COMMENTARY

Regulation of p34^{cdc2} protein kinase: new insights into protein phosphorylation and the cell cycle

PAUL R. CLARKE and ERIC KARSENTI

Cell Biology Programme, European Molecular Biology Laboratory, Postfach 10.2209, D6900 Heidelberg, Germany

Introduction

The reversible phosphorylation of specific residues on proteins is a ubiquitous mechanism for the regulation of cellular processes. Recently there has been a tremendous expansion of interest in the role of protein phosphorylation in the eukaryotic cell cycle. This has been a consequence of the discovery that the *cdc2* gene in the fission yeast *Schizosaccharomyces pombe*, which is required at the onset of mitosis and also at the 'start' control point at the G_1/S transition, encodes a 34 kDa protein-serine/threonine kinase catalytic subunit (p34^{cdc2}). Homologous genes have been identified in *Saccharomyces cerevisiae* (CDC28) and in all other eukaryotes examined. p34^{cdc2} is a component of MPF (maturation- or M-phase promoting factor), and it is now apparent that p34^{cdc2} protein kinase is central to the control mechanism of the cell cycle in all eukaryotic cells (reviewed by Nurse, 1990).

The mitotic function of p34^{cdc2} protein kinase has been somewhat better characterized than its role at 'start'. At the G_2/M transition, p34^{cdc2} protein kinase is specifically and dramatically activated, and catalyses the phosphorylation of key proteins (reviewed by Moreno and Nurse, 1990; Pines and Hunter, 1990*b*) to bring about the cellular changes that occur in mitosis. The level of p34^{cdc2} protein is constant throughout the cell cycle, and its protein kinase activity (usually assayed *in vitro* using histone H1 as substrate) requires the association of p34^{cdc2} with cyclins, proteins that are synthesised and degraded in a cell cycle-dependent manner (Evans *et al.* 1983). Activation also involves a complex series of phosphorylation/dephosphorylation events catalysed by a network of protein kinases and protein phosphatases. Although the picture is still incomplete, important developments have occurred recently in our understanding of these molecular events. Some of the protein kinases and protein phosphatases involved in controlling the phosphorylation of p34^{cdc2} itself have been tentatively identified and found to recognise both serine/threonine and tyrosine residues. This unexpected finding calls for a revision of our previous assumptions about the substrate specificity of such enzymes. In fact, the study of the cell cycle is now yielding new insights into the intricate mechanisms by which protein phosphorylation provides molecular switches and precise temporal control of cellular processes.

Phosphorylation of p34^{cdc2} protein kinase

p34^{cdc2} is itself phosphorylated at multiple sites in a cell cycle-dependent manner. Although the phosphorylation sites have not been directly sequenced, four have been identified by a combination of genetic methods and phosphopeptide mapping. Putative phosphorylated residues were mutated to non-phosphorylatable residues and the resulting phenotype examined. In parallel, phosphopeptides derived from p34^{cdc2} were compared with synthetic peptides phosphorylated *in vitro*. The first phosphorylation site to be identified in this way was Tyr15 in *S. pombe* p34^{cdc2} (Fig. 1). Mutation of Tyr15 to phenylalanine causes the cells to enter mitosis prematurely, suggesting that this mutation causes premature activation of p34^{cdc2} protein-kinase (Gould and Nurse, 1989). Furthermore, a purified human protein-tyrosine phosphatase can remove the tyrosine phosphate from wild-type p34^{cdc2} isolated from cells in G_2, and activate its histone H1 kinase activity (Gould *et al.* 1990). These results demonstrate that dephosphorylation on Tyr15 alone is sufficient to activate the G_2 form of *S. pombe* p34^{cdc2} protein kinase.

In vertebrates, the regulation of p34^{cdc2} protein kinase activity by phosphorylation on Tyr15 is conserved. However, there is an additional inhibitory phosphorylation site on the adjacent residue, Thr14 (Fig. 1). p34^{cdc2} phosphorylated on both Thr14 and Tyr15 can be isolated from chicken (Krek and Nigg, 1991*a*) and mouse (Norbury *et al.* 1991) cultured cells in S and G_2 phases. Norbury *et al.* (1991) have examined the activation of wild-type and mutant human p34^{cdc2} expressed in *Xenopus* extracts in the presence of cyclin B. The activity of the wild-type human p34^{cdc2} protein kinase is suppressed like the endogenous p34^{cdc2} protein kinase, only activating after a long lag. Only when both Thr14 and Tyr15 are mutated to non-phosphorylatable residues (Phe and Ala, respectively) is the transient suppression of kinase activity relieved (Norbury *et al.* 1991). Similar results have been obtained by Krek and Nigg (1991*b*), who have expressed wild-type and mutant chicken *cdc2* cDNAs in HeLa cells. They find that mutation of Thr14 and Tyr15 together to non-phosphorylatable residues induces premature mitotic events. However, the two sites are not equivalent:

Key words: cdc2 protein kinase, cell cycle, protein phosphatase, protein phosphorylation.

