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# The role of cdc25 in checkpoints and feedback controls in the eukaryotic cell cycle

**Ingrid Hoffmann and Eric Karsenti**

Cell Biology Programme, European Molecular Biology Laboratory, Meyerhofstr. 1, D-69117 Heidelberg, Germany

## SUMMARY

**Major checkpoints that gate progression through the cell cycle function at the $G_1$/S transition, entry into mitosis and exit from mitosis. Cells use feedback mechanisms to inhibit passage through these checkpoints in response to growth control signals, incomplete DNA replication or spindle assembly. In many organisms, transition points seem to involve regulation of the activity of cyclin-dependent kinases (cdks) not only through their interactions with various cyclins, but also by phosphorylation-dephosphorylation cycles acting on the kinase activity of the cdks. These phosphorylation cycles are modulated by the regulation of the opposing kinases and phosphatases that act on cdks and form feedback loops. In this article, we discuss the role of positive and negative feedback loops in cell cycle timing and checkpoints, focusing more specifically on the regulation of the dual specificity cdc25 phosphatase.**

Key words: cdk, cdc25, cell cycle, phosphorylation, feedback control

## INTRODUCTION

The past five years have seen a prodigious advance in our understanding of how the cell cycle is regulated. Separate lines of studies in different organisms, like yeast, flies, frogs and cultured mammalian cells have converged, allowing identification of the molecular mechanisms controlling the cell cycle, the so-called 'cell cycle engine'. Key components of the cell-cycle engine are members of the cyclin-dependent kinase (cdks) family. These kinases are complexes between a catalytic subunit (a member of the cdk family) and a regulatory subunit (a cyclin). It has become clear that many, if not all, eukaryotic cells contain multiple forms of this kinase, some concerned with commitment to the cell cycle, others with S phase, and yet others with various aspects of mitosis. The best characterized of these kinases is maturation promoting factor (MPF), a complex of $p34^{cdc2}$ (cdk1) and cyclinB. The activation of MPF results in entry into mitosis and its inactivation in exit from mitosis. Cdc2 kinase activity is regulated through its association with cyclins and by reversible phosphorylation events (Draetta and Beach, 1988; Draetta et al., 1988; Gautier et al., 1989; Morla et al., 1989). The phosphorylation of cdc2 on Tyr15 (in vertebrates also Thr14) negatively regulates kinase activity. Phosphorylation on Thr161/167 is required for tight association with cyclin and activity of the kinase (Ducommun et al., 1991; Gould et al., 1991; Krek and Nigg, 1991; Solomon et al., 1992). The residues whose phosphorylation is significant for vertebrate cdc2 activity seem to be conserved throughout the family of cyclin-dependent kinases and the regulation of the phosphorylation of these residues in various cdks may be similar to what has been described for cdc2 (Meyerson et al., 1992).

We begin to understand how these phosphorylation reactions are used to generate checkpoints at the $G_1$/S and $G_2$/M transitions, when the cell makes decisions about progression through the division cycle. In this review we report recent knowledge accumulated on the biochemistry of the control of these critical transitions. We try to show that beyond the apparent diversity of players, there might be a functional homogeneity in the molecular mechanisms involved in decision making at the $G_1$/S, $G_2$/M and M/$G_1$ transitions.

## MITOTIC FEEDBACK CONTROLS

Many of the genes controlling entry into mitosis were first identified in the fission yeast *Schizosaccharomyces pombe*. They involve at least four gene functions acting together in a network. A schematic illustration of the control of mitosis in eukaryotic cells is shown in Fig. 1. The product of the $cdc2^+$ gene in fission yeast, p34, is essential for entry into mitosis. The $nim1^+$ and $wee1^+/mik1^+$ (Russell and Nurse, 1987a,b; Feilotter et al., 1991; Lundgren et al., 1991) gene products, which encode serine/threonine protein kinases are negative regulators of cdc2 gene function whereas the $cdc25^+$ gene product, which encodes a protein phosphatase is a positive regulator (Russell and Nurse, 1986). The balance between these two pathways regulates cdc2 function and advances or delays onset of mitosis (Fantes, 1979; Russell and Nurse, 1986, 1987a,b). The wee1 protein kinase catalyzes the phosphorylation of cdc2 at Tyr15 (McGowan, 1993; Parker et al., 1992) and inhibits the activation of the kinase. The $nim1^+$ ($cdr1$) gene product is a putative protein kinase (Russell and Nurse, 1987b; Feilotter et al., 1991) that negatively regulates wee1 (Coleman

