Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 9107–9111, October 1991
Cell Biology

A minimal cascade model for the mitotic oscillator involving cyclin and cdc2 kinase

(cell cycle/maturation-promoting factor/phosphorylation cascade/thresholds/biochemical oscillations)

ALBERT GOLDBETER

Faculté des Sciences, Université Libre de Bruxelles, Campus Plaine, C.P. 231, B-1050 Brussels, Belgium

Communicated by I. Prigogine, July 12, 1991 (received for review June 12, 1991)

ABSTRACT **A minimal model for the mitotic oscillator is presented. The model, built on recent experimental advances, is based on the cascade of post-translational modification that modulates the activity of cdc2 kinase during the cell cycle. The model pertains to the situation encountered in early amphibian embryos, where the accumulation of cyclin suffices to trigger the onset of mitosis. In the first cycle of the bicyclic cascade model, cyclin promotes the activation of cdc2 kinase through reversible dephosphorylation, and in the second cycle, cdc2 kinase activates a cyclin protease by reversible phosphorylation. That cyclin activates cdc2 kinase while the kinase triggers the degradation of cyclin has suggested that oscillations may originate from such a negative feedback loop [Félix, M. A., Labbé, J. C., Dorée, M., Hunt, T. & Karsenti, E. (1990) *Nature (London)* 346, 379–382]. This conjecture is corroborated by the model, which indicates that sustained oscillations of the limit cycle type can arise in the cascade, provided that a threshold exists in the activation of cdc2 kinase by cyclin and in the activation of cyclin proteolysis by cdc2 kinase. The analysis shows how mitotic oscillations may readily arise from time lags associated with these thresholds and from the delayed negative feedback provided by cdc2-induced cyclin degradation. A mechanism for the origin of the thresholds is proposed in terms of the phenomenon of zero-order ultrasensitivity previously described for biochemical systems regulated by covalent modification.**

Recent advances in the characterization of the biochemical events underlying the cell cycle point to the existence of a universal mechanism regulating the onset of mitosis. Convergent results from studies on yeast (1) and embryonic cells (2) indicate that the various processes which bring about mitosis, such as breakdown of the nuclear envelope, chromosome condensation, or spindle assembly, are triggered by the periodic activation of a protein kinase, product of the gene *cdc2* in fission yeast or of its homologs in other eukaryotes (1–4).

The simplest form of mitotic trigger mechanism is found in early amphibian embryos, where the accumulation of a protein signal, named cyclin because of its periodic variation (5), suffices to drive the cell cycle (2, 6, 7): as cyclin progressively increases beyond a threshold, it causes the activation of cdc2 kinase with which it forms a complex, referred to as maturation- or M-phase-promoting factor (MPF). The latter complex triggers mitosis as well as cyclin degradation (8); the subsequent inactivation of cdc2 kinase resets the cell for a new division cycle (2, 6–8). In yeast as well as in somatic cells, the mechanism involves additional checkpoints (1, 9). What remains common to the various types of cell cycle mechanisms, however, is the fact that they rely on the periodic activation of cdc2 kinase (1–4). Control of the kinase is achieved by reversible covalent modification: activation of cdc2 kinase before the onset of mitosis involves its dephosphorylation, whereas the enzyme is inactivated after mitosis through rephosphorylation (10–15).

What is the molecular mechanism whereby the activation of cdc2 kinase occurs in a periodic manner? The fact that in extracts of amphibian eggs cyclin accumulation drives the activation of cdc2 kinase while the latter promotes cyclin degradation suggests that a minimum cell-cycle oscillator might be based on such a negative feedback loop (2, 8, 16). On the other hand, thresholds are often invoked for the activation of cdc2 kinase by cyclin (1–4, 8, 14) and for the rapid degradation of cyclin after mitosis (7, 8, 16); the origin of such thresholds, however, remains unclear. The purpose of the present work is to show, by means of a simple theoretical model, how thresholds in cdc2 kinase activation and in cyclin degradation may naturally arise as a result of post-translational modification, and how the mitotic cascade involving cyclin and cdc2 kinase can oscillate as a result of both the time delays associated with these thresholds and the triggering by cdc2 kinase of rapid cyclin degradation.

Although the model is based on a number of simplifying assumptions, its analysis highlights the conditions in which the cyclin–cdc2 kinase system can operate as a continuous autonomous oscillator. Besides the situation encountered in early embryogenesis, the results also bear on the more complex mechanisms that control the cell cycle in yeast and somatic cells. By pinpointing ways in which oscillations might be arrested, the model suggests how the products of other genes could play a role in inducing or suppressing mitosis, through the positive or negative regulation of various steps in the cascade that modulates the activity of cdc2 kinase.

