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Research Articles

Arresting the mitotic oscillator and the control of cell proliferation: insights from a cascade model for cdc2 kinase activation

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Abstract. We consider a minimal cascade model previously proposed[11] for the mitotic oscillator driving the embryonic cell division cycle. The model is based on a bicyclic phosphorylation-dephosphorylation cascade involving cyclin and cdc2 kinase. By constructing stability diagrams showing domains of periodic behavior as a function of the maximum rates of the kinases and phosphatases involved in the two cycles of the cascade, we investigate the role of these converter enzymes in the oscillatory mechanism. Oscillations occur when the balance of kinase and phosphatase rates in each cycle is in a range bounded by two critical values. The results suggest ways to arrest the mitotic oscillator by altering the maximum rates of the converter enzymes. These results bear on the control of cell proliferation.
Key words. Cell cycle; G_1/S and G_2/M transitions; metaphase arrest; cell proliferation; biochemical oscillations; phosphorylation-dephosphorylation cascade.

Genetic studies using yeast[1,2] and biochemical studies using amphibian eggs[3,4] have converged in recent years to indicate that mitosis in eukaryotic cells is controlled by a continuous biochemical oscillator. This oscillator, driven by the synthesis of proteins named cyclins[5], relies on a cascade of phosphorylation-dephosphorylation cycles that ultimately results in the activation of a protein kinase named cdc2 kinase[4,6], which brings about the onset of mitosis. The simplest form of mitotic oscillator is found in activated amphibian eggs[3-5,7], where oscillations in cdc2 kinase activity have also been observed in cell extracts[8].

The kinase cdc2 is activated through dephosphorylation by the tyrosine phosphatase cdc25[9]. The fact that cyclin promotes the activation of cdc2 kinase by cdc25 and that cdc2 kinase promotes the degradation of cyclin by a protease has suggested that such a negative feedback loop may provide the basis for a minimum cell cycle oscillator[3,5,10]. This conjecture was corroborated by the study of a theoretical model based on a minimal cascade containing two phosphorylation-dephosphorylation cycles[11-13]. In the first one, cyclin promotes the activation of cdc2 kinase through dephosphorylation by the phosphatase cdc25, while in the second cycle cdc2 kinase activates the cyclin protease.

The purpose of this note is to determine how changes in the maximum rates of the converter enzymes may stop the rhythmic operation of the mitotic cascade. We present diagrams showing regions of periodic behavior as a function of the maximum rates of the converter en-zymes in the minimal cascade model. From a dynamic point of view, halting the mitotic oscillator corresponds to the transition from sustained oscillations into a stable, steady (quiescent) state upon changing a control parameter beyond some critical value. The results suggest various ways to control cell proliferation by preventing the periodic operation of the phosphorylation-dephosphorylation cascade.

Dealing with the simplest structure of the mitotic oscillator, the minimal cascade model[11] focuses on the early cell cycles in amphibian embryos, where the two main actors are cyclin B and cdc2 kinase[3,5,7]. The basic assumption (see fig. 1) is that cyclin is synthesized at a constant rate and triggers the activation of cdc2 kinase. For simplicity, the formation of a complex between cyclin and cdc2 kinase[4,6] is not taken into account. Instead, it is assumed that in the first cycle of the cascade, cyclin drives cdc2 activation by enhancing the velocity of the cdc25 phosphatase[9]; inactivation of cdc2 kinase through tyrosine phosphorylation is catalyzed by the kinase wee1[14]. In line with the observation that the kinase activity of cdc2 promotes cyclin degradation[10], it is assumed that in the second cycle cdc2 kinase activates a cyclin protease, designated as X, by reversible phosphorylation (see fig. 1). There is evidence that cyclin degradation, which involves the ubiquitin pathway[15,16], itself occurs in a multicyclic phosphorylation cascade, the first step of which would be controlled by cdc2 kinase[10]. Consideration of a multicyclic rather than monocyclic cascade

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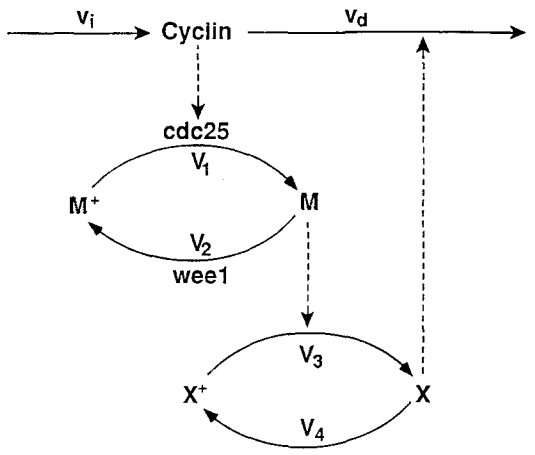

