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The kinetic origins of the restriction point in the mammalian cell cycle

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Abstract. A detailed model mechanism for the G1/S transition in the mammalian cell cycle is presented and analysed by computer simulation to investigate whether the kinetic origins of the restriction point (R-point) can be identified. The R-point occurs in mid-to-late G1 phase and marks the transition between mitogen-dependent to mitogen-independent progression of the cell cycle. For purposes of computer simulations, the R-point is defined as the first point in time after mitosis where cutting off mitogen stimulation does not prevent the cell reaching the threshold activity of cyclin-E/cdk2 required for entry into S phase. The key components of the network that generate a dynamic switching behaviour associated with the R-point include a positive feedback loop between cyclin-E/cdk2 and Cdc25A, along with the mutually negative interaction between the cdk inhibitor $p27^{Kip1}$ and cyclin-E/cdk2. Simulations of the passage through the R-point were carried out and the factors affecting the position of the R-point in G1 are determined. The detailed model also shows various points in the network where the activation of cyclin-E/cdk2 can be initiated with or without the involvement of the retinoblastoma protein.

INTRODUCTION

Normal cells require sustained stimulation from growth factors up to a certain point in the cell division cycle when the commitment for another round of DNA replication is made. This point in time occurs in mid-to-late G1 of the mammalian cell cycle and is called the restriction point (R-point), a name proposed by Pardee (1974) who conducted experiments demonstrating the R-point as a unique switching point between quiescent and proliferative states of normal animal cells. A precise determination of the location of the R-point was also carried out by Zetterberg & Larsson (1985) using time-lapse video analysis to observe individual mouse embryonic fibroblasts and showed that the R-point occurs within a remarkably constant time period (within 3–4 h) after mitosis. The R-point has also been referred to as the 'master break' of the cell cycle or a G1 checkpoint that must be crossed before the intrinsic cell division machinery becomes mitogen-independent.

The suggestion of Pardee (1974, 1989) that the R-point plays a key role in preventing malignant transformation has gained substantial support from detailed genetic and molecular

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studies reported in recent years. It has been claimed that almost all cancers are associated with one or more mutations of genes implicated in R-point control (Moroy 1998); examples include the following: inactivation of the retinoblastoma (RB) tumour suppressor gene in a variety of human tumours; loss of the tumour suppressor $p16^{INK4a}$ in certain human cancers including those of the pancreas; amplification of the cyclin D1, cyclin E or cdk4 genes as seen in breast, head or neck cancers; loss of the cyclin-dependent kinase (cdk) inhibitor $p27^{Kip1}$ has been implicated in a wide range of human cancers including those of the breast, prostate, colon, lung, and oesophagus (Tsihlias, Kapusta & Slingerland 1999).

A detailed picture of the molecular mechanisms orchestrating the G1/S transition in the mammalian cell cycle has emerged in the last few years and the literature has been reviewed by several authors (Zetterberg, Larsson & Wiman 1995, Bartek, Bartkova & Lukas 1996, Bartek, Bartkova & Lukas 1997,Kohn 1998, Moroy 1998). Kohn (1998) recognized the necessity of a network analysis and computer simulation of this highly non-linear and complex kinetic system. However, he did not discuss the kinetic origins of the R-point and ignored components of the network such as the cdk inhibitor (cki), $p27^{Kip1}$ and the phosphatase Cdc25A whose crucial roles in R-point control will be examined in detail in this paper. Here, a detailed model mechanism of the G1/S transition is presented (Figure 1 and Figure 2) and what and how components of the network co-operate to generate a sharp switch in the activity of a cdk that triggers S phase entry will be discussed. It is suggested that this trigger is cyclin-E/cdk2 (Lukas *et al.* 1997). Therefore, for purposes of computer simulations, an operational definition of the R-point is proposed as the first point in time

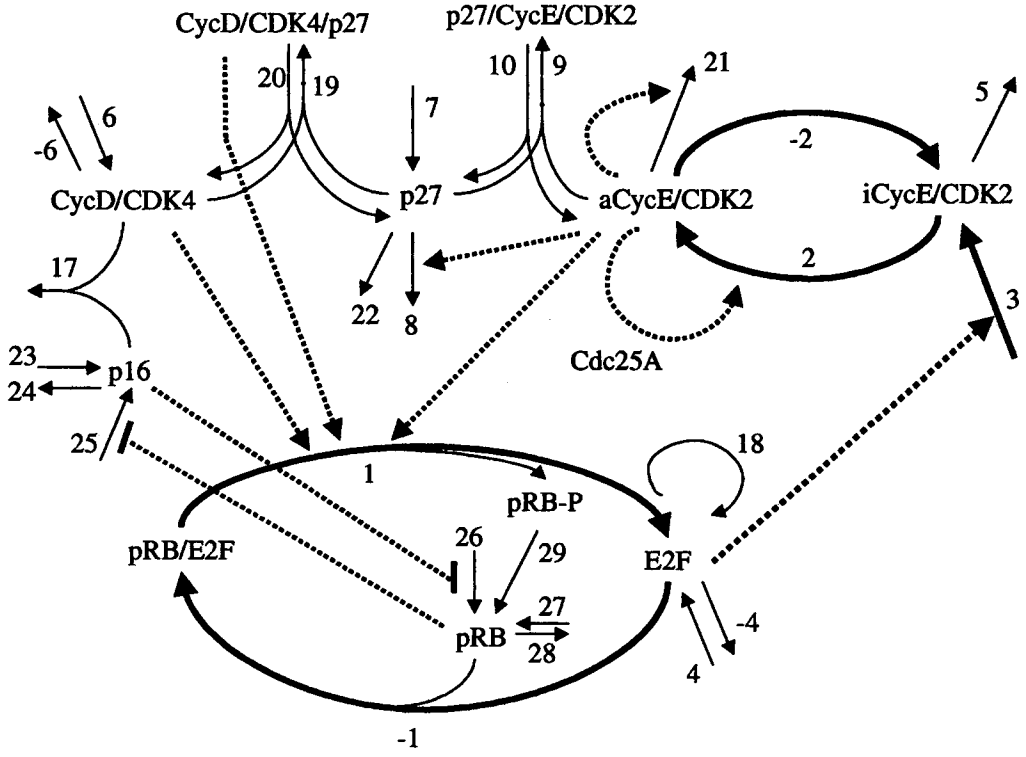

Figure 1. A detailed mechanistic model of the G1/S transition in the mammalian cell cycle.