Minimal Cascade Model Involving Cyclin and cdc2 Kinase

So as to deal with the minimal structure of the mitotic oscillator, the present analysis focuses on the cell cycle in early amphibian embryos, where the two main actors are cyclin and cdc2 kinase (2, 6–8). The basic assumption (see Fig. 1) is that cyclin is synthesized at a constant rate and triggers the activation of cdc2 kinase. The mechanism of this regulation is not fully understood and is currently the subject of active investigation (1–4, 10–15, 17–20). It appears that cdc2 kinase is inactivated by phosphorylation on a tyrosine (and possibly a threonine) residue located in the ATP-binding domain; dephosphorylation of these residues results in activation of the enzyme (1–4, 13, 15), but the phosphorylation of another (probably threonine) residue might be required for full activity (14, 15). The initial, inactivating, phosphorylation as well as the subsequent activation of cdc2 kinase appears to follow the formation of a complex with cyclin (21).

To avoid entering into the detailed description of a process which is not yet completely clarified, a phenomenological description for the action of cyclin will be retained. Thus, to

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

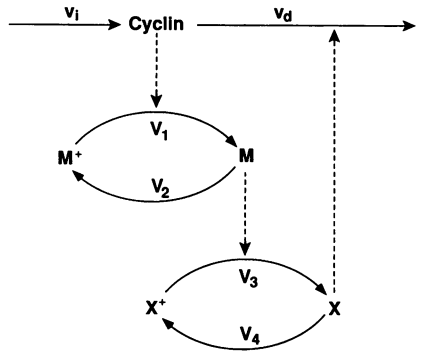

FIG. 1. Minimal cascade model for mitotic oscillations. Cyclin is synthesized at a constant rate (v_i) and triggers the transformation of inactive (M^+) into active (M) cdc2 kinase by enhancing the rate of a phosphatase (E_1); a kinase (E_2) reverts this modification. In the second cycle of the phosphorylation–dephosphorylation cascade, cdc2 kinase (identical to E_3) elicits the transition from the inactive (X^+) into the active (X) form of a protease that degrades cyclin; the activation of cyclin protease is reverted by a phosphatase (E_4). V_i (i = 1–4) denotes the effective maximum rate of each of the four converter enzymes; v_d denotes the maximum rate of cyclin degradation by protease X. As shown in Fig. 3, this minimal cascade is capable of autonomous oscillatory behavior.

keep the model simple and to allow for the straightforward generation of thresholds (see below), the formation of a complex between cyclin and cdc2 kinase will not be taken into account; instead, it is assumed that cyclin drives cdc2 activation by enhancing the velocity of an "activase" which (see the above discussion) might primarily represent a tyrosine (and, possibly, threonine) phosphatase. Such a direct activation of the phosphatase acting on phosphorylated cdc2 kinase is one of the hypothetical mechanisms originally put forward for cyclin action (7, 22). A further assumption is that the maximum activity of the kinase inactivating cdc2—the cdc2 "inactivase" (7)—remains constant throughout the cell cycle.

That okadaic acid, an inhibitor of phosphatase 2A, behaves as a mitotic inducer has suggested that the phosphatase acting on cdc2 might be activated through phosphorylation and inactivated by phosphatase 2A (23–26). This minimal model will not take into account the possible modification of the activase, nor will it differentiate the roles of cyclins A and B, which appear to cooperate in the activation of cdc2 kinase (27, 28).

In line with the observation that the kinase activity of the cdc2 protein promotes cyclin degradation (8), it is assumed that cdc2 kinase activates a cyclin protease, designated as X (as in ref. 8), by reversible phosphorylation (Fig. 1); the maximum activity of the phosphatase inactivating that protease is taken as constant throughout the cycle. There is evidence that the pathway of cyclin degradation is itself a bicyclic phosphorylation cascade, the first step of which would be controlled by cdc2 kinase (8, 16, 25, 26). Consideration of a multicyclic rather than monocyclic cascade leading to the activation of the protease by cdc2 kinase would, however, not significantly affect the results presented here. Cyclin was recently shown to be degraded by the ubiquitin pathway (29); activation of cyclin degradation by cdc2 kinase could accordingly result from the phosphorylation of a protein that would promote the conjugation of ubiquitin to cyclin, leading to rapid cyclin destruction (29).