Journal of Cell Science 100, 409–414 (1991)
Printed in Great Britain

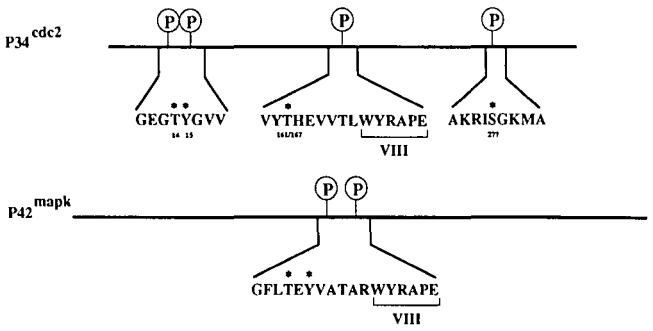

Fig. 1. Phosphorylation sites on $p34^{cdc2}$ and $p42^{mapk}$. The position of the phosphorylation sites are marked on linear representations of the two molecules. The amino acid sequences (single letter code) close to the phosphorylated residues (marked with an asterisk * and their number in the primary sequence) are shown below. The residues that form part of the conserved kinase domain VIII are also indicated.

mutation at Thr14 alone did not produce a phenotype, while mutation of Tyr15 alone partially induces premature mitosis.

Thr14 and Tyr15 lie in the putative ATP-binding site of $p34^{cdc2}$ protein kinase, and the presence of a phosphate group probably hinders the binding of ATP, inactivating the kinase. Inhibitory phosphorylation within the ATP-binding site constitutes a novel mechanism for the regulation of a protein kinase, although isocitrate dehydrogenase of *Escherichia coli* is regulated in a similar manner (Hurley *et al.* 1990).

Another putative phosphorylation site in $p34^{cdc2}$ is a threonine residue, Thr167 in fission yeast, Thr161 in vertebrates (Krek and Nigg, 1991*a*) (Fig. 1). Recently, two groups have mutated Thr161/167 in $p34^{cdc2}$ to various other residues, and examined the effect of expression in *S. pombe* (Ducommun *et al.* 1991; Gould *et al.* 1991). Mutation to a neutral non-phosphorylatable residue results in failure to rescue temperature-sensitive *cdc2* mutants (ts-*cdc2*) and overexpression is lethal or leads to elongated cells containing a single nucleus. However, mutation to serine allows ts-*cdc2* rescue. Replacement with glutamic acid, which might mimic a phosphorylated residue, permits some growth when expressed in the ts-*cdc2* strain. Overexpression of this mutant $p34^{cdc2}$ produces cells that are multiply septated and with multiple mitotic-like nuclei. These results indicate that phosphorylation of Thr161/167 is required for $p34^{cdc2}$ activity, and indeed this site appears to be phosphorylated when the kinase is active during M-phase (Ducommun *et al.* 1991; Gould *et al.* 1991; Krek and Nigg, 1991*a*).

Phosphorylation at a further site, Ser277 (chicken cdc2) (Fig. 1) occurs during G_1, and decreases on entry into S phase (Krek and Nigg, 1991*a*). Although the effect of this phosphorylation is, at present, unknown, it might be involved in the proposed function of cdc2 at the G_1/S transition.

The form of $p34^{cdc2}$ protein kinase that is active in M-phase consists of a complex of $p34^{cdc2}$ and cyclin B (Draetta *et al.* 1989; Labbé *et al.* 1989). The cyclin subunit is also phosphorylated, although the possible function of this phosphorylation is still unclear. *Xenopus* cyclin B2 can be phosphorylated *in vitro* by the product of the *mos* proto-oncogene, a serine/threonine protein kinase (Roy *et al.* 1990). It has been proposed that this may stabilize the active $p34^{cdc2}$/cyclin B2 complex and account for the effect of mos as the 'cytostatic factor' responsible for the arrest of unfertilized eggs in second meiotic metaphase (Maller, 1991). Conversely, it is possible that phosphorylation of cyclin by $p34^{cdc2}$ protein kinase itself acts as a signal for cyclin degradation, $p34^{cdc2}$ protein kinase inactivation and exit from M-phase (Felix *et al.* 1990*b*).

Protein kinases and the regulation of $p34^{cdc2}$ protein kinase

A new class of protein kinase

Until very recently, known protein kinases could be divided into two classes on the basis of their substrate specificity, having a mutually exclusive preference for either tyrosine or serine/threonine residues. This specificity was reflected in the conservation of certain residues in the primary sequences of kinases belonging to each group (Hanks *et al.* 1988). However, several protein kinases have now been described that phosphorylate both classes of residues, although their sequences resemble serine/threonine-specific kinases (Ben-David *et al.* 1991; Dailey *et al.* 1991; Howell *et al.* 1991; Stern *et al.* 1991). One of these, the 107 kDa product of the $wee1^+$ gene in *S. pombe* ($p107^{wee1}$), is a negative regulator of entry into mitosis (Russell and Nurse, 1987*b*). $p107^{wee1}$ can autophosphorylate on tyrosine and serine residues, and phosphorylates an exogenous peptide substrate on tyrosine (Featherstone and Russell, 1991). $p107^{wee1}$ has not been shown to phosphorylate $p34^{cdc2}$ directly, but co-expression of $p34^{cdc2}$ with $p107^{wee1}$ in insect cells using a baculovirus vector results in phosphorylation of $p34^{cdc2}$ on tyrosine. Moreover, this phosphorylation is greatly stimulated by co-expressed cyclin (Parker *et al.* 1991). Although non-physiological phosphorylations could occur when kinases are vastly over-produced in this system, these results strongly suggest that $p107^{wee1}$ protein kinase catalyses the tyrosine phosphorylation of $p34^{cdc2}$, and that the $p34^{cdc2}$/cyclin complex is a better substrate than $p34^{cdc2}$ alone. Although $p107^{wee1}$ from *S. pombe* phosphorylates serine/threonine and tyrosine residues *in vitro*, Thr14 has not been found phosphorylated in *S. pombe* $p34^{cdc2}$. Tyr15 phosphorylation involves the cooperation of $p107^{wee1}$ with another putative protein kinase, the product of the $mik1^+$ gene (Lundgren *et al.* 1991). In vertebrates both Thr14 and Tyr15 are phosphorylated, but it remains to be seen whether phosphorylation of both sites is catalysed by a homologue of $p107^{wee1}$ or by two distinct kinases. Perhaps distinct inhibitory pathways could act on each site.