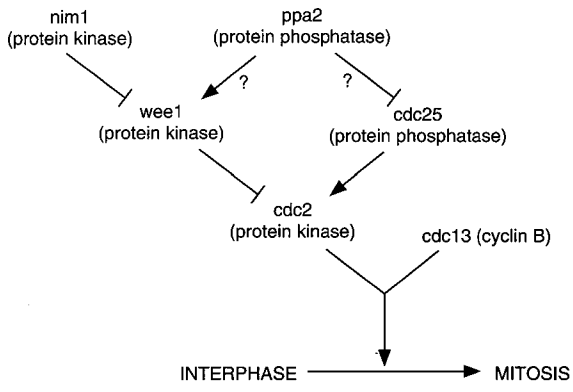


**Fig. 1.** Genes involved in the control of entry into mitosis in *Schizosaccharomyces pombe.*

et al., 1993; Wu and Russell, 1993). The cdc25 phosphatase removes the inhibitory phosphate from the Tyr15 residue of $p34^{cdc2}$ (Kumagai and Dunphy, 1991; Gautier et al., 1991; Millar et al., 1991a; Lee et al., 1992). The timing of entry into mitosis seems, therefore, to be largely determined by the temporal control of the phosphorylation of the Tyr15 residue on $p34^{cdc2}$. In the end, this timing must be determined by changes in the balance between the activities of wee1 and cdc25.

## Entry into mitosis is regulated by a positive feedback loop

In human cells, three homologues of the fission yeast cdc25 protein have been identified, denoted cdc25A, B and C (Sadhu et al., 1990; Galaktionov and Beach, 1991). The protein level of cdc25C does not vary greatly during the cell cycle (Millar et al., 1991b; Kumagai and Dunphy, 1992; Izumi et al., 1992), but this protein undergoes phosphorylation and activation during mitosis in HeLa cells (Hoffmann et al., 1993; Kumagai and Dunphy, 1992; Izumi et al., 1992). Depletion of cdc2 and cyclinB from mitotic extracts using specific antibodies showed that the phosphorylation and activation of cdc25C was dependent on active cdc2/cyclinB complex, and purified cdc2/cyclinB kinase was shown to catalyze directly the phosphorylation of cdc25C at sites identical to those observed in vivo (Hoffmann et al., 1993). This creates a positive feedback loop that may be responsible for the rapid activation of cdc2/cyclinB at the onset of mitosis (Fig. 2). Similar results in *Xenopus* have been reported by Izumi and Maller (1993), who also showed that the cdc25C phosphatase is inactivated if mutations are introduced at the consensus cdc2 phosphorylation sites present in the cdc25 protein.

The question of how the loop is initiated is still unresolved (Hoffmann et al., 1993). One interesting possibility is that the phosphatase that keeps cdc25C dephosphorylated and inactive in interphase is not constitutively active and that its down regulation is required for entry into mitosis. Cdc25C is dephosphorylated and inactivated by a type 2A phosphatase (PP-2A) that is down regulated during mitosis (Clarke et al., 1993; Kumagai and Dunphy, 1992). It is possible that in vivo, the positive feedback loop controlling the activation of cdc2/cyclinB fires when signals coming from the DNA replication machinery or from the state of chromatin organization lead to inhibition of the type 2A phosphatase that dephosphorylates cdc25C (Clarke et al., 1993) (Fig. 2). The timing of cdc2 kinase activation at this check point may also involve regulation of the wee1 pathway (Smythe and Newport, 1992; see also Atherton-Fessler et al., 1993, for a recent review). We propose that the existence of a positive feedback loop regulating the activation of cdc2 kinase at the onset of mitosis provides both a timing device that adjusts the rate of cdc2 kinase activation and a switch on which sensory mechanisms can act to determine when cdc2 kinase should activate. The reversibilty of phosphorylation-dephosphorylation reactions is perfectly suited to build this kind of regulatory network (Novak and Tyson, 1993). These functions may not be essential in all organisms and at all times during development (Edgar et al., 1994), but they are certainly important in many cases to synchronize the successive events of the cell cycle. Interestingly, it has been reported that the Tyr phosphorylation pathway is not functional in budding yeast (Sorger and Murray, 1992; Amon et al., 1992) and in this organism, the boundary between S phase and mitosis is not very clear to say the least (Nurse, 1985). So it is perhaps not surprising that the Tyr phosphorylation pathway is deficient. In fact this may be taken as an argument supporting the idea that positive feedback loops are essential to create sharp boundaries between two cell cycle phases.

