



Instabilities in phosphorylation-dephosphorylation cascades and cell cycle checkpoints

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The G2-M checkpoint in the cell cycle is identified with a set of phosphorylation-dephosphorylation (PD) cycles involving Cdc25 and the maturation-promoting factor (MPF); these PD cycles are coupled in a way that generates an instability. This instability arises out of a transcritical bifurcation which could be exploited by the G2 DNA damage checkpoint pathway in order to arrest or delay entry into mitosis. The coupling between PD cycles involving Wee1 and MPF does not lead to an instability and therefore Wee1 may not be a crucial target of the checkpoint pathway. A set of PD cycles exhibiting transcritical bifurcation also possesses the integrative ability of a checkpoint for ‘checking’ that prerequisites are satisfied prior to the next cell cycle event. Such a set of coupled PD cycles is suggested to be a core mechanism of cell cycle checkpoints.

Keywords: cell cycle; phosphorylation-dephosphorylation cascades; G2-M checkpoint

Introduction

Details of the biochemical reactions and pathways orchestrating the orderly progression of cell cycle events are currently being elucidated at a dizzying pace in various laboratories all over the world. Although we may just have begun to unravel the complexity of the cell cycle, it is now widely agreed upon that the key regulators of cell cycle transitions are the so-called *Cdk*'s (for *cyclin-dependent kinases*). The sequence of activation and deactivation of these *Cdk*'s, corresponding to the sequence of cell cycle phases, is sometimes referred to as the ‘cell cycle engine’ (Murray and Hunt, 1993). In mammalian cells, different *Cdk*'s are known to be active during the different phases of the cell cycle (Pines and Hunter, 1995); these *Cdk*'s include Cdc2 (or Cdk1), Cdk2, Cdk3, Cdk4, Cdk5 and Cdk6. Activation of these *Cdk*'s absolutely requires binding with cyclins (e.g. cyclin D, E, A, B); in addition, positive and negative regulation of *Cdk* activity involves phosphorylation-dephosphorylation (PD) processes (Morgan, 1995). These PD cycles are ubiquitous in the cell cycle and it is not uncommon to see cascades of PD cycles where, for example, an activating phosphorylation of one kinase is carried out by a second kinase which, in turn, could be regulated by the first kinase. One of the

objectives of this paper is to demonstrate that understanding the kinetics of coupled PD cycles can help us understand how cell cycle checkpoints operate.

Checkpoints are stages in the cell cycle where the cycle is arrested or slowed down when at least one of the prerequisites for the next event is not satisfied (Elledge, 1996). The usual criteria for checkpoint control include the demonstration of the dependence between two cell cycle events (i.e. the initiation of one event depends upon the satisfactory completion of a preceding event) and that this dependence can be relieved by mutation (Hartwell and Weinert, 1989). For example, mitosis is prevented if the DNA is not properly duplicated during S phase or if the DNA is damaged; the checkpoint function in this case is to delay the cell cycle (until the DNA is repaired) or arrest the cycle altogether. When there is DNA damage, there is a sensing mechanism (called a checkpoint pathway) that detects this damage and transduces the information to the cell cycle machinery. The G1 DNA damage checkpoint pathway has been studied extensively (Carr, 1996) and, recently, more details about the G2 DNA damage checkpoint (G2DDC) pathway are being elucidated (Nurse, 1997; Weinert, 1997). A second objective of this paper is to study how the G2DDC pathway impinges on the cell cycle and to propose a resolution of the issue on the identity of the cell cycle protein targeted by the G2DDC pathway (Osmani and Ye, 1997). We base our result on a mathematical analysis presented below on the stability of the steady states of coupled PD cycles. It will be shown that if the G2DDC pathway perturbs the cell cycle where there is an inherent instability, then a checkpoint response can be elicited, i.e. arrest or delay the cell cycle. We will provide more details of the known mechanism of the G2DDC pathway and the G2-M cell cycle transition below.