Tyrosine/threonine dual phosphorylation as a special regulatory motif

There are certain parallels between the phosphorylation of $p34^{cdc2}$ and some other protein-serine/threonine kinases. Mitogen-activated protein (MAP) kinases $p42^{mapk}$ and $p44^{mapk}$ (Sturgill and Wu, 1991) are members of a family of protein-serine/threonine kinases that includes the extracellular signal-regulated kinases (ERK) (Boulton *et al.* 1991), yeast kinases KSS1 (Courchesne *et al.* 1989) and FUS3 (Elion *et al.* 1990), and probably several other vertebrate kinases usually assayed using microtubule-associated protein-2 (MAP-2) or myelin-basic protein (MBP) as exogenous substrates, e.g. (Ahn *et al.* 1990;

Gotoh *et al.* 1991; Sanghera *et al.* 1990). These kinases, which are related to the *cdc2* family (26–41 % identity for ERKs; Boulton *et al.* 1991), play an important role in the cellular response to a variety of extracellular signals, forming part of the signal transduction pathway from receptor tyrosine kinases to serine/threonine phosphorylation of target proteins. They may also be involved in regulation of the cell cycle, being activated during M-phase (Sturgill and Wu, 1991). Like $p34^{cdc2}$, the activity of MAP kinases is controlled by both tyrosine and threonine phosphorylation, although in this case the phosphorylations are not inhibitory, but required for activation. Recently, the regulatory phosphorylation sites on $p42^{mapk}$ have been identified by peptide sequencing using mass spectrometry (Payne *et al.* 1991). Two phosphorylated residues, one tyrosine and one threonine, are separated by a single glutamic acid residue and situated near to the conserved kinase subdomain VIII (Hanks *et al.* 1988) (Fig. 1). The corresponding region in $p34^{cdc2}$ contains the Thr161/167 phosphorylation site (Fig. 1). In fact, the double activating phosphorylations in $p42^{mapk}$ may be considered homologous to the single activating (Thr161/167) phosphorylation in $p34^{cdc2}$. In some other protein kinases auto-phosphorylation sites are found in this region. Dephosphorylation of $p42^{mapk}$ on either threonine or tyrosine, using protein serine/threonine phosphatase 2A or a tyrosine-specific phosphatase, respectively, inactivates the kinase *in vitro* (Anderson *et al.* 1990). However, the phosphatase(s) and kinase(s) that act *in vivo* on these sites in $p42^{mapk}$ are unknown. Growth factor-stimulated protein kinases related to $p42^{mapk}$ may be phosphorylated on both tyrosine and threonine residues and activated in response to treatment with a single factor (Ahn *et al.* 1991; Gomez and Cohen, 1991). It has been suggested that this factor may not itself be a kinase, but rather stimulates autophosphorylation of MAP kinases (Seger *et al.* 1991).

Tight control of the activity of $p34^{cdc2}$ and MAP kinases is essential to prevent the cell from unregulated growth and division. In the case of $p42^{mapk}$, phosphorylation at both threonine and tyrosine sites is required for activity, so that unregulated phosphorylation by kinases specific for tyrosine or threonine/serine residues alone will not result in activation. For vertebrate $p34^{cdc2}$, it is dephosphorylation on both threonine and tyrosine residues that is required for activation, providing a double check against unwarrented activation. *S. pombe* $p34^{cdc2}$ does not seem to have the dual tyrosine/threonine inhibitory phosphorylations, however, and is activated by dephosphorylation on tyrosine alone (Gould and Nurse, 1989). Tyrosine phosphorylation may be very rare in *S. pombe*, and it is possible that the extra safety catch of inhibitory threonine phosphorylation on $p34^{cdc2}$ is not required.

MAP kinases, like $p34^{cdc2}$, may recognise certain serine/threonine residues followed by a proline in their substrates, although a second proline, two residues N-terminal to the phosphorylated residue, may also be required (Sturgill and Wu, 1991). It has been suggested that 'proline-directed' phosphorylations may constitute a sub-set of phosphorylation sites on proteins involved in growth control and mitotic induction (Hall and Vulliet, 1991). There are some clear differences in the ability of $p34^{cdc2}$ and MAP kinases to phosphorylate certain proteins *in vitro*; however, conclusions about the identity of the kinase that phosphorylates a particular serine/threonine-proline site *in vivo* must be interpreted with caution until we know the precise specificity determinants.