## Exit from mitosis is regulated by a negative feedback loop

Exit from metaphase requires inactivation of MPF, which follows cyclin degradation (Murray, 1992). This process is triggered by active cdc2 kinase, which activates the cyclin degradation machinery (at least in *Xenopus* egg extracts (Félix et al., 1990) (Fig. 3). The signal for cyclin degradation is given only when cdc2 kinase activity reaches a threshold that corresponds roughly to the mitotic level of cdc2 kinase activity. Moreover, there is a lag of about 10 minutes between the time when cdc2 kinase activity has reached the threshold and the time when cyclin is degraded (Félix et al., 1990). This creates a negative feedback loop that can be used to time the length of mitosis. The enzyme(s) responsible for the recognition of cyclin and initiation of its proteolysis have not been identified. There is evidence implicating a ubiquitin-dependent protease in cyclin degradation (Glotzer et al., 1990; Hershko et al., 1991). It is not clear, however, whether poly-ubiquitination is the sole signal for cyclin destruction. The biochemical route of ubiquitinated cyclin destruction is also unknown, although the multifunctional proteasome is likely to be involved. Therefore, it is still difficult to propose a mechanism accounting for the requirement of a threshold level of cdc2 kinase activity to induce cyclin degradation and for the lag observed before cyclin is actually degraded. The threshold and lag suggest anyway that a switch built of cdc2 kinase and an opposing phosphatase acting on a substrate essential for cyclin degradation is involved. The lag may also involve complex ubiquitination rates in addition to phosphorylation cycles. Other feedback loop mechanisms may also be important in the inactivation of MPF as, for example, the activation of the Thr161 phosphatase. All available data, however, indicate that dephosphorylation of cdc2 on Thr161 occurs concomitantly with cyclin degradation (Draetta, 1993). It seems that the feedback

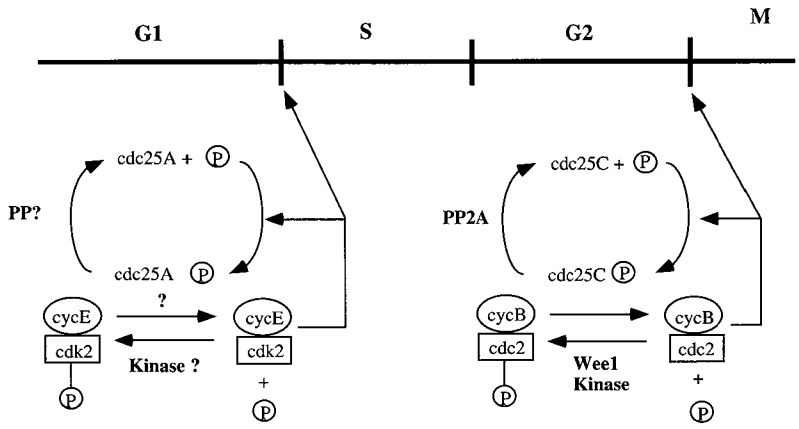


**Fig. 2.** Positive feedback loops at the $G_1$/S and $G_2$/M transition. The existence of a positive feedback loop at the $G_2$/M transition has been demonstrated (see text). This involves the increase in activity of cdc25C after its phosphorylation by the active cyclin B/cdc2 kinase. This leads to an increased rate of cdc2 activation. When fully active, the cdc2/cyclinB induces entry into M-phase. We have shown recently that cdc25A is phosphorylated and activated by cyclinE/cdk2 during S phase, suggesting that there is another positive feedback loop at this transition, built as described in the model. This remains speculative however, because we have not demonstrated yet that cyclinE/cdk2 is an in vivo substrate for the phosphorylated form of cdc25A. CyclinE/cdk2 is probably important to induce S phase. We think that both loops are important to generate sharp and irreversible cell cycle transitions and to provide switches on which cell cycle signals can impinge to allow or forbid progression in the next phase of the cycle.

loop of cdc2 kinase on cyclin degradation could very well contain a switch on which sensors detecting misaligned chromosomes or badly assembled spindles could act to block cyclin degradation.