Since the primary thesis of this paper is that the control of cell cycle checkpoints lies in the kinetics of PD cycles and their coupling, a thorough analysis of the properties of PD cycles is necessary. The kinetics of cyclic enzyme reactions, such as reversible covalent modifications involving phosphorylation-dephosphorylation, acetylation-deacetylation, methylation-demethylation, etc., have been studied in detail previously (Shacter *et al.*, 1986). It was shown, for example, that there is an amplification of signals through cascades of these cyclic reactions and, furthermore, if the enzymes have low values of Michaelis constants then the cyclic reactions exhibit ‘zeroth-order ultrasensitivity’ that allows them to function as switches (Goldbeter and Koshland, 1981). However, this switching property of a single PD cycle is sensitive to the form of the kinetics (i.e. whether of the first-order type, the Michaelis–Menten type, etc.) and the rate parameters such as rate

constants. I propose in this paper that the function of a cell cycle checkpoint is most probably not carried out by a single PD cycle but, instead, by a set of PD cycles coupled to each other in a way that an inherent instability exists which enables the system to function as a switch; furthermore, the coupled system possesses the integrative ability to check whether all prerequisites are satisfied to go on to the next cell cycle event. In the next section, we will show examples of unstable couplings of PD cycles.

How instabilities arise in PD cycles

Although we are particularly interested in PD cycles, the analysis in this section applies to any set of cyclic pathways. We start with a stability analysis of the trivial case of a cyclic reaction between two species X and Y. The forward direction $X \xrightarrow{f} Y$ is, in general, not an elementary reaction and the overall rate v_f is a function of the concentration of X and possibly of Y if some feedback loops are involved. Similarly, the reverse direction $Y \xrightarrow{r} X$ could also have a rate v_r that is a function of both Y and X concentrations. We assume that the cyclic pathway operates away from equilibrium and that the reverse direction does not retrace (in the opposite direction) the same steps undergone by the forward direction. Since the reaction is cyclic, the sum of the concentrations of X and Y, symbolized by x and y respectively, is a constant and given the symbol E_{tot} . The rate of change of the concentration of Y is then

$$\frac{dy}{dt} = v_f(x, y) - v_r(x, y) = f(y) \quad (1)$$

where $x = E_{tot} - y$. If y_s is a steady state of Y, i.e. $f(y_s) = 0$, then the eigenvalue λ for the linearization of equation (1) is

$$\lambda = \frac{df}{dy} = \left(\frac{\partial v_f}{\partial y} + \frac{\partial v_r}{\partial x}\right) - \left(\frac{\partial v_f}{\partial x} + \frac{\partial v_r}{\partial y}\right) \quad (2)$$

where the derivatives are evaluated at $y = y_s$. The steady state is unstable if $\lambda > 0$, i.e.

$$\frac{\partial v_f}{\partial y} + \frac{\partial v_r}{\partial x} > \frac{\partial v_f}{\partial x} + \frac{\partial v_r}{\partial y} \quad (3)$$

The inequality (3) above can be satisfied when at least one of the species is autocatalytic as in the case of the following rate expressions: $v_f = k_f xy$ and $v_r = k_r y$, where k_f and k_r are rate constants. With these rate functions, the steady state y_s is zero if $E_{tot} < k_r/k_f$; if $E_{tot} > k_r/k_f$ then two steady states are possible: $y_s = 0$ and $y_s = E_{tot} - k_r/k_f$. The steady states versus E_{tot} and their corresponding stabilities are shown in Figure 1. This diagram depicts an interesting result: although the pathway is cyclic and species Y is autocatalytic, Y vanishes at steady state when E_{tot} is less than k_r/k_f ; in other words, even if there is a nonzero initial concentration of Y, this concentration will eventually drop to zero when the total initial concentration ($x_0 + y_0$) is less than k_r/k_f .

The parameter value $E_{tot} = k_r/k_f$ is referred to as a *transcritical bifurcation point* (Strogatz, 1994). This is the point where the zero steady state loses stability and where the positive stable steady state is born (Figure 1). Note that the existence of a transcritical bifurcation point is not restricted to the particular rate functions

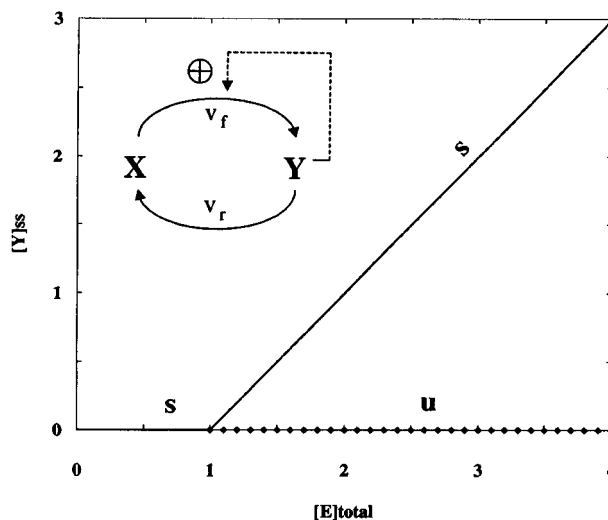


Figure 1 Steady state concentration $[Y]_s$ as a function of E_{total} , the sum of $[X]$ and $[Y]$, for the cyclic reaction between species X and Y (shown in inset). Species Y is autocatalytic. The expressions for the reaction rates v_f and v_r are given in the text. Branches of stable steady states (solid lines) are labeled **s** and unstable steady states are labeled **u** (dotted line). A transcritical bifurcation occurs at $E_{tot} = 1$. Parameters: $k_f = k_r = 1$