Protein phosphatases and the regulation of $p34^{cdc2}$ protein kinase

cdc25 is probably a $p34^{cdc2}$-specific phosphatase

Protein phosphatases have been divided, like protein kinases, into two major classes: those that dephosphorylate tyrosine residues and those with a preference for serine/threonine residues (Cohen, 1989; Tonks and Charbonneau, 1989). Unlike protein kinases, however, the two classes of phosphatase do not show sequence homology (Cohen *et al.* 1990; Tonks, 1990). Recently, the situation has changed somewhat with the discovery that vaccinia virus VH1 gene encodes a protein phosphatase that shows some sequence homology with tyrosine phosphatases, although it can dephosphorylate well both phosphotyrosine and phosphoserine residues (Guan *et al.* 1991). So, as for protein kinases, a third class of protein phosphatase may be emerging.

The product of the *cdc25* gene in *S. pombe* is a positive regulator of *cdc2*. Related genes have been identified in *Saccharomyces cerevisiae, Drosophila, Xenopus* and humans. Genetic evidence has suggested that cdc25 could function in the dephosphorylation and activation of $p34^{cdc2}$ at the G_2–M transition. Recently, this has been substantiated by the finding that a *Drosophila* homologue of cdc25 activated $p34^{cdc2}$ when added to *Xenopus* egg extracts. Activation was concomitant with dephosphorylation of $p34^{cdc2}$ on tyrosine (Kumagai and Dunphy, 1991). In fact, purified $p34^{cdc2}$/cyclin B complex isolated in an inactive form can be activated and dephosphorylated on tyrosine by incubation with a purified human cdc25 homologue (Stausfeld *et al.* 1991). These results strongly suggest that cdc25 can catalyse the dephosphorylation of $p34^{cdc2}$ directly.

Initially, cdc25 was not thought to be a $p34^{cdc2}$-tyrosine phosphatase, because no sequence similarity with known tyrosine phosphatases could be discerned (Nurse, 1990). However, the vaccinia VH1 gene product contains only a few residues in common with other tyrosine phosphatases. A closer inspection of the amino acid sequence of cdc25 does reveal some sequence similarity with VH1 phosphatase and other tyrosine phosphatases (Moreno and Nurse, 1991; Fig. 2). In particular, a motif His-Cys-(5 residues)-Arg is present in all of them. In VH1 phosphatase (Guan *et al.* 1991) and other tyrosine phosphatases (Sreuli *et al.* 1990) the Cys and Arg residues in this motif are essential for activity. Mutation of the corresponding residues in cdc25 prevents its activation of $p34^{cdc2}$ (M. W. Kirschner, personal communication), indicating that cdc25 also has phosphatase activity. cdc25 has not been shown to produce the efficient dephosphorylation of any substrate other than $p34^{cdc2}$ (on tyrosine) when it is complexed to cyclin, and so may be highly substrate-specific. Whether cdc25 is able to dephosphorylate the inhibitory phosphorylated threonine residue in vertebrate $p34^{cdc2}$ is not certain, although there is some evidence to support this notion (Stausfeld *et al.* 1991).

Role of protein phosphatase 2A in $p34^{cdc2}$ kinase activation

Okadaic acid, which specifically inhibits type 1 and 2A serine/threonine phosphatases, causes the premature activation of cdc2 in extracts of *Xenopus* eggs. The type 1 phosphatase-specific protein inhibitors 1 and 2 do not, indicating that the effect is due to the inhibition of

```
Drosophila stg   (348) ILDEFLTVQQTELQQQQNAESGHKRNIIIFHCEFSSERGPKMSRF-LRNLDRERNT
Human cdc25      (353) LFNFFLKKPIVPLDTQ-------KRIIIVFHCEFSSERGPRMCRC-LREEDRSLNQ
S. pombe cdc25   (461) IVDAFLSKPLTH----------RVALVFHCEHSAHRAPHLALH-FRNTDRRMNS
S. cerevisiae mih1 (293) LEYEFIHKVL----HSDTSNNNTLPTLLIIHCEFSSHRGPSLASH-LRNCDRIINQ
Vaccinia VH1      (91) DVTAFLSKC----------DQRNEPVLVHCAAGVNRSGAMILA----YLMSKNK
Human TPTP       (191) SFLNFLFKV------RESGSLNPDHGPAVIHCSAGIGRSGTFCLADTCLVLMEKGD

Drosophila stg   (403) NAYPALHYPEIYLLHNGYKEFFESHVELCEPHAYRTMLDPAYNEAYR (30aa)
Human cdc25      (399) --YPALYYPELYILKGGYRDFFPEYMELCEPQSYCPMHHQDHKTELL (27aa)
S. pombe cdc25   (504) HRYPFLYYPEVYILHGGYKSFYENHKNRCDPINYVPMNDRSHVMTCT (30aa)
S. cerevisiae mih1 (344) DHYPKLFYPDILILDGGYKAVL-TFPELCYPRQYVGMNSQENLLNCE (85aa)
Vaccinia VH1     (131) ESLPMLYFLYVYHSMRDLRGAFVENPSFKR----QIIE--KYVIDKN
Human TPTP       (241) D----INIKQVLLNMRKYRMGLIQTPDQLRFSYMAIIEGAKCIKGDS (132aa)
```

Fig. 2. Comparison of part of the amino acid sequences (single letter code) of cdc25 homologues, vaccinia virus VH1 phosphatase and human T-cell protein tyrosine phosphatase (Cool *et al.* 1989; Edgar and O'Farrell, 1989; Guan *et al.* 1991; Russell *et al.* 1989; Russell and Nurse, 1986; Sadhu *et al.* 1990). Residues conserved in all six sequences are shown with a dark background, those conserved in at least four sequences in bold type. The conserved histidine and arginine residues required for activity are indicated by asterisks (*).