Figure 1. Minimal cascade model for the mitotic oscillator[11]. Cyclin is synthesized at a constant rate (v_i) and triggers the transformation of inactive (M^+) into active (M) cdc2 kinase, by enhancing the rate of phosphatase cdc25 (E_1); kinase wee1 (E_2) reverses this modification. In the second cycle 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 reversed by a phosphatase (E_4). V_i ($i = 1, \ldots, 4$) 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 figure 2, this minimal cascade model is capable of autonomous oscillatory behavior.

leading to the activation of the protease by cdc2 kinase should enlarge the domain of periodic behavior. Adding phosphorylation-dephosphorylation cycles to the minimal cascade model indeed favors the occurrence of sustained oscillations[17].

The three variables of the minimal cascade model described in figure 1 are cyclin (C), the fraction of active cdc2 kinase (M), and the fraction of active cyclin protease (X). The dynamics of the bicyclic cascade are governed by the following system of three kinetic equations[11]:

$$\frac{dC}{dt} = v_i - v_d X \frac{C}{K_d + C} - k_d C \tag{1a}$$

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

$$\frac{dX}{dt} = V_3 \frac{(1 - X)}{K_3 + (1 - X)} - V_4 \frac{X}{K_4 + X}, \tag{1c}$$

with

$$V_1 = \frac{C}{K_c + C} V_{M1}, \quad V_3 = M V_{M3}. \tag{2a, b}$$

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 cdc25 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 contribu-

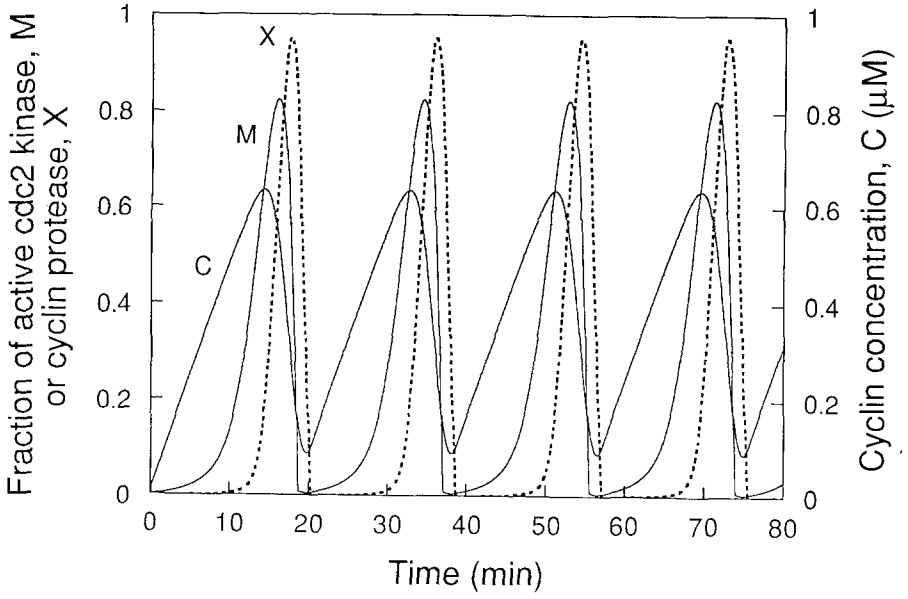

Figure 2. Sustained oscillations in the minimal cascade model involving cyclin and cdc2 kinase (see fig. 1). The time evolution of the cyclin concentration (C), the fraction of active cdc2 kinase (M), and the fraction of active cyclin protease (X), are obtained by numerical integration of eqs. (1). Parameter values (in min^{-1}) are: $V_{M1} = 3$, $V_2 = 1.5$, $V_{M3} = 1$, $V_4 = 0.5$; moreover, $K_c = 0.5$ μM, $v_i = 0.05$ μM min^{-1}, $v_d = 0.25$ μM min^{-1}, $K_d = 0.02$ μM, $K_i = 0.01$ ($i = 1, \ldots, 4$), and $k_d = 0.01$ min^{-1}.

tion is generally 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 is inhibited).

The remaining parameters V_i and K_i ($i = 1, \ldots, 4$) denote the effective maximum rate and the Michaelis constant for each of the enzymes E_i ($i = 1, \ldots, 4$) involved in the two cycles of post-translational modification: on the one hand, the phosphatase cdc25 (E_1) and the kinase wee1 (E_2) acting on the cdc2 molecule, and on the other hand, the kinase cdc2 (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 divided by the total amount of relevant target protein, i.e. M_T (total amount of cdc2 kinase) for E_1 and E_2, and X_T (total amount of cyclin protease) for E_3 and E_4. Both M_T and X_T are considered as constant.