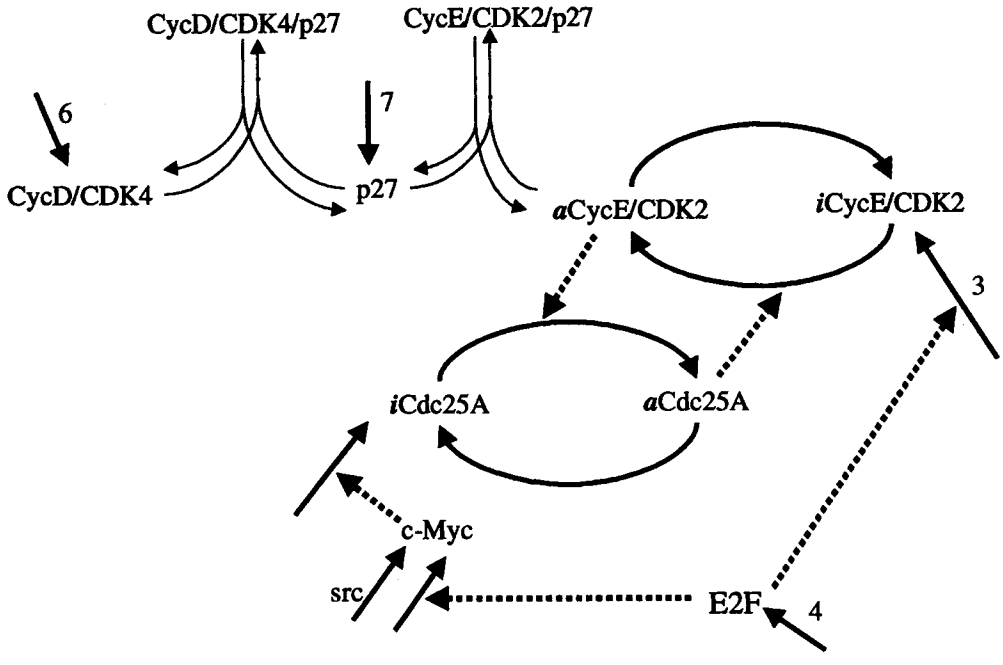

Figure 2. pRB-independent pathways leading to activation of CycE/cdk2. Details of the coupling between the PD cycles of Cdc25A and CycE/cdk2 are shown.

after mitosis where cutting off growth factor stimulation does not prevent the cell to achieve the threshold of cyclin-E/cdk2 activity required for entry into S phase.

In the present analysis of the G1/S network in Figure 1 and Figure 2, it is proposed that there exists a core mechanism involving a minimal set of components that exhibits a switching behaviour. With various justifications that will be discussed in this paper, it is suggested that the core mechanism involves the coupled phosphorylation–dephosphorylation (PD) cycles between cyclin-E/cdk2 and Cdc25A. This proposal is mainly based on the intrinsic switching behaviour of the positive feedback loop between the PD cycles of cylin-E/cdk2 and Cdc25A, and experimental evidence for the presence of R-point controls that are independent of the retinoblastoma protein (pRB) pathway (Herrera *et al.* 1996, Takuwa & Takuwa 1997). In this paper, computer simulations are presented demonstrating that the detailed network in Figure 1 could account for the existence of the R-point; furthermore, the factors affecting the location of the R-point are discussed and it is shown that the sharpness and timing of the switch for cyclin-E/cdk2 activity is largely influenced by the level of $p27^{Kip1}$ in agreement with experiments (Sheaff *et al.* 1997). Computer simulations carried out by others (Obeyesekere *et al.* 1997) did not consider detailed models of the G1/S transition and the crucial participation of Cdc25A and $p27^{Kip1}$.

MODEL NETWORK FOR G1/S CONTROL

A network diagram is shown in Figure 1 summarizing the important processes involved in G1/S transition in the mammalian cell cycle. To obtain an overview of the operation of this network, the individual reactions are discussed as the commonly observed temporal

variations of the major proteins involved during the transition from quiescence to proliferation are followed (Takuwa & Takuwa 1996, Roussel 1998). Quiescent cells possess high levels of $p27^{Kip1}$, which binds reversibly with cyclin-D/cdk4 (or cdk6) and cyclin-E/cdk2 complexes as represented by processes 9, 10, 19, and 20 in Figure 1. To simplify the simulations, it is assumed that p27 binds only with the active cyclin–cdk complex symbolized by aCycE/cdk2 in Figure 1 and not with the inactive iCycE/cdk2; this assumption does not lead to any qualitative change in the results. Process 7 represents the expression of p27 (Hengst & Reed 1996) whose downregulation is given by process 22 which is independent of cdk2 kinase activity and process 8 which is dependent on cdk2 (Sheaff *et al.* 1997, Vlach, Hennecke & Amati 1997). In quiescent cells, the levels of the D-type cyclins and cyclin E, and the kinase activities of cdk4,6 and cdk2 are low. Upon mitogenic stimulation, a complex signal transduction system generates an increase in cyclin D and is depicted in Figure 1 simply as process 6 (Bartek *et al.* 1996 and references therein). This increase in cyclin D contributes to the activation of cdk4 and cdk6 kinases which phosphorylate pRB. In early G1, pRB is in a hypophosphorylated (active) form and is able to bind members of the E2F family of transcription factors (process −1). In mid-to-late G1, pRB becomes hyperphosphorylated. Possible models of pRB phosphorylation, including sequential and co-operative modes of phosphorylation carried out by cdk4,6 and cdk2 kinases, have been reviewed (Lundberg & Weinberg 1998, Mittnacht 1998). The dashed arrows impinging on process 1 represent pRB phosphorylation, with the assumption that the contributions of the different cdks are additive. Note that the trimeric complex cycD/cdk4/p27 is still known to be active, hence the dashed arrow ending at process 1. Hyperphosphorylation of pRB leads to its inactivation and the release of E2F. pRB is dephosphorylated during mitosis (process 29). Process 26 is a schematic representation of the negative regulation by $p16^{INK4a}$ of the expression of pRB (Fang *et al.* 1998). Process 27 represents p16-independent expression of pRB, and process 28 represents the degradation of pRB.