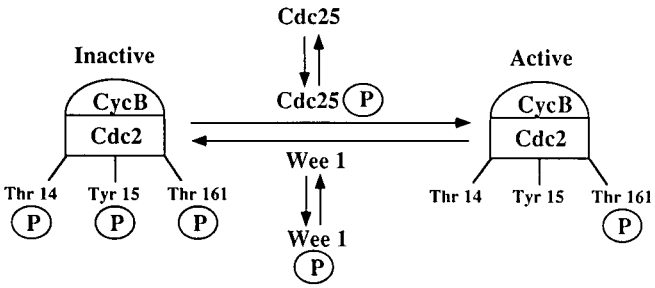

Fig. 3. A highly simplified model of the regulation of $p34^{cdc2}$/cyclin B complex by phosphorylation. In G_2, $p34^{cdc2}$ is associated with cyclin B and kept in an inactive form by phosphorylation on Thr14 and Tyr15. We assume that Thr161 is also phosphorylated, since its phosphorylation is required for tight interaction between $p34^{cdc2}$ and cyclin. The phosphorylation of $p34^{cdc2}$ also involves mik1 kinase and probably other proteins, not shown here for clarity. The active form of $p34^{cdc2}$/cyclin B is produced when the rate of dephosphorylation of Thr14 and Tyr15, probably catalysed by cdc25, exceeds the rate of their phosphorylation. The regulation of cdc25 phosphatase and wee1 kinase by phosphorylation is hypothetical, but in this model dephosphorylation of one or both by phosphatase 2A would result in the inhibition of cdc2 activity. Alternatively, phosphatase 2A could act directly on $p34^{cdc2}$ itself, possibly at Thr161.

phosphatase 2A (Felix *et al.* 1990*a*; reviewed by Karsenti *et al.* 1991). In extracts without endogenous cyclins, but to which bacterially expressed cyclin B has been added, okadaic acid abolishes the normal requirement for a threshold of cyclin concentration and reduces the time lag between the addition of cyclin and kinase activation (Solomon *et al.* 1990). Recently, a factor that inhibits the activation of $p34^{cdc2}$ (termed INH) has been purified and found to contain the catalytic subunit of phosphatase 2A as well as other components (Lee *et al.* 1991). However, the target of phosphatase 2A is not certain. Because inhibition of phosphatase 2A stimulates the *dephosphorylation* of $p34^{cdc2}$ on tyrosine, it has been proposed that the activity of a tyrosine phosphatase (probably a cdc25 homologue) is reduced by dephosphorylation catalysed by phosphatase 2A (Fig. 3; reviewed by Karsenti *et al.* 1991). Although it is not clear whether cdc25 is regulated by phosphorylation, it is a phosphoprotein (Moreno *et al.* 1990). *In vitro*, phosphatase 2A can also dephosphorylate and inactivate $p34^{cdc2}$ directly (Lee *et al.* 1991; Gould *et al.* 1991). Since Thr161/167 phosphorylation seems to be required for tight association between $p34^{cdc2}$ and cyclin (Ducommun *et al.* 1991), dephosphorylation of that site by phosphatase 2A could cause dissociation of the complex. However, it is not at all certain that phosphatase 2A acts in this way *in vivo*. Phosphatase 2A might also activate a tyrosine kinase, probably $p107^{wee1}$, to maintain $p34^{cdc2}$ in an inactive state (Fig. 3). Indeed, genetic evidence suggests that wee1 is repressed by phosphorylation due to another kinase, the product of the *nim1* gene (Russell and Nurse, 1987*a*). Further investigation is required to determine the actual component(s) of the system on which phosphatase 2A acts in the cell. This is an important question, because phosphatase 2A seems to be part of the G_2 checkpoint pathway that monitors when $p34^{cdc2}$/cyclin B can be activated.

G_2-M checkpoint and $p34^{cdc2}$ protein kinase activation

The mechanism of activation of $p34^{cdc2}$ complexed to cyclin B is intricate and not yet completely understood. The Thr161/167 phosphorylation site in $p34^{cdc2}$ is required for tight cyclin binding (Ducommun *et al.* 1991) and activation of the kinase (Gould *et al.* 1991). It is not yet clear whether phosphorylation of this site precedes cyclin B association or is a consequence of it, but the two events may be cooperative. For instance, cyclin binding to $p34^{cdc2}$ could increase the rate of Thr161/167 phosphorylation, which may then stabilize the complex. Cyclin B binding also induces inhibitory tyrosine phosphorylation (Tyr15) (Solomon *et al.* 1990; Meijer *et al.* 1991), but the timing of Thr14 phosphorylation and its possible dependence on cyclin B binding is uncertain. As cyclin B is synthesised it associates with $p34^{cdc2}$ in an inactive complex that accumulates before the final activation leading to metaphase. Why should it be necessary to have inhibitory phosphorylation of $p34^{cdc2}$ after cyclin binding? One answer is that each regulatory phosphorylation on the complex can be used as a checkpoint, sensing the