that we have considered so far; a similar scenario occurs when the kinetics are assumed to be of the Michaelis–Menten type with Y catalyzing the forward reaction, i.e. $v_f = k_f y x / (K_{Mf} + x)$ and $v_r = k_r y / (K_{Mr} + y)$ where K_{Mf} and K_{Mr} are Michaelis constants; in this case, the transcritical bifurcation point occurs earlier at $E_{tot} = (k_r/k_f) - K_{Mr}$.

Next, we consider a linear cascade of two PD cycles as shown in Figure 2. There are now two conservation conditions: $x_1 + y_1 = E_1$ and $x_2 + y_2 = E_2$ where E_1 and E_2 are constants. The kinetic equations for the two chosen independent species, y_1 and y_2 , are

$$\frac{dy_1}{dt} = v_{1f} - v_{1r}, \quad \frac{dy_2}{dt} = v_{2f} - v_{2r} \quad (4)$$

where $v_{1f} = v_{1f}(x_1)$, $v_{1r} = v_{1r}(y_1)$, $v_{2f} = v_{2f}(x_2, y_1)$, and $v_{2r} = v_{2r}(y_2)$. The linearized matrix in this case is $\mathbf{M} = [m_{ij}]$ where $m_{ij} = \partial y_i / \partial y_j$, $\dot{y}_i = dy_i / dt$ ($i, j = 1, 2$). The eigenvalues of \mathbf{M} are

$$\lambda_1 = -\left(\frac{\partial v_{1f}}{\partial x_1} + \frac{\partial v_{1r}}{\partial y_1}\right) \quad \text{and} \quad \lambda_2 = -\left(\frac{\partial v_{2f}}{\partial x_2} + \frac{\partial v_{2r}}{\partial y_2}\right).$$

Thus, as expected, the steady state is stable (i.e. the eigenvalues are negative) since

$$\left(\frac{\partial v_{1f}}{\partial x_1} + \frac{\partial v_{1r}}{\partial y_1}\right) > 0 \quad \text{and} \quad \left(\frac{\partial v_{2f}}{\partial x_2} + \frac{\partial v_{2r}}{\partial y_2}\right) > 0$$

when the rate functions satisfy the requirement that an increase in reactant concentration increases the reaction's rate.

One possible way that system (4) becomes unstable is when

$$\frac{\partial(v_{1f} - v_{1r})}{\partial y_2} = \frac{\partial v_{1f}}{\partial y_2} - \frac{\partial v_{1r}}{\partial y_2} \neq 0 \quad (5)$$

which means that there must be a feedback from the second cycle to the first cycle (see Figure 2). The possible destabilizing feedback loops are all shown in Figure 3. Cases 1a and 1b in this figure correspond to

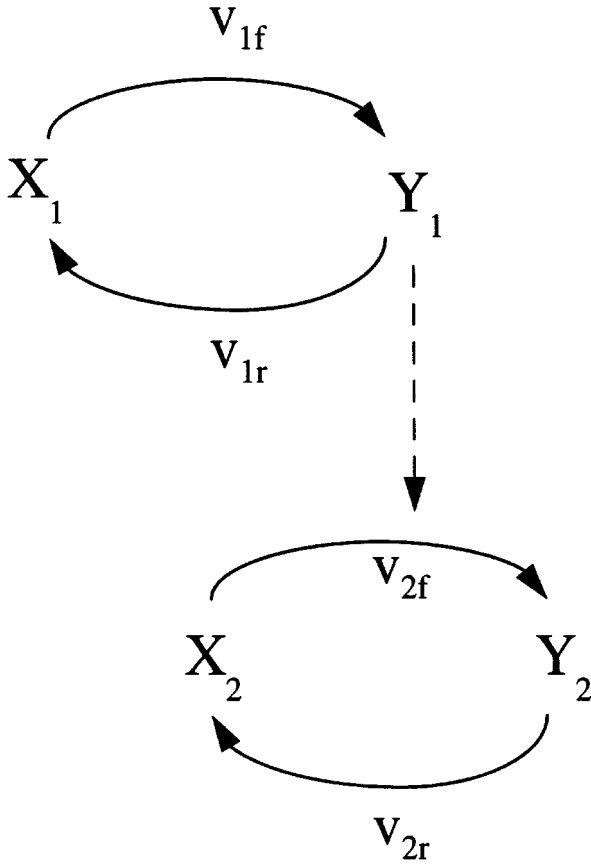


Figure 2 A linear cascade of two PD cycles. The rate of reaction $2f$ is a function of $[Y_1]$ because species Y_1 affects that reaction without being consumed or produced (as indicated by the dash line)