E2F transcription factors have several important target genes that drive cells into S phase and one of these is cyclin-E (Ohtani, Degregori & Nevins 1995, Geng *et al.* 1996). Process 3 represents the E2F-dependent induction of cyclin-E and its subsequent binding with cdk2. Processes 4 and −4 represent expression and degradation of E2F, respectively (see Bernards (1997) for a review on E2F regulation).

An important property of the network in Figure 1 is the presence of various positive feedback loops. Process 18 represents the report that E2F stimulates its own expression (Neuman *et al.* 1994). There is a positive feedback loop composed of E2F, process 3, process 2, and pRB-phosphorylation by aCycE/cdk2. A very important feedback loop involves the phosphatase Cdc25A (symbolized by the dashed curved arrow that catalyses process 2 in Figure 1). Figure 2 gives more details of the assumed positive feedback loop between CycE/cdk2 and Cdc25A (Hoffmann, Draetta & Karsenti 1994, Sheaff 1997). There are recent questions, however, whether Cdc25A acts directly on cdk2 (Sexl *et al.* 1999) and this feedback loop should be considered as a tentative feature of the model. All these aforementioned positive feedback loops contribute, in varying degrees, to the sudden increase in CycE/cdk2 activity which is assumed to trigger entry into S phase. In the next few sections, it will be shown precisely how this CycE/cdk2 trigger is generated. Processes 5 and 21 represent the degradation of cyclin-E protein; it is assumed that aCycE/cdk2 induces its own degradation (curved arrow in process 21; see Reed (1996) and references therein). The regulation of cdk2 and cdc25A activities as shown in Figure 1 is simplified and do not show the participation of CAK (cdk-activating kinase) in process 2 and Wee1/Myt1 kinases in process −2.

Another component in Fig. 1 is the cki $p16^{INK4a}$ which is considered to be a *bona fide* tumour suppressor (Serrano 1997) and specifically binds cdk4 and cdk6. The regulation of p16 activity is not well understood at this time. Some studies have shown an inverse relationship between the levels of pRB and p16 (Li *et al.* 1994, Fang *et al.* 1998). Thus, as is shown in Figure 1, p16 negatively regulates the expression of pRB (process 26), and *vice versa* (process 25). Processes 23 and 24 represent the pRB-independent expression and degradation of p16, respectively.

The assumed expressions for the rates of the individual processes in Figure 1 and the kinetic equations used in the present simulations are given in Table 1.

SWITCHING PROPERTIES OF COUPLED PD CYCLES

To understand the origin of the R-point, one may first look at the nature of the coupling between the phosphorylation–dephosphorylation (PD) cycle involving pRB and the PD cycle involving the activation/inactivation of cyclin-E/cdk2 (Processes 1, −1, 2, and −2 of Figure 1). It is assumed initially that Cdc25A is not involved. The positive feedback loop between these PD cycles (through dashed arrow pointing to process 3 and the dashed arrow pointing to process 1) has an inherent switching property (similar to the switching behaviour exhibited by the coupling between the PD cycles of cyclin-B/cdc2 and Cdc25C in G2/M control (Aguda 1999, Poon *et al.* 1997)). The switching threshold exhibited by these coupled PD cycles could be seen in Figure 3 (curve **a**) where it is shown that the activity of cyclin-E/cdk2 starts increasing only after some time lag of about 12 time units (indicated by the arrow); however, it is clear that this is not a robust switch in the sense that the increase in cyclin-E/cdk2 activity is quite gradual and the switch-on time is quite sensitive to the values of the parameters used. Other important components in the network are involved to create a robust switch.

It is suggested that a crucial component of the R-point switch is the positive feedback loop between the PD cycles of cyclin-E/cdk2 and Cdc25A. This effectively autocatalytic activation of cyclin-E/cdk2 is indicated by the dashed curved arrow impinging on process 2 (Figure 1) and represented in the rate $v_2 = k_2 Y_1 Y_2$ (see Table 1). With this autocatalytic feature added to the two coupled PD cycles (processes 1, −1, 2, −2), a much sharper increase in active cyclin-E/cdk2 is obtained (see curve **b** in Figure 3). Because other reactions such as 9, 10 and 21 are not included in Figure 3b (and Figure 4), the activity of cyclin-E/cdk2 seems to increase without bounds; this will not be the case when the whole reaction network is considered. Figure 2 gives more details of the coupling of the PD cycles between Cdc25A and Cyclin-E/cdk2. Next, it is discussed what factors determine the time when the activity of cyclin-E/cdk2 is switched on.

ROLE OF $P27^{KIP1}$

The levels of p27 and cyclin-E/cdk2 activity have been observed to be inversely correlated (Coats *et al.* 1996). To see how p27 affects the switch-on time for the activity of cyclin-E/cdk2, a simplified network composed of processes 1, −1, −2, 2, 7, 8, 9, 10 in Figure 1 is considered. Figure 4 shows the effect of changing the dissociation constant of the p27/CycE/cdk2 trimer on the switch-on time of active cyclin-E/cdk2. It can be seen that as the dissociation constant decreases (k_{10}/k_9), i.e. the tighter p27 binds to cycE/cdk2, the switch-on time is increased.