In summary, the oscillator driving the mitotic cycle is based on a succession of positive and negative feedback loops. These loops can act as timing devices to determine the length of the cell cycle phases they control and as switches on which sensors can impinge to adjust the period of the oscillator to the rate of DNA replication and spindle assembly. The building blocks of these loops appear to be couples of kinases and phosphatases that oppose each other by acting on specific substrates.

## $G_1$/S FEEDBACK CONTROLS

The proliferation of all eukaryotic cells is primarly regulated by a decision that occurs during the $G_1$ phase of the cell cycle - to remain in the cell cycle and divide or to withdraw from the cell cycle and adopt an alternative cell fate (Pardee, 1989). In budding yeast this decision, called START, is the physiological process whereby specific extracellular and intracellular signals combine to promote either cell cycle progression or cell cycle arrest and preparation for conjugation (Reed, 1991; Pringle and Hartwell, 1981). Proliferation of mammalian fibroblasts is also regulated by mitogenic signals during the $G_1$ phase of the cell cycle, and cells can switch between quiescence and proliferation at a unique point in $G_1$ (Pardee, 1989). Experiments in a number of model systems support the idea that, like yeast, higher eukaryotes require a cdk for the completion of $G_1$ and the onset of DNA replication.

### A positive feedback loop for cyclin transcription in budding yeast

The apparently simple picture of a cyclin-kinase complex assembling at START belies a complicated array of cyclins and cdks that function during $G_1$ and S phases. The budding yeast $G_1$ cyclins CLN1, 2 and 3, all activate the CDC28 kinase at START. In the absence of all three CLNs, the cell cycle arrests at START (Richardson et al., 1989). While CLN1 and CLN2-associated kinase activities fluctuate during the cell cycle, CLN3/CDC28 activity does not, suggesting that it might have

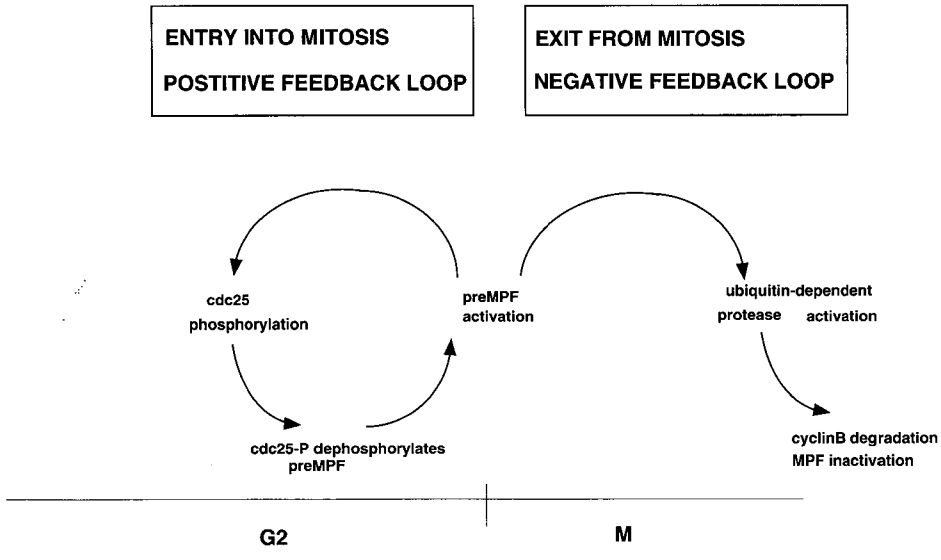


**Fig. 3.** Regulation of MPF activity by feedback loops during the cell cycle. Two feedback loops seem to produce the rapid activation and inactivation of MPF. The positive feedback loop is triggered by the phosphorylation of cdc25. Activation of a ubiquitin-dependent proteolytic pathway is part of the negative feedback loop.

a different function (Tyers et al., 1993). Another family, composed of B-type cyclins encoded by CLB1, 2, 3 and 4 genes, is necessary for the formation and function of the mitotic apparatus (Fitch et al., 1992; Richardson et al., 1992; Surana et al., 1991). CLB5 and CLB6, encoding another pair of B-type cyclins have a function in S phase (Epstein and Cross, 1992; Schwob and Nasmyth, 1993).