Thus, the three variables of the minimal model are cyclin, the active (i.e., dephosphorylated) form of cdc2 kinase, and the active (i.e., phosphorylated) form of cyclin protease. The dynamics of the bicyclic cascade of post-translational modification is governed by the following system of kinetic equations:

$$\frac{dC}{dt} = v_i - v_d X \frac{C}{K_d + C} - k_d C,$$

$$\frac{dM}{dt} = V_1 \frac{(1 - M)}{K_1 + (1 - M)} - V_2 \frac{M}{K_2 + M},$$

$$\frac{dX}{dt} = V_3 \frac{(1 - X)}{K_3 + (1 - X)} - V_4 \frac{X}{K_4 + X} \quad [1]$$

with

$$V_1 = \frac{C}{K_c + C} V_{M1}, \; V_3 = M V_{M3}. \quad [2]$$

In the above equations, C denotes the cyclin concentration, while M and X represent the fraction of active cdc2 kinase and the fraction of active cyclin protease; $(1 - M)$ thus represents the fraction of inactive (i.e., phosphorylated) cdc2 kinase, while $(1 - X)$ represents the fraction of inactive (i.e., dephosphorylated) cyclin protease. As to parameters, v_i and v_d denote, respectively, the constant rate of cyclin synthesis and the maximum rate of cyclin degradation by protease X reached for $X = 1$; K_d and K_c denote the Michaelis constants for cyclin degradation and for cyclin activation of the phosphatase acting on the phosphorylated form of cdc2 kinase; k_d represents an apparent first-order rate constant related to nonspecific degradation of cyclin (this facultative reaction, whose contribution is much smaller than that of cyclin degradation by protease X, is not needed for oscillations; its sole effect is to prevent the boundless increase of cyclin in conditions where the specific protease would be inhibited).

The normalized parameters V_i and K_i (i = 1–4) characterize the kinetics of the enzymes E_i (i = 1–4) involved in the two cycles of post-translational modification: on one hand, the phosphatase (E_1) and the kinase (E_2) acting on the cdc2 molecule, and on the other hand, the cdc2 kinase (E_3) and the phosphatase (E_4) acting on the cyclin protease (see Fig. 1). For each converter enzyme, the two parameters V_i and K_i are the effective maximum rate and the Michaelis constant, divided by the total amount of relevant target protein—i.e., M_T (total amount of cdc2 kinase) for enzymes E_1 and E_2, and X_T (total amount of cyclin protease) for enzymes E_3 and E_4; both M_T (4, 11, 12) and X_T will be considered as constant throughout the cell cycle. The expressions for the effective maximum rates V_1 and V_3 are given by Eq. 2. These expressions reflect the assumption that cyclin activates phosphatase E_1 in a Michaelian manner; V_{M1} denotes the maximum rate of that enzyme reached at saturating cyclin levels. On the other hand, the effective maximum rate of cdc2 kinase is proportional to the fraction of active enzyme; V_{M3} denotes the maximum velocity of the kinase reached for $M = 1$.

All nonlinearities in the model are of the Michaelian type. In other words, no form of positive cooperativity is assumed, neither in the proteolysis of cyclin or in the activation by cyclin of the phosphatase acting on cdc2 nor in any of the reactions of covalent modification. The self-amplification effect due to the possible activation of cdc2 kinase by the active form of the cdc2 product (2, 14) has not been considered (see *Discussion*). One of the main goals of the present analysis is, indeed, to determine whether oscillations can arise solely as a result of the negative feedback provided by cdc2-induced cyclin degradation and of the thresholds and time delays built into the cyclin–cdc2 cascade of covalent modification.

Thresholds and Time Delays Arising from Post-Translational Modification

Of key importance for oscillations in the model is the existence of sharp thresholds both in the triggering by cyclin of cdc2 kinase activation through dephosphorylation and in the triggering by cdc2 kinase of cyclin degradation. In the absence of any form of allosteric cooperativity, such thresholds arise naturally in the model from the phenomenon of zero-order ultrasensitivity previously described for covalent modification (30). The basic feature of this phenomenon is that the dependence of the fraction of protein modified (e.g., phosphorylated) at steady state varies in a very steep manner as a function of the ratio of kinase to phosphatase maximum rates when the two converter enzymes possess relatively low Michaelis constants and therefore function in the zero-order kinetic domain. The existence of thresholds associated with zero-order ultrasensitivity has been predicted theoretically (30, 31) and verified experimentally in several enzymatic systems controlled by covalent modification, such as isocitrate dehydrogenase (32) and glycogen phosphorylase (33).

The occurrence of such thresholds in the mitotic cascade model is illustrated by the curves showing the dependence at steady state of the fraction of active cdc2 kinase on cyclin (Fig. 2*A*) and of the fraction of active cyclin protease on cdc2 kinase (Fig. 2*B*). When the value of the reduced Michaelis constants K_i (i = 1–4) is significantly smaller than unity, the variation of M and X as a function of the ratio of effective maximum modification rates (V_1/V_2) or (V_3/V_4) is extremely steep (curves a in Fig. 2). In contrast, the transition curves for M and X become much smoother, and finally acquire a hyperbolic nature, as the constants K_i increase up to and above unity (curves b in Fig. 2). The values of C and M corresponding to the thresholds in the activation of M and X (see curves a in Fig. 2) will be referred to as C^* and M^*.