Table 1. Reaction rates and kinetic equations of the G1/S network in Figure 1

Molecular Species		
Y_1 = aCyclin-E/CDK2	Y_2 = iCyclin-E/CDK2	Y_3 = pRB/E2F
Y_4 = E2F	Y_5 = pRB	Y_6 = CycD/CDK4
Y_7 = p27	Y_8 = CycE/CDK2/p27	Y_9 = CycD/CDK4/p27
Y_{10} = p16	Y_{11} = pRB-P	
Reaction Rates		
$v_1 = k_1'Y_6Y_3 + k_1''Y_9Y_3 + k_1Y_1Y_3$	$v_2 = k_2Y_1Y_2$	$v_{-2} = k_{-2}Y_1$
$v_3 = k_3Y_4 + k_3'$	$v_4 = k_4$	$v_{-4} = k_{-4}Y_4$
$v_5 = k_5Y_2$	$v_6 = k_6$	$v_{-6} = k_{-6}Y_6$
$v_7 = k_7$	$v_8 = k_8Y_7Y_1$	$v_9 = k_9Y_7Y_1$
$v_{10} = k_{10}Y_8$	$v_{17} = k_{17}Y_{10}Y_6$	$v_{18} = k_{18}Y_4$
$v_{19} = k_{19}Y_6Y_7$	$v_{20} = k_{20}Y_9$	$v_{21} = k_{21}Y_1^2$
$v_{22} = k_{22}Y_7$	$v_{23} = k_{23}$	$v_{24} = k_{24}Y_{10}$
$v_{25} = k_{25}/(1 + k_{25}'Y_5)$	$v_{26} = k_{26}/(1 + k_{26}'Y_{10})$	$v_{27} = k_{27}$
$v_{28} = k_{28}Y_5$	$v_{29} = k_{29}Y_{11}$	
Kinetic Equations		
$dY_1/dt = v_2 + v_{10} - (v_{-2} + v_9 + v_{21})$	$dY_2/dt = v_3 + v_{-2} - (v_2 + v_5)$	
$dY_3/dt = v_{-1} - v_1$	$dY_4/dt = v_1 + v_4 + v_{18} - (v_{-1} + v_{-4})$	
$dY_5/dt = v_{27} + v_{26} + v_{29} - (v_{-1} + v_{28})$	$dY_6/dt = v_6 + v_{20} - (v_{-6} + v_{19} + v_{17})$	
$dY_7/dt = v_7 + v_{10} + v_{20} - (v_8 + v_9 + v_{19} + v_{22})$	$dY_8/dt = v_9 - v_{10}$	
$dY_9/dt = v_{19} - v_{20}$	$dY_{10}/dt = v_{23} + v_{25} - (v_{17} + v_{24})$	
$dY_{11}/dt = v_1 - v_{29}$		

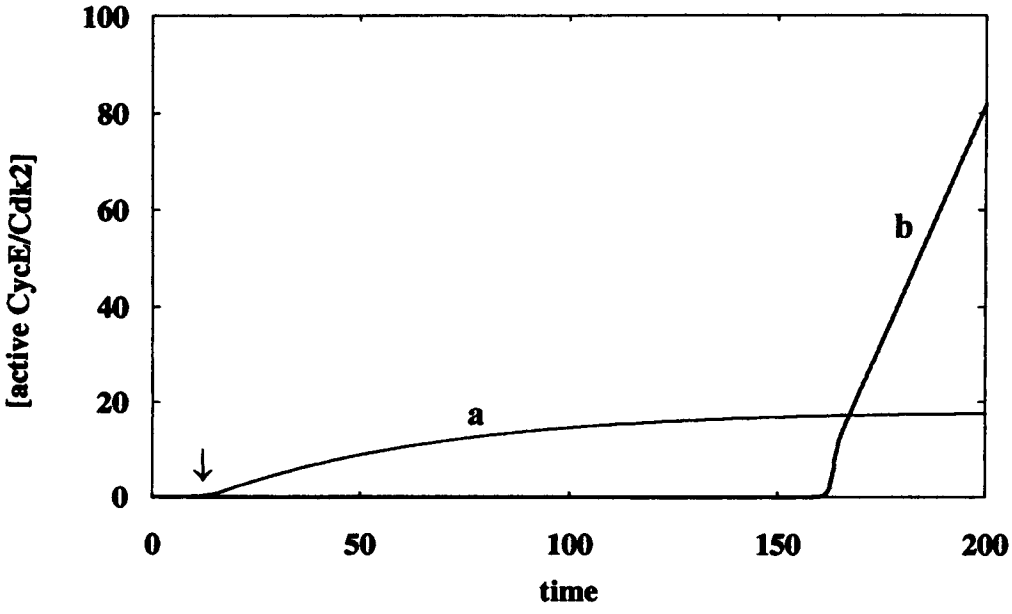

Figure 3. The effect of the autocatalytic nature of active CycE/cdk2. A subset of reactions from Figure 1 with the following numbers used: 1, −1, 2, −2, 3, 4, −4, 5, 6, −6 and 29. Parameter values: $k_1 = k_3 = k_{-1} = k_{-2} = k_{-6} = k_1' = 1$, $k_2 = 0.2$, $k_1'' = k_3' = 0$, $k_4 = k_6 = k_{-4} = k_{29} = 10^{-3}$, $k_5 = 0.02$, [total pRB] = 2. Curve **a**: v_2 in Table 1 is replaced with $v_2 = k_2Y_2$. Curve **b**: same parameters as curve **a** except $v_2 = k_2Y_1Y_2$ as in Table 1. Initial conditions: $Y_1 = Y_4 = Y_6 = 0$, $Y_2 = 0.01$, $Y_3 = 1.95$, $Y_5 = 0.05$.

Generating a pulse of cyclin-E/cdk2 activity prior to S phase entry (Koff *et al.* 1992) requires more components of the network to be included and choosing appropriate parameters especially for cyclin-E and E2F degradation (Campanero & Flemington 1997). The full network in Figure 1 and the kinetic equations in Table 1 are used to carry out a series of simulations to see the effect of different initial levels of p27. The results are shown in Figure 5. For all graphs in Figure 5, the activity of *a*CycE/cdk2 (referred to as E/cdk2 in the figure) is almost zero until p27 levels decrease to almost zero after which the activity of E/cdk2 increases sharply. The simulations also show that the initial p27 level ($[p27]_o$) influences the switch-on time for E/cdk2 activity; the lower the $[p27]_o$, the shorter the time needed to activate E/cdk2. This result could explain the reduced dependency on serum mitogens in culture fibroblasts when p27 is downregulated (Coats *et al.* 1996). Note that the maximum values of E/cdk2 in Figure 5 are: 4.2 (a), 5.4 (b), 10 (c), 3 (d); in fact, by increasing levels of $[p27]_o$ further, it can be demonstrated (simulations not shown) that there exists a threshold value of $[p27]_o$ beyond which no peak of E/cdk2 is generated. From the maximum values of E/cdk2, it can be concluded that there exists a particular value of $[p27]_o$ which gives a maximum response in E/cdk2 activity. Figure 5 also shows that there are two distinct kinds of E/cdk2 peaks: at lower $[p27]_o$ levels (Figure 5a), only a smooth, slowly varying peak (i.e. larger range of times) of E/cdk2 activity is seen; at higher $[p27]_o$ levels (see Figure 5b,c), a distinctly sharp pulse of E/cdk2 activity is generated. The E2F time profiles are also different for low and high values of $[p27]_o$; for Figure 5(a),(b), the peaks of E2F and E/cdk2 activities coincide, whereas for higher $[p27]_o$ (Figure 5c,d), the peaks are separated.