$(\partial v_{1f}/\partial y_2) \neq 0$ and $(\partial v_{1r}/\partial y_2) = 0$, while cases 2a and 2b correspond to $(\partial v_{1r}/\partial y_2) \neq 0$ and $(\partial v_{1f}/\partial y_2) = 0$.

We provide here a specific example of case 1a in Figure 3 to gain a better understanding of the instability that is intrinsic to this coupling. This example will have a direct application to the cell cycle checkpoint discussed in the next section. Let the steps in the mechanism have the following rate expressions:

$$v_{1f} = k_{1f}x_1y_2, \quad v_{1r} = k_{1r}y_1, \quad v_{2f} = k_{2f}x_2y_1, \quad \text{and} \quad v_{2r} = k_{2r}y_2.$$

The steady states of Y_1 and Y_2 can be solved explicitly in terms of the parameters:

$$y_1^s = \frac{k_{1f}k_{2f}E_1E_2 - k_{1r}k_{2r}}{k_{1f}k_{2f}E_2 + k_{1r}k_{2f}}, \quad y_2^s = \frac{k_{1f}k_{2f}E_1E_2 - k_{1r}k_{2r}}{k_{1f}k_{2f}E_1 + k_{1f}k_{2r}}. \quad (6)$$

Thus, non-vanishing values of y_1^s and y_2^s can exist only if

$$E_1E_2 > \frac{k_{1r}k_{2r}}{k_{1f}k_{2f}}. \quad (7)$$

Plots of y_2^s versus E_2 and y_2^s versus E_1 are shown in Figure 4. As in the single-PD cycle case with an autocatalytic species (Figure 1), there is a transcritical bifurcation at

$$E_2 = \frac{k_{1r}k_{2r}}{k_{1f}k_{2f}} \frac{1}{E_1}$$

which is the minimum value of E_2 needed to ‘switch on’

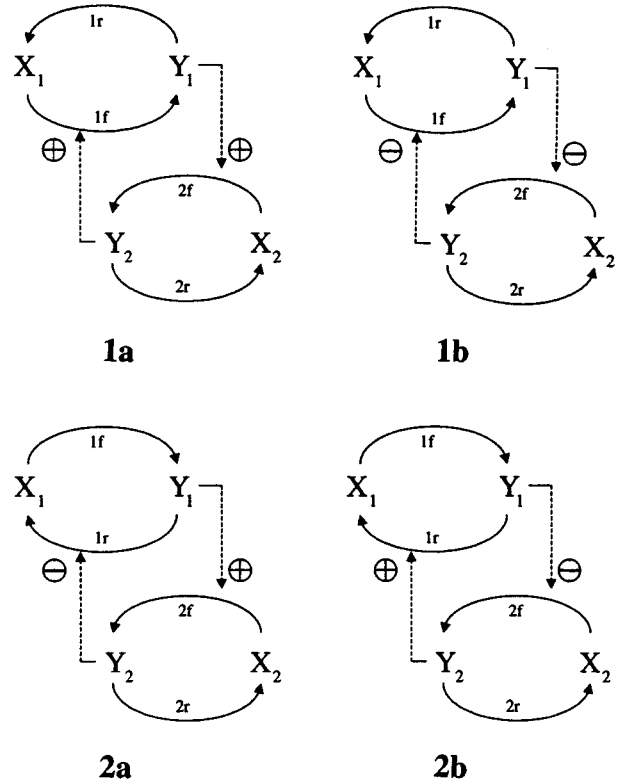


Figure 3 Four cases of unstable coupling between two PD cycles. The (+) symbol indicates that species Y_1 catalyzes the reaction while (-) means inhibition