accomplishment of S–prophase processes before permitting activation of the mitotic form of the kinase (Enoch and Nurse, 1991). Recent genetic (Enoch and Nurse, 1990) and biochemical (Dasso and Newport, 1990; Kumagai and Dunphy, 1991) evidence indicates that the tyrosine phosphorylation of $p34^{cdc2}$ is affected by the state of replication of DNA; if DNA replication is blocked then tyrosine phosphorylation occurs normally but dephosphorylation does not take place and the $p34^{cdc2}$ protein kinase remains inactive. cdc25 is somehow involved in this check point (Enoch and Nurse, 1990). Unreplicated DNA could act by inhibiting the activity of cdc25, or by activating the opposing tyrosine kinase (Fig. 3). Intriguingly, in *Xenopus* egg extracts to which a large number of nuclei have been added, when traffic through nuclear pores is blocked, $p34^{cdc2}$ is tyrosine phosphorylated normally but not dephosphorylated (Kumagai and Dunphy, 1991). This may suggest that a regulator of cdc25 or cdc25 itself shuttles between nucleus and cytoplasm, coupling the tyrosine dephosphorylation of $p34^{cdc2}$ to the completion of DNA replication.

However, tyrosine phosphorylation may not be an obligatory step in the pathway of $p34^{cdc2}$ protein kinase activation. In the cleaving *Xenopus* embryo, after the 1st mitotic cycle, the cell divides rapidly from the 2nd to 12th mitotic cell cycles without G_1 or G_2 phases. During this period, $p34^{cdc2}$ protein kinase oscillates between inactive and active forms, although tyrosine phosphorylation is not detected (Ferrell *et al.* 1991). The lack of inhibitory tyrosine phosphorylation could explain why the activation of $p34^{cdc2}$ protein kinase is not dependent on the completion of DNA replication until the midblastula transition.

Multiple cyclins and cyclin-dependent protein kinases

In this commentary, we have discussed the regulation of the activity of $p34^{cdc2}$ protein kinase by its association with regulatory subunits, in particular B-type cyclins, and by phosphorylation/dephosphorylation of the $p34^{cdc2}$ catalytic subunit. However, catalytic subunits of other putative cyclin-dependent protein kinases, closely related to $p34^{cdc2}$, are being identified (Lehner and O'Farrell, 1990; Paris *et al.* 1991; Pines and Hunter, 1990*a*). In addition to the specific regulation of $p34^{cdc2}$/cyclin B at the G_2–M transition, cell cycle control probably involves other protein kinases consisting of combinations of $p34^{cdc2}$-like catalytic subunits with different cyclins. Cyclin-like proteins specific of the G_1 phase have been identified in different species (Forsburg and Nurse, 1991; Richardson *et al.* 1989; Wittenberg *et al.* 1990), and during the G_2–M period, there are both A- and B-type cyclins (Draetta, 1990). These subunits may confer specific functions to a protein kinase and target it to particular subcellular compartments. Each kinase may be recognised differently by regulatory kinases and phosphatases, and hence be differentially controlled. Each kinase may prime the activation of the next kinase in the sequence (Minshull *et al.* 1990), so that a successsion of phases is assured. In fact, we are only just beginning to uncover the complexity of protein phosphorylation in the cell cycle.

We thank those authors who communicated their results prior to publication. Work in this laboratory is supported by a grant from the Human Frontiers Science Programme. P.R.C. is the recipient of a research fellowship of The Wellcome Trust.

References

Ahn, N. G., Seger, R., Bratlien, R. L., Diltz, C. D., Tonks, N. K. and Krebs, E. G. (1991). Multiple components in an epidermal growth factor-stimulated protein kinase cascade. *J. biol. Chem.* **296**, 4220–4227.

Ahn, N. G., Weiel, J. E., Chan, C. P. and Krebs, E. G. (1990). Identification of multiple epidermal growth factor-stimulated protein serine/threonine kinases from Swiss 3T3 cells. *J. biol. Chem.* **265**, 11487–11494.

Anderson, N. G., Maller, J. L., Tonks, N. K. and Sturgill, T. W. (1990). Requirement for integration of signals from two distinct phosphorylation pathways for activation of MAP kinase. *Nature* **343**, 651–653.

Ben-David, Y., Letwin, K., Tannock, L., Bernstein, A. and Pawson, T. (1991). A mammalian protein kinase with potential for serine/threonine and tyrosine phosphorylation is related to cell cycle regulators. *EMBO J.* **10**, 317–325.

Boulton, T. G., Nye, S. H., Robbins, D. J., Ip, N. Y., Radziejewska, E., Morgenbesser, S. D., DePinho, R. A., Panayotatos, N., Cobb, M. H. and Yancopoulos, G. D. (1991). ERKs: A family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* **65**, 663–675.

Cohen, P. (1989). The structure and regulation of protein phosphatases. *Ann. Rev. Biochem.* **58**, 453–508.

Cohen, P. T. W., Brewis, N. D., Hughes, V. and Mann, D. J. (1990). Protein serine/threonine phosphatases; an expanding family. *FEBS Lett.* **268**, 355–359.

Cool, D. E., Tonks, N. K., Charbonneau, H., Walsh, K. A., Fischer, E. H. and Krebs, E. G. (1989). cDNA isolated from a human T-cell library encodes a member of the protein tyrosine phosphatase family. *Proc. natn. Acad. Sci. U.S.A.* **86**, 5257–5261.

Courchesne, W. E., Kunisawa, R. and Thorner, J. (1989). A putative protein kinase overcomes pheromone-induced arrest of cell cycling in *S. cerevisiae*. *Cell* **58**, 1107–1119.