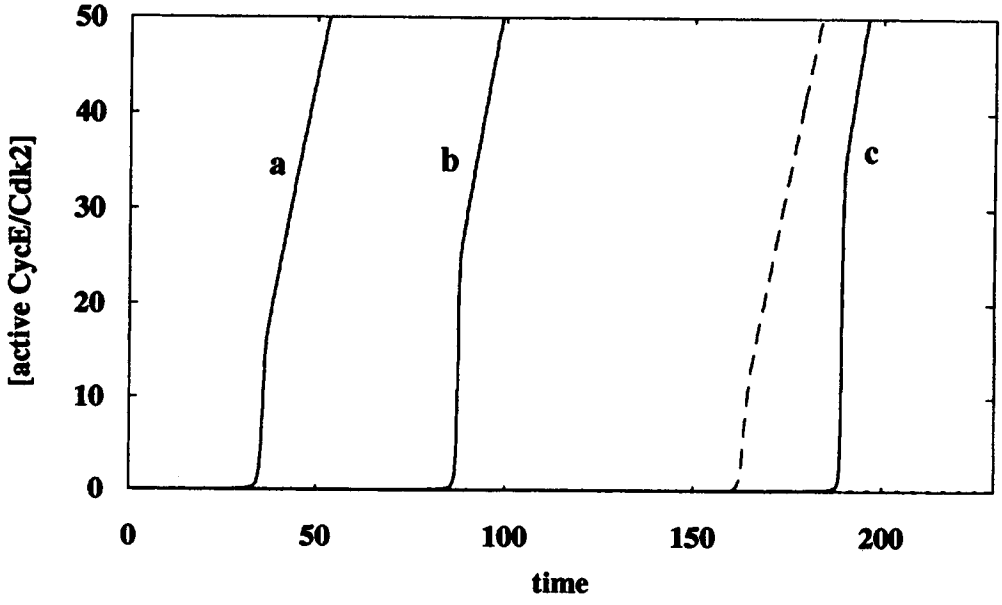

Figure 4. The effect of p27 on the switch-on time for the activity of cycE/cdk2. Reactions 7, 8, 9 and 10, and species Y_7 (p27) and Y_8 (cycE/cdk2/p27) are added to the model used in Figure 3. Values for k_{10}: 0.1 (curve **a**), 0.05 (curve **b**), 0.035 (curve **c**). Parameter values for curves **a, b and c**: $k_7 = 2$, $k_8 = 0.6$, $k_9 = 0.1$, and colleagues are the same as those of curve **b** in Figure 3. Initial conditions: $Y_1 = Y_4 = Y_6 = 0$, $Y_2 = 0.01$, $Y_3 = 1.95$, $Y_5 = 0.05$, $Y_7 = Y_8 = 1$. The dot-dash curve is the same as curve **b** in Figure 3.

SIMULATION OF THE PASSAGE THROUGH THE R-POINT

Using the network in Figure 1 and Table 1, the effect of cutting off mitogenic stimulation is represented by turning off process 6; the results of computer simulations are shown in Figure 6. Curve 1 in Figure 6a is the time course for active E/cdk2 level when there is sustained mitogenic stimulation (process 6 is on). Curves 2, 3, 4 and 6 are the levels of active E/cdk2 when cyclin D is cut off at t_{cut} = 80, 50, 30, and 28 time units, respectively. In Figure 6b, curves 4 and 6 (same as those in Figure 6a) are redrawn along with curve 5 where t_{cut} is 29 time units. It is clear that there exists a cut-off time between t_{cut} = 28 and t_{cut} = 29 where the activity of E/cdk2 is turned off. Should the R-point be then identified as the point in time between 28 and 29 time units? The answer to this question depends on the threshold level of E/cdk2 activity that is required for S phase entry which, unfortunately, is not known. Looking at Figure 6c, it can be seen the time course of E2F levels where curves 4′, 5′, and 6′ correspond to the t_{cut} values for curves 4, 5 and 6 in Figure 6b. Figure 6c shows that the second peak of E2F is in-phase with the peak of E/cdk2. It would be of interest to see if experiments can be designed to test this damped oscillatory activity of E2F during the G1 and S phases of the cell cycle.

PRB-INDEPENDENT R-POINT CONTROL

It is shown here that there could still be a sudden switch in the activity of E/cdk2 without the participation of pRB. These results are in accordance with reports that, unlike cyclin D, cyclin E is rate-limiting for S phase entry in both pRB-negative and pRB-positive cells