Also essential for the onset of oscillations in the model are time delays whose existence is closely correlated with the occurrence of the steep transitions in M and X. A first delay indeed originates from the slow accumulation of cyclin up to the threshold value C^*, beyond which the fraction of active cdc2 kinase abruptly increases up to a value close to unity (see curve a in Fig. 2*A*). A second delay comes from the time required for M to reach the threshold M^*, beyond which the cyclin protease is switched on (curve a in Fig. 2*B*). Moreover, the transitions in M and X do not occur instantaneously once C and M reach the threshold values predicted by the steady-state curves; the time lag in each of the two modification processes contributes to the delay that separates the rise in C from the increase in M and the latter increase from the rise in X. Finally, the mirror time lag on the decreasing phase of M and X is no less important: the fact that the cyclin protease is not directly inactivated when the level of cyclin drops below C^* prolongs the phase of cyclin degradation, with the consequence that M and X will both become inactivated to a further degree as C drops well below C^*.

Oscillatory Dynamics of the Bicyclic Cascade Involving Cyclin and cdc2 Kinase

Provided that thresholds exist in the dependence of M on C and of X on M, as for curves a in Fig. 2, the evolution of the bicyclic cascade readily proceeds in a periodic manner (Fig. 3). Starting from a small value of C corresponding to a low initial concentration of cyclin, the latter accumulates at a constant rate due to protein synthesis; during that phase, both M and X have a small value, since C is well below the threshold of activation of M and, consequently, M is well below the threshold of activation of X. As the cyclin level continues to rise, the value of C approaches the sharp threshold C^*, beyond which M abruptly starts to increase (Fig. 3). The increase in M is such that the cdc2 kinase soon exceeds the threshold M^* for activation of X. The level of the active cyclin protease therefore begins to rise, after a lag due to the time required for M to increase above M^* and to the time taken by the converter enzymes to change the level of X in the second cycle of the cascade.

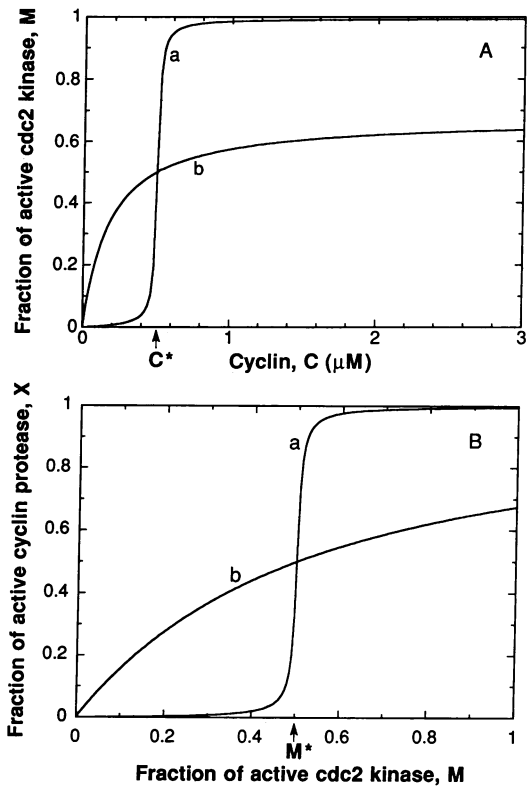

FIG. 2. Dependence of the fraction of active cdc2 kinase, M, on cyclin (*A*), and of the fraction of active cyclin protease, X, on M (*B*). The curves in *A* are generated by means of an equation (see refs. 30 and 31) yielding the steady-state value of M as a function of the ratio of maximum modification rates V_1/V_2, which is itself a function of cyclin concentration (see Eq. 2); the curves in *B* are generated with a similar equation yielding the steady-state value of X as a function of the ratio V_3/V_4, which is proportional to M (see Eq. 2). C^* and M^* refer to the thresholds apparent in curves a of *A* and *B*, respectively. The curves are established for the following parameter values (in min^{-1}): $V_{M1} = 3$, $V_2 = 1.5$, $V_{M3} = 1$, $V_4 = 0.5$; moreover, $K_c = 0.5$ μM. In both *A* and *B*, curves a are obtained for $K_i = 0.005$ while curves b are obtained for $K_i = 10$ (i = 1–4). The actual values of the maximum rates and Michaelis constants of the converter enzymes E_1 and E_2 are obtained by multiplying V_{M1}, V_2, K_1, and K_2 by M_T (= 4 μM) (11, 22). The actual values of the corresponding parameters for enzymes E_3 and E_4 in the second modification cycle are obtained by multiplying V_{M3}, V_4, K_3, and K_4 by X_T, for which a value of 4 μM is taken; the resulting value, of the order of 10^{-5} M·min^{-1}, for the maximum rate of cdc2 kinase matches that observed experimentally (see, e.g., ref. 24). The value of K_c is in the range of experimentally determined cyclin concentrations (22).