Dailey, D., Schieven, G. L., Young Lim, M., Marquart, H., Gilmore, T., Thorner, J. and Martin, G. S. (1991). Novel yeast protein kinase (*YPK1* gene product) is a 40-kilodalton phosphotyrosyl protein associated with protein-tyrosine kinase activity. *Molec. cell. Biol.* **10**, 6244–6256.

Dasso, M. and Newport, J. W. (1990). Completion of DNA replication is monitored by a feedback system that controls the initiation of mitosis *in vitro*: studies in *Xenopus*. *Cell* **61**, 811–823.

Draetta, G. (1990). Cell cycle control in eukaryotes: molecular mechanisms of cdc2 activation. *Trends biochem. Sci.* **15**, 378–383.

Draetta, G., Luca, F. J. W., Brizuela, L., Ruderman, J. and Beach, D. (1989). cdc2 protein kinase is complexed with both cyclin A and B: Evidence for proteolytic inactivation of MPF. *Cell* **56**, 829–838.

Ducommun, B., Brambilla, P., Félix, M.-A., Franza, B. R., Karsenti, E. and Draetta, G. (1991). Cdc2 phosphorylation is required for its interaction with cyclin. *EMBO J.* (in press).

Edgar, B. A. and O'Farrell, P. H. (1989). Genetic control of cell division patterns in the *Drosophila* embryo. *Cell* **57**, 177–187.

Elion, E. A., Grisafi, P. L. and Fink, G. R. (1990). FUS3 encodes a $cdc2^{+}$/CDC28-related kinase required for the transition from mitosis into conjugation. *Cell* **60**, 649–664.

Enoch, T. and Nurse, P. (1990). Mutation of fission yeast cell cycle control genes abolishes dependence of mitosis on DNA replication. *Cell* **60**, 665–673.

Enoch, T. and Nurse, P. (1991). Coupling M phase and S phase: controls maintaining the dependence of mitosis on chromosome replication. *Cell* **65**, 921–923.

Evans, T., Rosental, E. T., Youngblom, J., Distel, D. and Hunt, T. (1983). Cyclin: a protein specified by maternal mRNA in sea urchin eggs that is destroyed at each cleavage division. *Cell* **33**, 389–396.

Featherstone, C. and Russell, P. (1991). Fission yeast $p107^{wee1}$ mitotic inhibitor is a tyrosine/serine kinase. *Nature* **349**, 808–811.

Felix, M. A., Cohen, P. and Karsenti, E. (1990*a*). Cdc2 H1 kinase is negatively regulated by a type 2A phosphatase in the *Xenopus* early embryonic cell cycle: evidence from the effects of okadaic acid. *EMBO J.* **9**, 675–683.

Felix, M. A., Labbe, J. C., Doree, M., Hunt, T. and Karsenti, E. (1990*b*). Triggering of cyclin degradation in interphase extracts of amphibian eggs by cdc2 kinase. *Nature* **346**, 379–382.

Ferrell, J. E., Wu, M., Gerhart, J. C. and Martin, G. S. (1991). Cell cycle tyrosine phosphorylation of $p34^{cdc2}$ and a microtubule-associated protein kinase homolog in *Xenopus* oocytes and eggs. *Molec. cell. Biol.* **11**, 1965–1971.

Forsburg, S. L. and Nurse, P. (1991). Identification of a G1-type cyclin $puc1^{+}$ in the fission yeast *Schizosaccharoyces pombe*. *Nature* **351**, 245–248.

Gomez, N. and Cohen, P. (1991). *Nature* (in press).