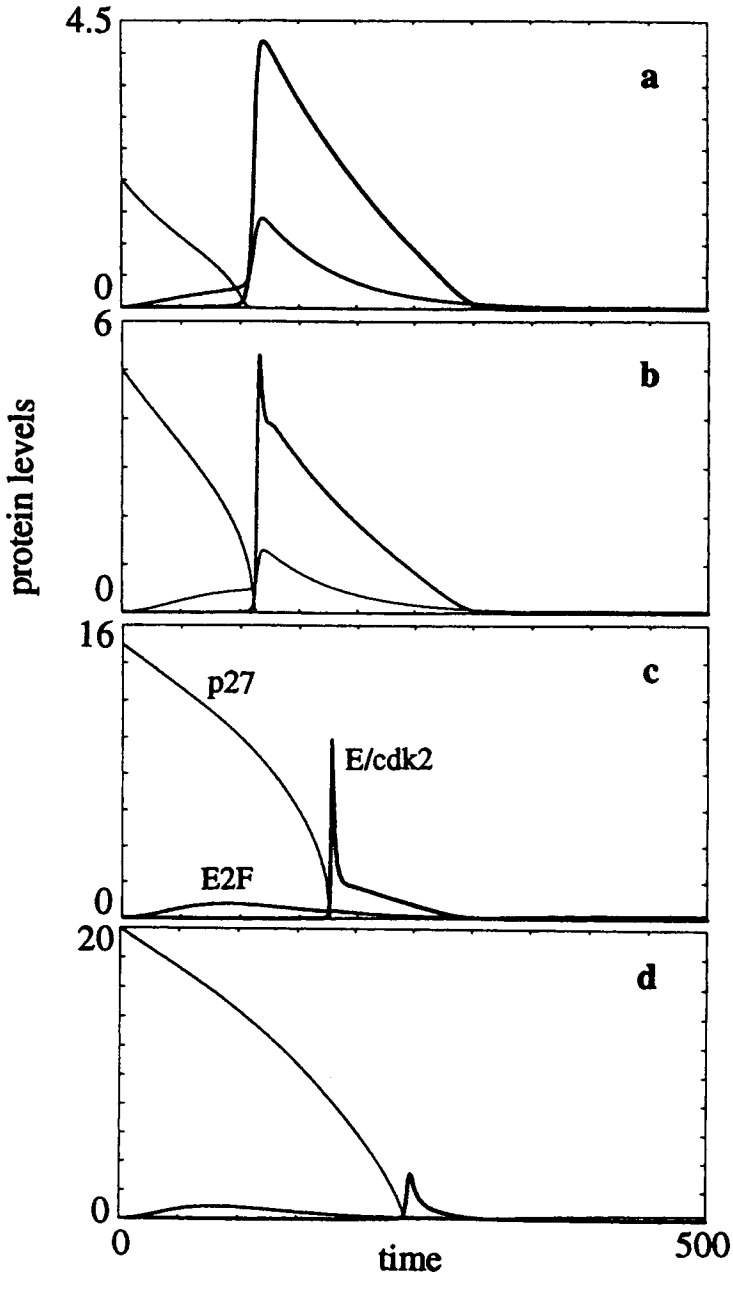

Figure 5. The effect of p27 level on peak activities of E2F and cycE/cdk2. The proteins corresponding to the three curves are shown in (c). Initial levels of p27: (a) 2 (b) 5 (c) 15 (d) 20. Parameters: $k_1 = k_2 = 0.1$, $k_3 = 1.42$, $k_4 = 10^{-6}$, $k_5 = 0.02$, $k_6 = 0.018$, $k_7 = 10^{-5}$, $k_8 = k_9 = 2$, $k_{10} = 0.035$, $k_{-1} = 10^{-3}$, $k_{-2} = 1$, $k_{-4} = 0.016$, $k_{-6} = 5$, $k_{29} = 10^{-3}$, $k_1' = k_1'' = 0.5$, $k_{17} = 3.5$, $k_{18} = 10^{-5}$, $k_{19} = 0.05$, $k_{20} = 0.01$, $k_{21} = k_{24} = k_{26}' = 0.1$, $k_{22} = 10^{-3}$, $k_{23} = 0.2$, $k_{25} = k_{26} = k_{27} = k_{28} = 0.01$, $k_{25}' = 0.02$, $k_3' = 0$. Initial conditions: $Y_1 = Y_4 = Y_6 = Y_9 = 0$, $Y_2 = Y_{11} = 0.01$, $Y_3 = 1.95$, $Y_5 = 0.05$, $Y_8 = 1$, $Y_{10} = 5$.