Much of the order and timing of cell cycle events seems to be determined by transcriptional control, involving positive feedback loops. A postive feedback loop has been invoked to explain the appearance of CLN1 and CLN2 proteins in late $G_1$. It has been proposed that in daughter cells, CLN/CDC28 kinase activates a transcription factor, SBF, which in turn activates CLN1 and CLN2 transcription, thereby closing the loop by further activating CDC28 (Ogas et al., 1991; Dirick and Nasmyth, 1991). The existence of a positive feedback loop may help to understand the apparent irreversibility of START, which might be determined by the transition from low to high CLN-dependent kinase activity (Nasmyth, 1993).

### Feedback controls regulating $G_1$ and S phases in mammalian cells

Cell cycle progression in vertebrates seems also to be driven by successive waves of cyclins. The regulation of SBF is similar to that of the mammalian transcription factor E2F. Both are involved in the activation of gene expression during late $G_1$ or early S phase (Pagano et al., 1992; Devoto et al., 1992). In vertebrate cells, progression through the cell cycle is blocked by the binding of retinoblastoma protein (Rb) to E2F (Chellappan et al., 1991). This block is released when Rb is phosphorylated and dissociated from E2F by a cdk/$G_1$ cyclin. The free E2F induces transcription of both early and delayed response genes including $G_1$ cyclin genes. Thus, progression through the restriction point in mammalian cells may also be regulated by an autocatalytic positive feedback loop acting at the transcriptional level.

Regulation of the $G_1$ to S phase transition may also involve positive feedback loops regulating the kinase activity of cdks at the post-translational level. In human cells, the cdk2 kinase is involved in the regulation of S phase progression when associated with cyclinA (Pagano et al., 1993). Its activity is negatively regulated by tyrosine (and probably also threonine) phosphorylation (Gu et al., 1992), suggesting that cdk2/cyclinA activation also requires dephosphorylation by cdc25 (Gu et al., 1992). Recently, we found that human cdc25A is phosphorylated and activated during S phase (Hoffmann et al. 1994, unpublished data). However, cdc25A phosphorylation and activation does not seem to be mediated by cdk2/cyclinA but rather by the cdk2/cyclinE complex. We still do not know what is the physiological substrate of cdc25A (cdk2/cyclinA or cdk2/cyclinE). However, these results strongly suggest that a positive feedback loop acting on the phosphorylation level of cdk2 associated to cyclinE and cyclinA plays an important role in the temporal control of the $G_1$/S transition and maybe in the progression through S phase (Fig. 2).

Why should such a level of regulation exist during $G_1$/S and maybe even during S phase progression? The positive feedback loop involving cdc25C at the $G_2$/M transition, seems to be a switch capable of sensing the state of DNA replication and therefore to tie entry into mitosis to the end of DNA replication. The situation is obviously very different during late $G_1$ and early S phase where the cdc25A-positive feedback loop would be operating. Our favorite hypothesis is that this loop senses late $G_1$ events, like centrosome duplication, or the level of components essential for the initiation of DNA replication. Cdc25A may also be involved in regulating the successive activation of cdk2/cyclinE and cdk2/cyclinA. Alternatively, it may simply be used to produce a sharp timing of cdk2 activation at the onset of S phase.

## CONCLUSION

Although the actors involved at each cell cycle transition point vary ($G_1$ and mitotic cyclins, $G_1$ and mitotic cdks), it seems that similar basic principles govern each transition: feedback loops. Beyond the identification of specific kinases and phosphatases, if we are to understand the logic of the cell cycle, we have to understand the kinetics of cdk activation and inactivation. We have to understand how the positive and negative feedback loops function to generate irreversible transition points. We also have to understand how such loops provide switches to be acted upon by sensory devices, allowing the cell to decide whether to go or not to go one step further in the cycle.

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