common mode of dynamic behavior, obtained under conditions of strong mutual inhibition, is that of alternating oscillations in cdk1 and cdk2, which correspond to the physiological situation of the ordered recurrence of the M and S phases. In addition, for weaker inhibition we obtain evidence for a variety of dynamic phenomena such as complex periodic oscillations, chaos, and the coexistence between multiple periodic or chaotic attractors. We discuss the conditions of occurrence of these various modes of oscillatory behavior, as well as their possible physiological significance.

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