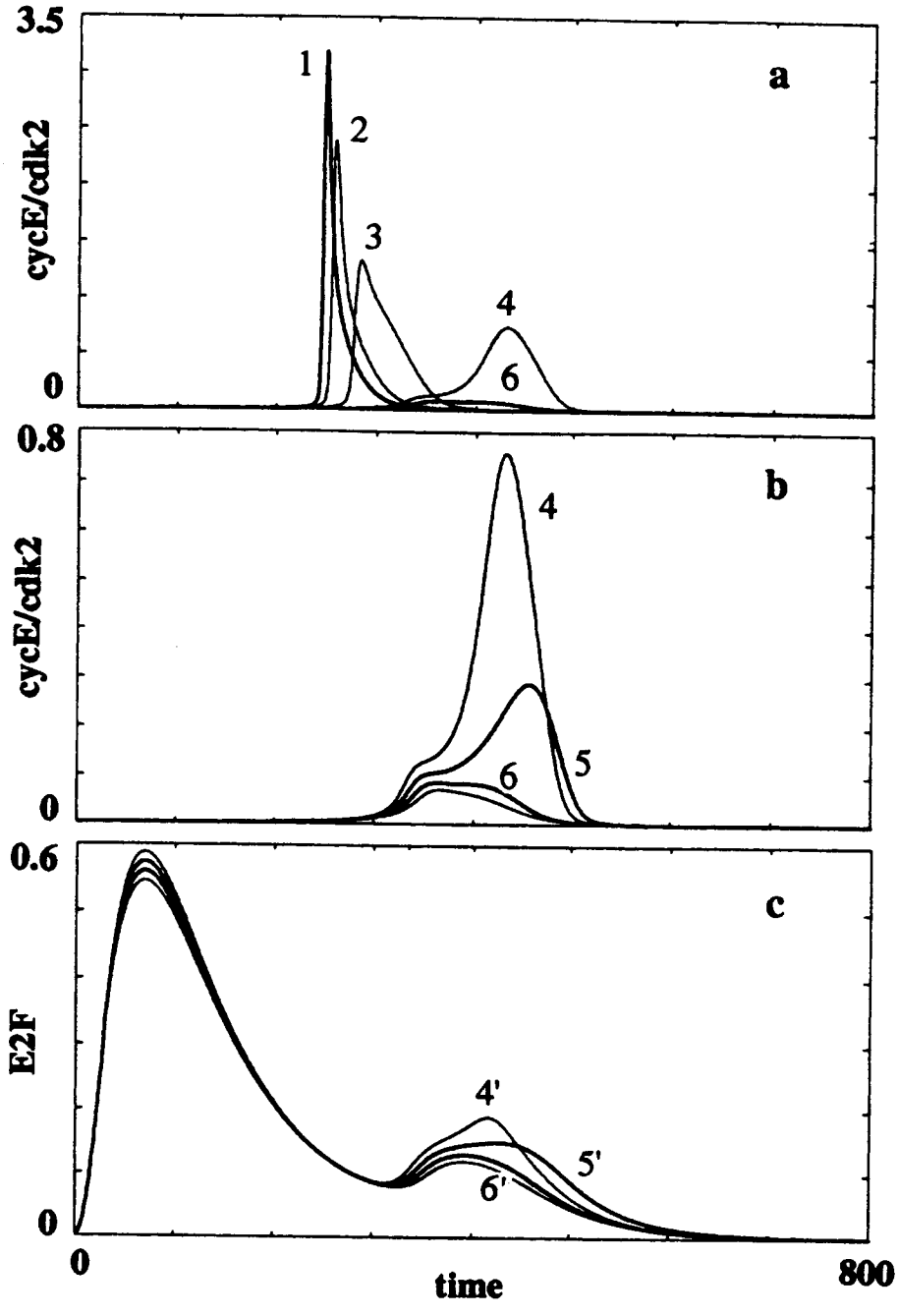

Figure 6. Activity of cycE/cdk2 (Y_1) and E2F (Y_4) when mitogens are cut off (by setting $k_6 = 0$) at various times. Curve 1 corresponds to sustained mitogenic stimulation. (a) Mitogens are cut off at t = 80 (curve 2), t = 50 (curve 3), t = 30 (curve 4), and t = 28 (curve 6). (b) Mitogens are cut off at t = 29 (curve 5); curves 4 and 6 as in (a). (c) E2F levels corresponding to curves 4, 5, 6 in (b). Parameters and initial conditions: identical to those of Figure 5.

(Bartek *et al.* 1996 and references therein). A simulation of the pRB-independent pathway is shown in Figure 7.

The simulations presented in Figure 7 demonstrate two important features of this pRB-independent network: (1) the crucial importance of the positive feedback loop between Cdc25A and E/cdk2 in generating a sharp switch and large spike for the activity of E/cdk2 (curve **a** in Figure 7), and (2) the timing of the spike of E/cdk2 is determined by the initial level of p27. For curves **a** (E/cdk2) and **b** (p27), the positive feedback loop between Cdc25A and E/cdk2 is considered while curves **a**′ and **b**′ do not include this positive feedback loop. Note that there is still a mild switching behaviour in curve **a**′ but no sharp spike in E/cdk2 is possible without the positive feedback loop.

Computer simulations were also performed to see the effect of cutting off mitogens (turning off process 6); the results (not shown) indicate that the switch-on time for E/cdk2 activity is more sensitive to p27 levels than it is to the expression of cyclin D. The downregulation of p27 as a result of mitogenic stimuli is perhaps a more important control mechanism for passage through the R-point in pRB-negative cells (see related experiments reported by Takuwa & Takuwa (1997) showing the effect of Ras activity in downregulation of p27).

The simulations presented above are also in agreement with the experiments reported by Herrera *et al.* (1996) showing a dramatic increase in cyclin-E levels in pRB-deficient mouse embryo fibroblasts. This dramatic increase in cyclin-E levels could be a result of the fact that pRB is no longer present to titrate or sequester the E2Fs which are known to induce the expression of cyclin-E. The present simulations also agree with the report of Almasan *et al.*

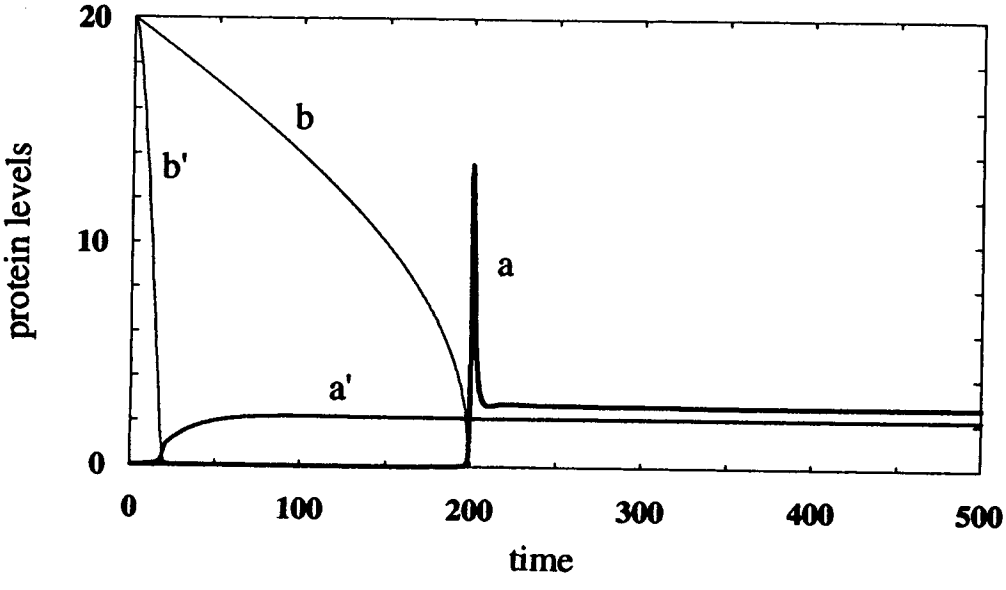

Figure 7. pRB-independent activation of cycE/cdk2 and the importance of the positive feedback loop between Cdc25A and cycE/cdk2. All the reactions involving pRB and E2F in Figure 1 are turned off. Curves **a** (active cycE/cdk2) and **b** (p27) correspond to the rate $v_2 = k_2[a\text{cycE/cdk2}][i\text{cycE/cdk2}]$ while curves **a**′ and **b**′ correspond to the nonautocatalytic rate $v_2 = k_2[i\text{cycE/cdk2}]$. Parameters: $k_6 = 0.018$, $k_{-6} = 5$, $k_{17} = 3.5$, $k_{23} = 0.2$, $k_{24} = 0.1$, $k_{20} = 0.01$, $k_{19} = 0.05$, $k_7 = 10^{-5}$, $k_{22} = 10^{-3}$, $k_8 = k_9 = 2$, $k_{10} = 0.035$, $k_5 = 0.02$, $k_{21} = 0.1$, $k_2 = 0.1$, $k_{-2} = k_3' = 1$. Initial conditions: $Y_1 = Y_6 = Y_9 = 0$, $Y_2 = 0.01$, $Y_7 = 20$, $Y_8 = 1$, $Y_{10} = 5$.