The expressions for the effective, maximum rates V_1 and V_3 are given by eqs (2). Equation (2a) reflects the assumption that cyclin activates the cdc25 phosphatase in a Michaelian manner; V_{M1} denotes the maximum rate of that enzyme at saturating cyclin levels. On the other hand, eq. (2b) expresses the proportionality of the effective maximum rate of cdc2 kinase to the fraction M of active enzyme; V_{M3} denotes the maximum velocity of the kinase reached for $M = 1$.

The cascade of figure 1 provides a minimal model for mitotic oscillations based on cdc2-induced cyclin degradation. As well as the possible extension of the cascade controlling cyclin proteolysis, additional phosphorylation-dephosphorylation cycles could be considered. Thus, cdc25[18] and wee1[19,20] are themselves regulated by reversible phosphorylation. Incorporation of these processes requires an extension of the minimal cascade model[12,13,17]. The minimal model, to which the present study is restricted, does not consider the further activation of cdc2 kinase by threonine phosphorylation[21], nor the self-amplification due to the direct or indirect activation of cdc2 kinase by the active form of the enzyme[3,22]. The effect of such autocatalysis, which is to favor oscillations, has been addressed in more complex models[12,17,23,24]. The qualitative results presented below for the minimal model are based on the general characteristics of domains of oscillations in parameter space and remain largely independent of the specific details of the model considered for the mitotic oscillator.

Typical oscillations generated by the minimal cascade model are shown in figure 2 (see reference 11 for a discussion of the mechanism underlying periodic behavior). Among the main parameters affecting the oscillatory dynamics of the cascade are the maximum rates of the converter enzymes E_1, E_2 and E_3, E_4. Shown in panels A and B of figure 3 are the stability diagrams established as a function of V_{M1} vs V_2, and V_{M3} vs V_4. Conspicuous in these figures is the shape of the oscillatory domain which extends diagonally just above the first bisectrix, $V_{M1} = V_2$ in (A), and $V_{M3} = V_4$ in (B). This is because the activation thresholds in M and X, which play a key role in the oscillations[11-13], occur at values of the ratios V_{M1}/V_2 and V_{M3}/V_4 equal to unity, under the conditions of figure 3.

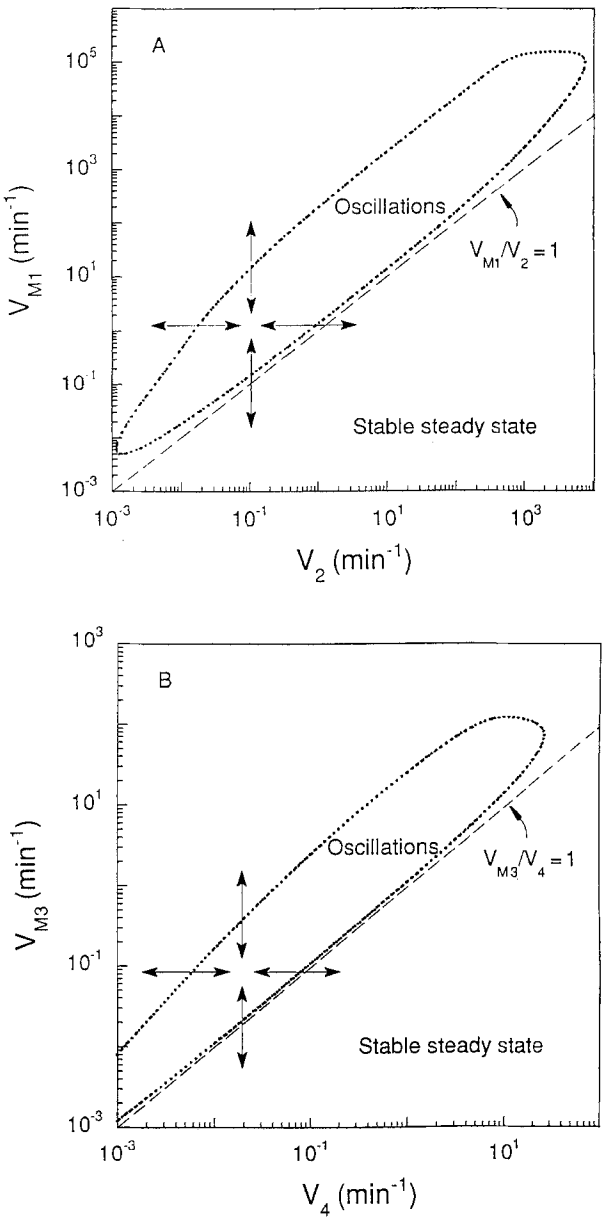

Figure 3. Stability diagrams showing the behavior of the minimal cascade model for the mitotic oscillator as a function of the maximum rates of the converter enzymes in the first *(A)* and second *(B)* phosphorylation-dephosphorylation cycles. The arrows indicate different ways to quit or enter the oscillatory domain following a change in the rate of any of the four converter enzymes. The results are obtained by means of linear stability analysis of the steady state solution admitted by eqs. (1). Shown in each diagram are the regions where the system evolves toward a stable steady state or to sustained oscillations. Parameter values are the same as in figure 2, with K_i ($i = 1, \ldots, 4$) $= 5 \times 10^{-3}$. Other parameter values are $v_i = 0.025$ μM min^{-1}, $v_d = 0.25$ μM min^{-1}, $K_d = 0.02$ μM, and $k_d = 0.01$ min^{-1}.