Thus, while the abrupt rise in the active cdc2 kinase M brings about the various biochemical events leading to mitosis, it also causes a sharp increase in the activity of the cyclin protease X. Once the latter becomes activated in the monocyclic (or multicyclic) cascade of covalent modification controlled by cdc2 kinase, the rate of cyclin degradation rapidly exceeds its rate of synthesis, so that the level of cyclin begins to drop precipitously and C falls below C^*. As a result of the steep dependence of M on C (curve a in Fig. 2*A*), the cdc2 kinase is rapidly inactivated, so that M drops below M^*. The sharp dependence of X on M (curve a in Fig. 2*B*) is such that the cyclin protease rapidly becomes inactivated. These events thus lead, after a lag, to a sharp drop first in M and soon after in X; then the constant rate of cyclin synthesis can again

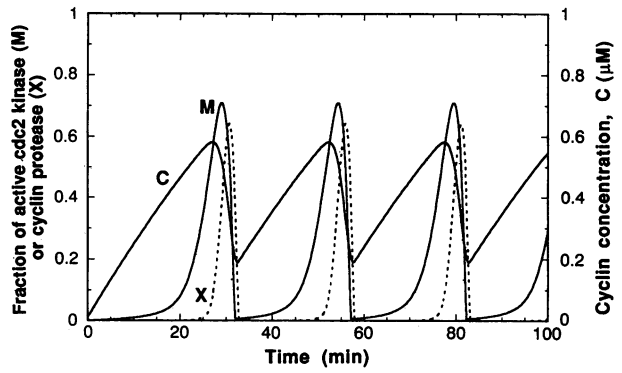

FIG. 3. Sustained oscillations in the minimal cascade model involving cyclin and cdc2 kinase (see Fig. 1). The time evolution of the cyclin concentration (C), the fraction of active cdc2 kinase (M), and the fraction of active cyclin protease (X) is obtained by numerical integration of Eqs. 1 in conditions where a threshold exists in the dependence of M on C and of X on M, namely, for the parameter values corresponding to curves a in Fig. 2, with $v_i = 0.025\ \mu M \cdot min^{-1}$, $v_d = 0.25\ \mu M \cdot min^{-1}$, $K_d = 0.02\ \mu M$, and $k_d = 0.01\ min^{-1}$. Initial conditions are $C = 0.01\ \mu M$, $M = X = 0.01$.

exceed the rate of cyclin degradation and a new cell cycle begins.

The waveform and period of the oscillations in Fig. 3 match those reported experimentally (2, 6, 8, 22). Moreover, the model accounts for the observation in extracts of amphibian embryos (6) that an increase in the rate of cyclin synthesis shortens the interphase preceding mitosis. The length of that phase is indeed dictated by the slow rise of C up to its threshold value C^*, in which abrupt activation of the cdc2 kinase begins.

In the phase space (C, M, X), regardless of initial conditions, the system reaches a unique, closed trajectory. Sustained oscillations in the cascade model therefore correspond to the evolution towards a limit cycle around a nonequilibrium, unstable steady state (34). The projection of this limit cycle in the cyclin–cdc2 kinase space is shown in Fig. 4. Oscillations of the limit cycle type are particularly stable, as they are characterized by an amplitude and period that are fixed for a given set of parameter values.

That thresholds play an essential role in the above-described mechanism for mitotic oscillations is demonstrated by the fact