the species Y_1 and Y_2 for some fixed value of E_1 (see Figure 4a). According to inequality (7), a ‘switch on’ value of E_1 also exists for a fixed value of E_2 (see Figure 4b). This is an interesting result because it says that the steady state value of Y_2 does not merely depend on Y_1 (the species directly catalyzing the production of Y_2) but actually depends on the *sum* of the concentrations of Y_1 and X_1 , as equation (6) above explicitly states. A similar statement applies to the dependence of the steady state of Y_1 on the sum of Y_2 and X_2 concentrations. Figure 5 shows a computer simulation of the time course of the concentration of Y_1 when E_1E_2 is below and above the transcritical bifurcation value. Although curves *a* and *c* in Figure 5 have identical initial conditions, curve *c* goes to zero because E_1E_2 in this case is below the transcritical bifurcation value; on the other hand, although curve *b* starts off with a small concentration of Y_1 , it will eventually rise to a steady state of larger magnitude because the parameters are beyond the transcritical bifurcation point.

The generalization of the above result is straightforward: let there be n PD cycles, composed of the set of species $\{(X_1, Y_1), (X_2, Y_2), \dots, (X_n, Y_n)\}$ with species Y_n catalyzing reaction $1f$; we call this network a ring of PD cycles (see Figure 6). If for the i -th PD cycle we have

$$v_{if} = k_{if}x_iy_{i-1} = k_{if}(E_i - y_i)y_{i-1} \quad \text{and} \quad v_{ir} = k_{ir}y_i,$$

then each and every species Y_i in the ring will vanish unless

$$\prod_{i=1}^n E_i > \prod_{i=1}^n \frac{k_{ir}}{k_{if}}. \quad (8)$$

If any member (X_i, Y_i) of the ring has an E_i that leads to the violation of inequality (8), then all species Y_i in the ring will eventually disappear. On the other hand, as long as all E_i 's are positive, any member (X_i, Y_i) of the ring has

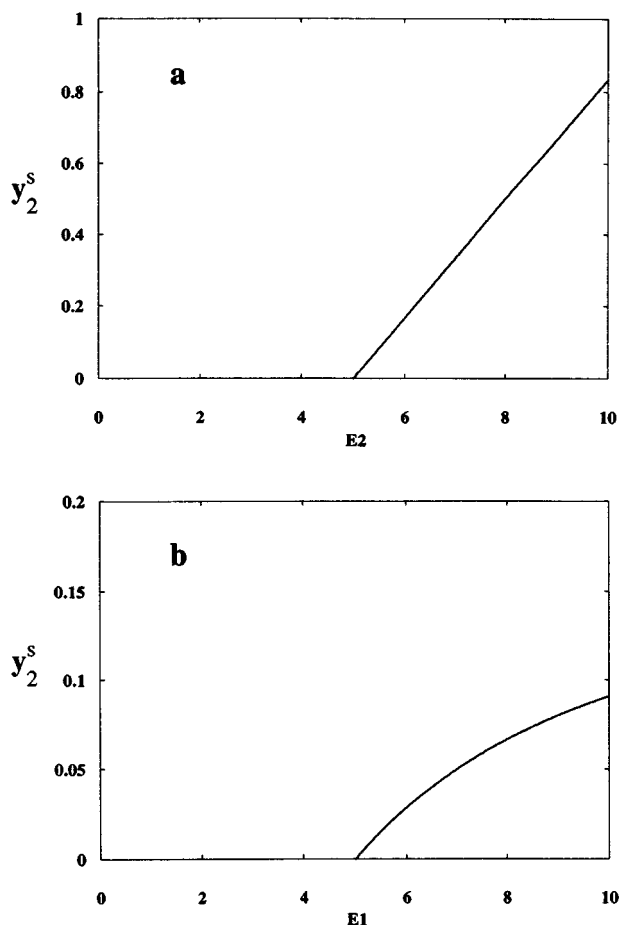


Figure 4 The steady state y_2^s versus total enzyme concentrations for case 1a in Figure 3 (see also equation 6 in the text). (a) E_1 is fixed at 0.2. (b) E_2 is fixed at 0.2. Other parameter values: $k_{1f}=k_{1r}=k_{2f}=k_{2r}=1$. The steady state y_2^s is zero below the transcritical bifurcation value of $E_2=5$ in (a) and $E_1=5$ in (b)