(1995) showing that deficiency in pRB leads to inappropriate S-phase entry and activation of E2F-responsive genes, and those of Lukas *et al.* (1997) who demonstrated cyclin-E-induced S phase entry without activation of the pRB pathway.

A more detailed network involving pRB-independent pathways for activation of E/cdk2 is shown in Figure 2. As already mentioned, there are several initiation points that could lead to the activation of E/cdk2: induction of cyclin-D, induction of Cdc25A by the Myc pathway (Galaktionov, Chen & Beach 1996), expression of cyclin-E or E2F, and the downregulation of p27 by the Ras pathway (Leone *et al.* 1997, Takuwa & Takuwa 1997). The experiments of Alevizopoulos *et al.* (1997) could be taken as evidence that, indeed, activation of E/cdk2 is initiated via expressions of Myc and cyclin-E which bypass the inhibitory effects of p16 and pRB.

That pRB is not essential for generating a spike in E/cdk2 activity leads to the question as to what role pRB plays in cell cycle progression. This role must be intimately linked with the ability of pRB to regulate levels of E2F transcription factors. Some authors (e.g. Ikeda, Jakoi & Nevins 1996) believe that the ratio of free E2F to Rb-complexed E2F is critical in the decision to continue proliferation or exit from the cell cycle. In terms of the mechanism for generating the spike of active E/cdk2, note that E2F influences the expression of both Cdc25A and cyclin-E as schematically shown in Fig. 2. Evidence had been presented suggesting that E2F induces expression of Myc (Hiebert, Lipp & Nevins 1989, Thalmeier *et al.* 1989). pRB has been implicated in the regulation of cell growth by repressing gene expression by RNA polymerases I and III (Bartek *et al.* 1996). With its key role of regulating expression of S-phase genes by RNA polymerase II, pRB is thus essential in the coordination of cell growth and DNA replication.

CONCLUSIONS

A detailed model mechanism of the G1/S control in the mammalian cell cycle has been presented. Detailed kinetics of the model have been suggested and computer simulations performed that agree with various experiments. The focus of the present study was to see whether the network is capable of generating a switch that could explain the origins of the R-point. Indeed, it has been shown that the network generates a sharp switching behaviour in the activity of E/cdk2 as a result of the coupling between the different PD cycles in the network. The core of this switch involves a positive feedback loop between the Cdc25A cycle and the E/cdk2 cycle, along with the mutually negative interaction between E/cdk2 and $p27^{Kip1}$. The timing of the increase in E/cdk2 activity coincides with the decay of $p27^{Kip1}$. In summary, the major factors affecting the switch-on time of active E/cdk2 include: (1) the level of p27, which could be influenced by its expression (process 7), its decay (processes 8 and 22) as well as the degree of binding with the cyclin–cdk complexes (processes 9 and 10); (2) the amount of Cdc25A which depends on how fast it is expressed and activated; and (3) the levels of cyclin-E which rely on E2F-dependent expression.

In the computer simulations using the entire network in Figure 1, it was shown that there is a particular time after cutting off mitogens when the network is 'committed' to the activation of E/cdk2. It is also emphasized the fact that there are various points in the network, in addition to the expression of the D-type cyclins, where the activation of E/cdk2 could be initiated, namely, the downregulation of p27 by the Ras pathway, induction of Myc which stimulates expression of Cdc25A, and E2F with its induction of cyclin-E expression. It would be interesting in the future to incorporate the details of known signal transduction

systems that impinge on the cell cycle machinery in G1/S. It is expected that these details will provide more quantitative predictions with respect to R-point control. An intriguing result of these computer simulations is the possibility of two waves of E2F activity (Figure 6c). The second wave of E2F activity is in phase with the increase in the activity of E/cdk2. It would be interesting to see if experiments could prove or disprove this predicted phenomenon.

The mechanism of G1/S transition is expected to be more complex than that presented in this paper. The detailed model shown in Figure 1 and Figure 2 must still be perceived as tentative and there are still some problems that need to be clarified; most important of these is directly relevant to the thesis of this paper, i.e. whether Cdc25A is indeed involved in a positive feedback loop with E/cdk2. Recently, Sexl *et al.* (1999) reported results that suggest that Cdc25A does not act directly on cdk2. Whether or not these results imply that there is no positive feedback loop (even indirectly) between Cdc25A and E/cdk2 still remains to be resolved. This issue may be crucial in understanding the operation of the R-point. Other crucial issues in modelling the R-point include the collaboration of E2F with cdk2 in inducing S phase (Leone *et al.* 1999) and how the cdk inhibitor $p16^{INK4a}$ interacts with the major players in G1. In the present detailed model, the details of the molecular mechanism involving $p16^{INK4a}$ are still sketchy and extensive simulations were not conducted beyond showing that increased levels of p16 lead to increased switch-on times for the activities of E2F and E/cdk2 (simulations not shown). It may also turn out that the participation of cyclin-A is important in R-point control because of the negative regulation by Cyclin-A/cdk2 of the DNA-binding capability of E2F (Krek *et al.* 1994).

There are problems involved in performing a kinetic analysis of a complex biochemical network such as the cell cycle. Almost all the actual rate expressions for the individual processes are unknown, and almost all the rate parameters are also unknown. It is, however, a good assumption that the R-point is robust with respect to the choices of parameter values, i.e. wide ranges of parameter variations should still generate a switching phenomenon. More importantly, some mathematical results in complex network analysis have shown that the qualitative behaviour of certain robust systems is mainly a result of the network structure (Feinberg & Horn 1974). The structure of the network of biochemical processes behind G1/S control is now coming into clearer view and an initial quantitative study such as this paper is quite reasonable at this time.

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