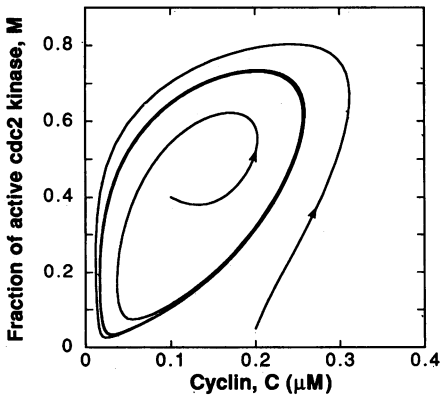

FIG. 4. Limit cycle behavior of the cascade model for the mitotic oscillator. The curves are obtained by projecting the trajectory of the three-variable system governed by Eqs. 1 onto the cyclin–cdc2 kinase (C, M) plane. Two sets of initial conditions are considered, one inside and the other outside the limit cycle; arrows indicate the direction of the time evolution. Parameter values are $K_i = 0.1$ (i = 1–4), $V_{M1} = 0.5\ min^{-1}$, $V_2 = 0.167\ min^{-1}$, $V_{M3} = 0.2\ min^{-1}$, $V_4 = 0.1\ min^{-1}$, $v_i = 0.023\ \mu M \cdot min^{-1}$, $v_d = 0.1\ \mu M \cdot min^{-1}$, $K_c = 0.3\ \mu M$, $K_d = 0.02\ \mu M$, $k_d = 3.33 \times 10^{-3}\ min^{-1}$; for these values, the period of the oscillations is equal to 36 min. The initial value of X for the two trajectories shown is 0.01.

that the cascade ceases to oscillate when the activation curves for M and X lack the steepness associated with zero-order ultrasensitivity. Thus, integration of Eqs. 1 in the conditions of curves b in Fig. 2 shows that the system reaches a stable steady state. These results indicate that the time lags associated with the thresholds in covalent modification are required for inducing oscillations in the cascade controlling mitosis. A further role of the thresholds is to provide increased responsiveness to small changes in cyclin concentration; such enhanced sensitivity underlies the abrupt changes that allow the cascade to function in a seesaw manner.

Discussion

The suggestion that the cell cycle is controlled by a continuous biochemical oscillator of the limit cycle type was put forward in a theoretical and experimental study of mitosis in *Physarum* (35). Because of the lack of biochemical information, however, the chemical nature of the oscillator in that study remained unspecified. The recent convergence of genetic and biochemical approaches has since led to significant breakthroughs in the search for the mechanism that regulates the onset of mitosis. On the basis of these experimental advances, a minimal molecular model for the mitotic oscillator is proposed. The results support the inclusion (34) of the mitotic control system among the chemical and biological processes capable of nonequilibrium self-organization in the form of limit cycle oscillatory behavior.

The model relies on the cascade of phosphorylation-dephosphorylation cycles involving cyclin and cdc2 kinase as uncovered in the early stages of frog embryogenesis, where cyclin accumulation suffices to drive the cell cycle. The progressive rise of cyclin beyond a threshold triggers the activation of cdc2 kinase; the latter in turn elicits cyclin degradation, which switches off the activation of cdc2 kinase and thereby resets the system for a new mitotic cycle.

A key result of the present analysis is the demonstration that sustained oscillations in cdc2 kinase and cyclin of appropriate period and waveform can arise from thresholds and time delays built into the cascade of post-translational modification controlling the activation of cdc2 kinase and cyclin proteolysis. The time delays are not introduced, as is often the case, in an *ad hoc* manner; rather, they are a natural consequence of the thresholds that characterize the dependence of cdc2 kinase activation on cyclin, and of cyclin proteolysis on cdc2 kinase. These thresholds originate in the model from the kinetic characteristics of covalent modification: any couple of converter enzymes such as a kinase and its associated phosphatase possesses the potential to function as a biochemical switch; a sharp transition will indeed occur in the steady-state level of phosphorylated protein whenever the converter enzymes are saturated by their substrate (30).

The results support the view, derived from experiments on extracts of amphibian embryos (2, 3, 6–8), that the cell cycle is driven by continuous biochemical oscillations and that the triggering by cdc2 kinase of cyclin degradation creates a negative feedback loop on which a minimum cell-cycle oscillator could be based (8). In particular, the model fully corroborates the conjecture put forward by Félix *et al.* (8) for whom "the system cannot reach equilibrium: it keeps oscillating, because threshold levels of both cyclin protein and cdc2 kinase activities trigger post-translational reactions with built-in time delays, and the destruction of cyclin causes the loss of cdc2 kinase activity." The present model represents a simple mathematical implementation of such a minimum cell-cycle oscillator.

Although the model provides an appealingly straightforward explanation for the origin of thresholds in the post-translational modification cycles controlling cdc2 kinase and cyclin proteolysis, the putative role of zero-order ultrasensitivity in the origin of these thresholds remains to be

established. Other mechanisms based, for example, on the action of an inhibitory phosphatase that reverts a cdc2 phosphorylation apparently required for activity have been invoked (14, 36) to explain the requirement for a critical level of cyclin as well as the lag in the activation of cdc2 kinase; alternatively, the target of that phosphatase might be cdc2 activase (26). Whatever the origin of the thresholds, the model shows how time delays arise from them to give rise to sustained oscillations around an unstable steady state.