The present results show that for oscillations to occur, the maximum rates of phosphorylation and dephosphorylation in each cycle have to be in an appropriate balance. If the ratio (V_{M1}/V_2) is too low, the system will be blocked in a stable steady state characterized by high levels of cyclin and low levels of active cdc2 kinase and cyclin protease. If, on the contrary, the ratio (V_{M1}/V_2) is too large, the system will be trapped in a stable steady state characterized by high levels of active cdc2 kinase and cyclin protease, and low levels of cyclin. Similarly, in the second cycle, a stable steady state in which M and C are high and X is low will be established when the ratio (V_{M3}/V_4) is too low. Such a stable steady state is analogous to the cytostatic factor (CSF)-induced state of metaphase arrest in vertebrate eggs prior to fertilization[3,7,25]. Conversely, the system will be trapped in a stable steady state characterized by significant levels of both active cyclin protease and cdc2 kinase and a low level of cyclin when the ratio (V_{M3}/V_4) becomes too large.

One of the properties characterizing the cancerous state is unrestrained proliferation due to loss of control of the cell division cycle. Arresting the mitotic oscillator is thus of key importance for bringing continuously dividing cells into a quiescent state. The model suggests that there are different ways to arrest the mitotic oscillator and thereby control cell proliferation. The most straightforward way to suppress oscillations in the cascade controlling cdc2 is to alter the maximum rate of either one of the four converter enzymes in the cascade of figure 1. Because each of these rates possesses a range, bounded by two critical values, in which sustained oscillations occur, there exist at least eight different ways to suppress oscillations through altering any one of these rates in the minimal cascade model; such changes are symbolized by arrows in figure 3.

Thus, oscillations can be suppressed by decreasing or increasing the maximum rate of cdc25 phosphatase (V_{M1}), wee1 kinase (V_2), cdc2 kinase (V_{M3}), or the phosphatase acting on the cyclin protease (V_4). If the inhibition of a converter enzyme can readily be thought of as a means of blocking the periodic operation of the cascade, the result that activating the same enzyme can also stop the oscillations is more counterintuitive. This is because it is not the absolute value of any rate that matters, but rather the balance between the rates of the kinase and phosphatase in each cycle of the cascade.

The addition of a single covalent modification cycle to the cascade introduces four new modes of arresting the mitotic oscillator, by increasing or decreasing the maximum rate of the two new converter enzymes. An important property of phosphorylation-dephosphorylation cascades is therefore to multiply the possibilities of controlling the final output and the dynamics of the cascade by modulating the activity of any of its converter enzymes.

Besides the G_2/M transition considered here, controls in the G_1 phase are also crucial for the regulation of cell proliferation[1,26–28]. The G_1/S transition is also governed by a biochemical oscillator, which involves a G_1 cyclin and a cdc2-related kinase known as cdk2[29]. Because this second oscillator appears to rely on a mechanism similar to the one involving cdc2, the present analysis of a minimal model for the control of the G_2/M transition also bears, *mutatis mutandis*, on the control of the G_1/S transition in the cell cycle.

Change in the rate of any of the converter enzymes can be brought about either by an allosteric effector or by phosphorylation or dephosphorylation. Both types of control play a role in the regulation of cell cycle progression. Thus cdc25 phosphatase[18] and wee1 kinase[19,20] are both regulated by reversible phosphorylation. Moreover, protein inhibitors of cyclin-dependent kinases also regulate progression through the cycle, particularly at the G_1/S transition[30,31]. Terminal cell cycle arrest and differentiation have recently been shown to be governed by changes in the levels of such protein inhibitors[32]. Controlling the converter enzymes of the phosphorylation cascades which govern the ordered progression from DNA replication to mitosis therefore represents an important physiological mode of cell cycle regulation.

Most anticancer drugs inhibit cell division by interfering with either DNA synthesis or microtubule polymerization. Because modulating the activity of the converter enzymes in the cascades has such profound consequences on the dynamics of the biochemical oscillators that govern the G_1/S and G_2/M transitions, these enzymes are key potential targets for new therapeutic approaches to the control of cell proliferation.

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