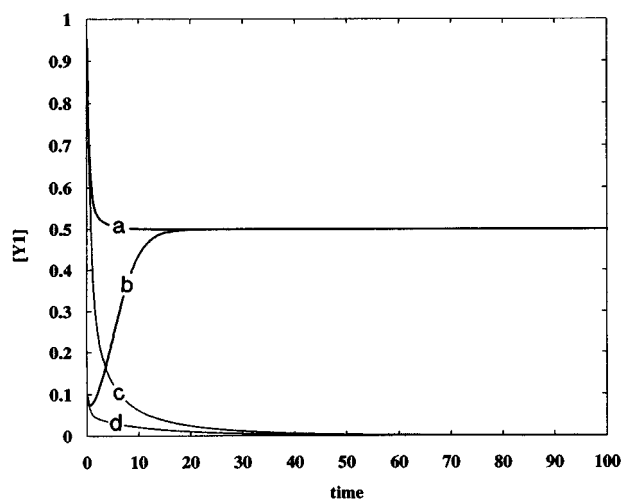


Figure 5 Evolution of the concentration of species Y_1 for case 1a in Figure 3. E_2 is fixed at 1.0. For curves a and b, $E_1=2.0$. For curves c and d, $E_1=0.9$. The transcritical bifurcation point is $E_1=1$. Initial conditions: $Y_1(0)=1$ (curves a and c); $Y_1(0)=0.1$ (curves b and d)

the ability to increase the product $(E_1 E_2 \dots E_n)$ through an increase in that member's E_i which then enables the entire system to cross the transcritical bifurcation point where all members simultaneously 'switch on' their corresponding Y species.

A further generalization of the above result applies to a system of coupled cycles where some of the component cycles have more than two species involved per cycle. One can readily show that a transcritical bifurcation point exists as long as one member in a cycle catalyzes a reaction in another cycle which, in turn, has a member that catalyzes a reaction in the first cycle.

An extension of the stability analysis performed in this section would be to consider how the steady states of cascades of PD cycles become unstable and give rise to oscillatory behaviour. In fact, a linear cascade of PD cycles is prone to oscillatory behaviour when the right feedback loops are included; an example of this is Goldbeter's minimal cascade model for the mitotic oscillator (Goldbeter, 1991). Since we focus on cell cycle checkpoints in this paper, oscillatory behaviour is not considered further.

Coupling of the PD cycles involving Cdc25, Wee1 and MPF

We now apply the results of the previous section to the analysis of a cell cycle checkpoint that operates in the G2 phase of the cell cycle. There is now a consensus that the G2-M transition is induced primarily by the activation of a protein kinase called MPF, the acronym for maturation promoting factor (for excellent reviews, see: Murray and Hunt, 1993; Novak and Tyson, 1993; Basi and Draetta, 1995). MPF is a heterodimer of Cdc2 and cyclin B. Activation of MPF requires the phosphorylation of the kinase subunit, Cdc2, at a threonine residue (Thr161); however, phosphorylation of Cdc2 at a tyrosine residue (Y15) or a threonine residue (Thr14) will render MPF inactive. The inhibitory phosphorylations at the Y15/Thr14 residues are carried out by Wee1/Myt1 kinases; these same residues are dephosphorylated by members of the Cdc25 family of dual-specificity phosphatases. The

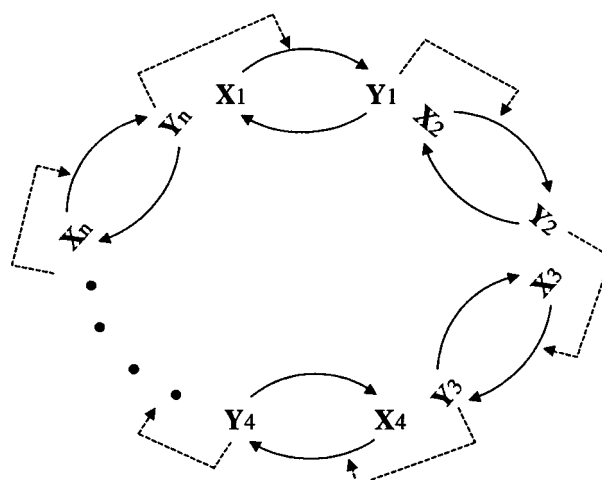


Figure 6 A ring of PD cycles

activities of Wee1 and Cdc25 are likewise regulated by phosphorylations, and it is important to note that activation of Cdc25 by phosphorylation is itself induced by active MPF (Hoffmann *et al.*, 1993; Poon *et al.*, 1997); furthermore, deactivation of Wee1 by phosphorylation is thought to be induced by active MPF (see references in: Murray and Hunt, 1993; Novak and Tyson, 1993). A schematic diagram of this network of coupled PD cycles is shown in Figure 7.