Another result of the analysis is the demonstration that positive feedback is not required for oscillations. This is in contrast with previous models (37, 38), which were based on the autocatalytic activation of cdc2 kinase but did not take into account the kinetic characteristics of covalent modification; oscillations do not occur in these models in the absence of autocatalysis. Self-amplification in activation of maturation-promoting factor has been described experimentally (14); the phenomenon could originate from the activation of the cdc2 activase (i.e., phosphatase) through phosphorylation by cdc2 kinase (16, 25–27), or from some autophosphorylation (15). Any form of autocatalysis would favor the occurrence of periodic behavior, as is well known in other biochemical (39) and chemical systems (34). The model nevertheless supports the conjecture (8) that the oscillatory mechanism primarily rests on both the delayed negative feedback provided by cdc2-induced cyclin degradation and the combined occurrence of thresholds and time lags in the cascade of post-translational modification. In this respect, the mechanism proposed for the periodic activation of cdc2 kinase differs from other examples of oscillatory behavior in biochemistry such as glycolytic oscillations in yeast (40), the periodic generation of cAMP pulses in *Dictyostelium* (41), or intracellular calcium oscillations (42, 43), which all originate from positive feedback amplified by the cooperativity of allosteric interactions.

The model presented for the mitotic oscillator is conspicuous by its robustness in producing oscillations. The latter, indeed, occur over a wide range of parameter values once sharp thresholds occur in the activation of cdc2 kinase and cyclin protease. When the thresholds are less sharp, as in the conditions of Fig. 4, the domain of periodic behavior is reduced and the oscillations are smoother than those of Fig. 3. While cdc2 kinase and cyclin protease are both controlled by a single cycle of phosphorylation–dephosphorylation in this minimal model, it is likely that additional cycles of covalent modification are involved (16, 25, 26). Such a lengthening of the cascade, besides introducing further delays, could produce sharper thresholds in covalent modification, since the sensitivity gained in one cycle can be amplified in subsequent cycles (30, 31). In such a way, the multiplicity of covalent modification cycles could increase the robustness of the mitotic clock.

That the reactions controlling the onset of mitosis are organized as a network of covalent modification cycles also multiplies the potential sites of regulation and thereby suggests different ways of arresting the cell cycle, besides a major alteration of the balance between cyclin synthesis and degradation. Thus the oscillations can be suppressed if the ratios of effective maximum rates of the converter enzymes, V_1/V_2 or V_3/V_4, are held below their threshold values.

The question arises as to the applicability of the model to cell cycles subject to more complex regulation than in early embryogenesis. In yeast, an additional control occurs at the "start" point before DNA replication (1, 9); two different states of cdc2 kinase are involved in the checkpoints before "start" and mitosis (44), corresponding to two different forms of covalently modified cdc2 per cycle (15, 44). Each of these two forms could be involved in an oscillator similar to the one described here, with its own cyclin (45), cyclin protease, and set of converter enzymes; the alternation between the two forms of active cdc2 kinase would result from the coupling of the two oscillators (2). The minimum oscillating cascade of Fig. 1 could thus provide a "building clock" for the more complex mechanisms underlying the cell cycle in yeast or somatic cells.

This work was supported by the Belgian National Incentive Program for Fundamental Research in the Life Sciences (Convention BIO/08), launched by the Science Policy Programming Services of the Prime Minister's Office (SPPS).