When DNA is damaged, the cell cycle can be arrested at the G1 phase or the G2 phase of the cell cycle. Recently, details of the G2 DNA damage checkpoint (G2DDC) pathway that impinges on the cell cycle have been elucidated (Nurse, 1997; Weinert, 1997). It is now being suggested that the G2DDC pathway perturbs the cell cycle via Cdc25 (Furnari *et al.*, 1996; Rhind *et al.*, 1997). There are, however, reports that also link the damage pathway with Wee1 (O'Connell *et al.*, 1997), and there is still some uncertainty as to which of Cdc25 or Wee1 is the target of the damage checkpoint pathway (Osmani and Ye, 1997). We now give a reason why, indeed, the essential target of the damage checkpoint pathway must be Cdc25; we suggest below that targeting Wee1 by itself, without the involvement of Cdc25, will not elicit a checkpoint response.

The coupling between the PD cycles involving Cdc25 and MPF corresponds to case 1a in Figure 3. As we have seen in the preceding section, this coupling of PD cycles gives rise to a transcritical bifurcation which requires that the product of total Cdc25 and total MPF must be larger than a threshold value before active MPF and active Cdc25 acquire non-zero steady states.

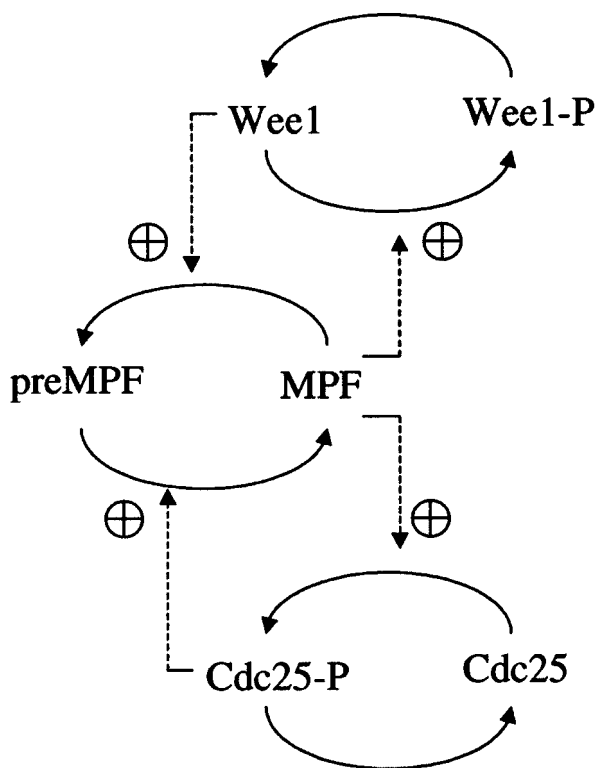


Figure 7 PD cycles involved in the regulation of the mitosis promoting factor (MPF) in the cell cycle. PreMPF is the tyrosine-phosphorylated inactive form of MPF. Wee1 is a kinase and Cdc25 is a phosphatase. See text for more details

If the DNA damage checkpoint pathway has the effect of decreasing the total Cdc25 level, then our analysis leads us to conclude that ultimately the activity of MPF will decrease and could vanish if Cdc25 is diminished to a level that leads to the condition below the transcritical bifurcation point. A demonstration of the feasibility of this idea is given in Figure 8 where we show that a transient increase in a species X that decreases Cdc25 can switch off the activity of MPF; we propose that X could represent 14-3-3 proteins that are known to bind with Cdc25 (Nurse, 1997; Weinert, 1997).

The case of the coupling of the Wee1 and MPF PD cycles (see Figure 7) does not correspond to any of the unstable couplings shown in Figure 3. This implies that if Wee1 is a target of the DNA damage checkpoint pathway then one would not expect a switch-like phenomenon due to transcritical bifurcation; instead, an increase in Wee1 kinase activity leads to a monotonic decrease in the activity of MPF without shutting it off. We suggest that this is the primary reason why Cdc25 must be the crucial target of the DNA damage checkpoint pathway and it now seems that this statement is supported by recent experiments (Nurse, 1997; Weinert, 1997).

Concluding remarks

The most interesting result of this paper is the demonstration that a transcritical bifurcation occurs when two PD cycles are coupled in a way that the product of one cycle catalyzes the formation of the product of the other cycle and vice versa, as in case 1a of Figure 3. The steady state profiles in Figure 4 show that there is a 'waiting' phase before both product species, Y_1 and Y_2 , are *simultaneously* 'switched on' at the transcritical bifurcation point. This bifurcation point is determined by the product of the total enzyme concentrations, E_1E_2 , according to equation (7).

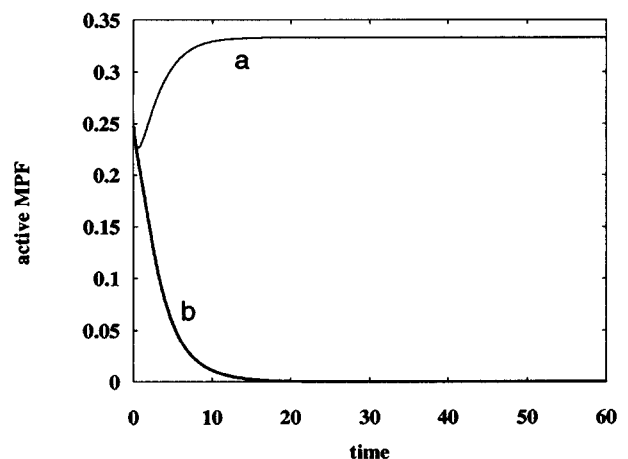


Figure 8 Computer simulations showing how the activity of MPF is switched off when the reaction $\{Cdc25 + X \xrightarrow{k_x} Cdc25-X\}$ that decreases the total Cdc25 is included. The rate expressions are the same as those used in Figure 5 with all rate constants equal to 1.0 (except $k_x=2.0$) and total MPF is 1.0. Initial conditions for curve a: $[Cdc25-P]_0=0.2$, $[Cdc25]_0=1.8$, $[active MPF]_0=0.2$, $[X]_0=0$. Initial conditions for curve b: same as curve a except $[X]_0=1.5$

The above result was used in the analysis of the coupling between the PD cycles involving Cdc25 and MPF (Figure 7). We can now conclude that MPF gets activated only after the total level of MPF (preMPF + active MPF) is beyond a certain threshold value which is inversely proportional to the total Cdc25; in other words, the larger the value of total Cdc25, the less the amount of total MPF needed to begin switching to positive values of active MPF. Thus, if the DNA damage pathway targets the cell cycle through Cdc25 then one would expect a checkpoint response (as we have demonstrated in Figure 8). On the other hand, the coupling between the PD cycles involving Wee1 and MPF does not possess an inherent switching kinetics; thus, if Wee1 is the only target of the DNA damage pathway then a threshold behaviour expected of a checkpoint response would not occur.

One of the aims of this paper is to contribute towards understanding the nature of cell cycle checkpoints. We now propose that a candidate checkpoint in the cell cycle is a set of PD cycles that are coupled in such a way that a transcritical bifurcation point exists. For the particular coupling considered in this paper, i.e. case 1a of Figure 3, two important checkpoint criteria are met, namely, the ability to arrest or delay the cell cycle and the ability to check whether all prerequisites for the next cell cycle event are satisfied. A signal transduction pathway that impinges on any member PD cycle of a checkpoint (according to our proposed definition above) could delay cell cycle progression by decreasing the total concentration of that particular member PD cycle below the transcritical bifurcation point (as in inequality (7)). Our proposed definition of a checkpoint as a set of coupled PD cycles has the integrative ability to 'check' whether all components are ready for the next cell cycle event; these components in our view would be the member PD cycles and their associated regulatory networks.

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It is almost certain that there are many other ways that member PD cycles of a checkpoint could be coupled so that an inherent instability exists that would satisfy the criteria for a cell cycle checkpoint. Mathematical analysis of other theoretical possibilities is needed. At the very least, the present work suggests features of the cell cycle mechanism that we should study in order to understand and perhaps manipulate cell cycle checkpoints.

Lastly, we should mention that other investigators (Tyson *et al.*, 1995; Thron, 1997) have proposed a different basis for checkpoint behaviour, namely, bistability which is defined as the coexistence of two stable steady states under the same set of parameters; the checkpoint in this case operates by switching the system from one stable steady state to another stable steady state. Note that the coupled PD cycles involving MPF, Cdc25 and Wee1 can be shown to generate bistability when other reactions are included (Novak and Tyson, 1993). As defined in this paper, a checkpoint mechanism is not necessarily the same as the mechanism intrinsic to the transition from one cell cycle event to the next; bistability, and the associated phenomenon of hysteresis, is an attractive kinetic model for cell cycle transitions because it 'ensures that commitment to the next phase of the cell cycle is complete and irrevocable' (Thron, 1997). However, we have limited the investigation presented in this paper to points in the cell cycle engine where extrinsic checkpoint pathways could operate. Bistability may well be the intrinsic mechanism for cell cycle transitions but this remains to be shown experimentally.

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