1. Nurse, P. (1990) *Nature (London)* **344,** 503–508.
2. Murray, A. W. & Kirschner, M. W. (1989) *Science* **246,** 614–621.
3. Murray, A. W. (1989) *Nature (London)* **342,** 14–15.
4. Draetta, G. (1990) *Trends Biochem. Sci.* **15,** 378–383.
5. Evans, T., Rosenthal, E. T., Youngblom, J., Distel, D. & Hunt, T. (1983) *Cell* **33,** 389–396.
6. Murray, A. W. & Kirschner, M. W. (1989) *Nature (London)* **339,** 275–280.
7. Murray, A. W., Solomon, M. J. & Kirschner, M. W. (1989) *Nature (London)* **339,** 280–286.
8. Félix, M. A., Labbé, J. C., Dorée, M., Hunt, T. & Karsenti, E. (1990) *Nature (London)* **346,** 379–382.
9. Hartwell, L. H. & Weinert, T. A. (1989) *Science* **246,** 629–634.
10. Gautier, J., Matsukawa, T., Nurse, P. & Maller, J. (1989) *Nature (London)* **339,** 626–629.
11. Labbé, J. C., Picard, A., Peaucellier, G., Cavadore, J. C., Nurse, P. & Dorée, M. (1989) *Cell* **57,** 253–263.
12. Morla, A. O., Draetta, G., Beach, D. & Wang, J. Y. J. (1989) *Cell* **58,** 193–203.
13. Gould, K. & Nurse, P. (1989) *Nature (London)* **342,** 39–45.
14. Solomon, M. J., Glotzer, M., Lee, T. H., Philippe, M. & Kirschner, M. W. (1990) *Cell* **63,** 1013–1024.
15. Krek, W. & Nigg, E. A. (1991) *EMBO J.* **10,** 305–316.
16. Hunt, T. (1989) *Nature (London)* **342,** 483–484.
17. Moreno, S., Nurse, P. & Russell, P. (1990) *Nature (London)* **344,** 549–552.
18. Gould, K. L., Moreno, S., Tonks, N. K. & Nurse, P. (1990) *Science* **250,** 1573–1576.
19. Kumagai, A. & Dunphy, W. G. (1991) *Cell* **64,** 903–914.
20. Strausfeld, U., Labbé, J. C., Fesquet, D., Cavadore, J. C., Picard, A., Sadhu, K., Russell, P. & Dorée, M. (1991) *Nature (London)* **351,** 242–245.
21. Meijer, L., Azzi, L. & Wang, J. Y. J. (1991) *EMBO J.* **10,** 1545–1554.
22. Minshull, J., Pines, J., Golsteyn, R., Standart, N., Mackie, S., Colman, A., Blow, J., Ruderman, J. V., Wu, M. & Hunt, T. (1989) *J. Cell Sci. Suppl.* **12,** 77–97.
23. Goris, J., Hermann, J., Hendrix, P., Ozon, R. & Merlevede, W. (1989) *FEBS Lett.* **245,** 91–94.
24. Félix, M.-A., Cohen, P. & Karsenti, E. (1990) *EMBO J.* **9,** 675–683.
25. Lorca, T., Fesquet, D., Zindy, F., Le Bouffant, F., Cerruti, M., Brechot, C., Devauchelle, G. & Dorée, M. (1991) *Mol. Cell. Biol.* **11,** 1171–1175.
26. Karsenti, E., Verde, F. & Félix, M. A. (1991) *Adv. Protein Phosphatases* **6,** 453–482.
27. Minshull, J., Golsteyn, R., Hill, C. S. & Hunt, T. (1990) *EMBO J.* **9,** 2865–2875.
28. Buendia, B., Clarke, P. R., Félix, M. A., Karsenti, E., Leiss, D. & Verde, F. (1991) *Cold Spring Harbor Symp. Quant. Biol.*, in press.
29. Glotzer, M., Murray, A. W. & Kirschner, M. W. (1991) *Nature (London)* **349,** 132–138.
30. Goldbeter, A. & Koshland, D. E., Jr. (1981) *Proc. Natl. Acad. Sci. USA* **78,** 6840–6844.
31. Goldbeter, A. & Koshland, D. E., Jr. (1982) *Q. Rev. Biophys.* **15,** 555–591.
32. LaPorte, D. C. & Koshland, D. E., Jr. (1983) *Nature (London)* **305,** 286–290.
33. Meinke, M. H., Bishop, J. S. & Edstrom, R. D. (1986) *Proc. Natl. Acad. Sci. USA* **83,** 2865–2868.
34. Nicolis, G. & Prigogine, I. (1977) *Self-Organization in Nonequilibrium Systems* (Wiley, New York).
35. Kauffman, S. & Wille, J. J. (1975) *J. Theor. Biol.* **55,** 47–93.
36. Lee, T. H., Solomon, M. J., Mumby, M. C. & Kirschner, M. W. (1991) *Cell* **64,** 415–423.
37. Hyver, C. & Le Guyader, H. (1990) *Biosystems* **24,** 85–90.
38. Norel, R. & Agur, Z. (1991) *Science* **251,** 1076–1078.
39. Goldbeter, A. (1990) *Rythmes et Chaos dans les Systèmes Biochimiques et Cellulaires* (Masson, Paris).
40. Boiteux, A., Goldbeter, A. & Hess, B. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 3829–3833.
41. Martiel, J. L. & Goldbeter, A. (1987) *Biophys. J.* **52,** 807–828.
42. Meyer, T. & Stryer, L. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 5051–5055.
43. Goldbeter, A., Dupont, G. & Berridge, M. J. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 1461–1465.
44. Broek, D., Bartlett, R., Crawford, K. & Nurse, P. (1991) *Nature (London)* **349,** 388–393.
45. Reed, S. I. (1991) *Trends Genet.* **7,** 95–99.