Gotoh, Y., Nishida, E., Matsuda, S., Shiina, N., Kosaka, H.,

SHIOKAWA, K., AKIYAMA, T., OHTA, K. AND SAKAI, H. (1991). *In vitro* effects on microtubule dynamics of purified *Xenopus* M phase-activated MAP kinase. *Nature* **349**, 251–254.
GOULD, K. L., MORENO, S., OWEN, D. J., SAZER, S. AND NURSE, P. (1991). Phosphorylation at Thr 167 is required for fission yeast $p34^{cdc2}$ function. *EMBO J.* (in press).
GOULD, K. L., MORENO, S., TONKS, N. K. AND NURSE, P. (1990). Complementation of the mitotic activator, $p80^{cdc25}$, by a human protein-tyrosine phosphatase. *Science* **250**, 1573–1576.
GOULD, K. L. AND NURSE, P. (1989). Tyrosine phosphorylation of the fission yeast $cdc2^{+}$ protein kinase regulates entry into mitosis. *Nature*. **342**, 39–45.
GUAN, K., BROYLES, S. S. AND DIXON, J. E. (1991). A Tyr/Ser protein phosphatase encoded by vaccinia virus. *Nature* **350**, 359–362.
HALL, F. L. AND VULLIET, P. R. (1991). Proline-directed protein phosphorylation and cell cycle regulation. *Curr. Opin. Cell Biol.* **3**, 176–184.
HANKS, S. K., QUINN, A. M. AND HUNTER, T. (1988). The protein kinase family: conserved features and deduced phylogeny of the catalytic domains. *Science* **241**, 42–52.
HOWELL, B. W., AFAR, D. E. H., LEW, J., DOUVILLE, E. M. J., ICELY, P. L. E., GRAY, D. A. AND BELL, J. C. (1991). STY, a tyrosine-phosphorylating enzyme with sequence homology to serine/threonine kinases. *Molec. cell. Biol.* **11**, 568–572.
HURLEY, J. H., DEAN, A. M., SOHL, J. L., KOSHLAND, D. E. AND STROUD, R. M. (1990). Regulation of an enzyme by phosphorylation at the active site. *Science* **249**, 1012–1016.
KARSENTI, E., VERDE, F. AND FÉLIX, M.-A. (1991). Role of type 1 and type 2A protein phosphatases in the cell cycle. *Adv. Protein Phosph.* **6**, 453–482.
KREK, W. AND NIGG, E. A. (1991*a*). Differential phosphorylation of vertebrate $p34^{cdc2}$ kinase at the G1/S and G2/M transitions of the cell cycle: identification of the major phosphorylation sites. *EMBO J.* **10**, 305–316.
KREK, W. AND NIGG, E. A. (1991*b*). Mutations of $p34^{cdc2}$ phosphorylation sites induce premature mitotic events in HeLa cells: evidence for a double block to $p34^{cdc2}$ kinase activation in vertebrates. *EMBO J.* (in press).
KUMAGAI, A. AND DUNPHY, W. G. (1991). The cdc25 protein controls tyrosine dephosphorylation of the cdc2 protein in a cell-free system. *Cell* **64**, 903–914.
LABBÉ, J.-C., CAPONY, J.-P., CAPUT, D., CAVADORE, J. C., DERANCOURT, J., KAGHAD, M., LELIAS, J.-M., PICARD, A. AND DORÉE, M. (1989). MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cyclin B. *EMBO J.* **8**, 3053–3058.
LEE, T. H., SOLOMON, M. J., MUMBY, M. J. AND KIRSCHNER, M. W. (1991). INH, a negative regulator of MPF, is a form of protein phosphatase 2A. *Cell* **64**, 415–423.
LEHNER, C. F. AND O'FARRELL, P. H. (1990). *Drosophila* cdc2 homologs: a functional homolog is coexpressed with a cognate variant. *EMBO J.* **9**, 3573–3581.
LUNDGREN, K., WALWORTH, N., BOOHER, R., DEMBSKI, M., KIRSCHNER, M. AND BEACH, D. (1991). mik1 and wee1 cooperate in the inhibitory tyrosine phosphorylation. *Cell* **64**, 1111–1122.
MALLER, J. L. (1991). Mitotic control. *Curr. Opin. Cell Biol.* **3**, 269–275.
MEIJER, L., AZZI, L. AND WANG, J. Y. J. (1991). Cyclin B targets $p34^{cdc2}$ for tyrosine phosphorylation. *EMBO J.* **10**, 1545–1554.
MINSHULL, J., GOLSTEYN, R., HILL, C. S. AND HUNT, T. (1990). The A- and B-type cyclin associated cdc2 kinases in *Xenopus* turn on and off at different times in the cell cycle. *EMBO J.* **9**, 2865–2875.
MORENO, S. AND NURSE, P. (1990). Substrates for $p34^{cdc2}$: *In vivo* veritas. *Cell* **61**, 549–551.
MORENO, S. AND NURSE, P. (1991). Clues to action of cdc25 protein. *Nature* **351**, 194.
MORENO, S., NURSE, P. AND RUSSELL, P. (1990). Regulation of mitosis by cyclic accumulation of p80cdc25 mitotic inducer in fission yeast. *Nature* **344**, 549–552.
NORBURY, C., BLOW, J. J. AND NURSE, P. (1991). Regulatory phosphorylation of the p34cdc2 protein kinase in vertebrates. *EMBO J.* (in press).
NURSE, P. (1990). Universal control mechanism regulating onset of M-phase. *Nature* **344**, 503–508.
PARIS, J., LE, G. R., COUTURIER, A., LE, G. K., OMILLI, F., CAMONIS, J., MACNEILL, S. AND PHILIPPE, M. (1991). Cloning by differential screening of a *Xenopus* cDNA coding for a protein highly homologous to cdc2. *Proc. natn. Acad. Sci. U.S.A.* **88**, 1039–1043.
PARKER, L. L., ATHERTON-FESSLER, S., LEE, M. S., OGG, S., FALK, J., SWENSON, K. I. AND PIWNICA-WORMS, H. (1991). Cyclin promotes the tyrosine phosphorylation of p34cdc2 in a $wee1^{+}$ dependent manner. *EMBO J.* **10**, 1255–1263.
PAYNE, D. M., ROSSOMONDO, A. J., MARTINO, P., ERICKSON, A. K., HER, J.-H., SHABANOWITZ, J., HUNT, D. F., WEBER, M. J. AND STURGILL, T. W. (1991). Identification of the regulatory phosphorylation sites in pp42/mitogen-activated protein kinase (MAP kinase). *EMBO J.* **10**, 885–892.
PINES, J. AND HUNTER, T. (1990*a*). Human cyclin A is adenovirus E1A-associated protein p60 and behaves differently from cyclin B. *Nature* **346**, 760–763.
PINES, J. AND HUNTER, T. (1990*b*). $p34^{cdc2}$: The S and M kinase. *The New Biol.* **2**, 389–401.
RICHARDSON, H. E., WITTENBERG, C., CROSS, F. AND REED, S. I. (1989). An essential G1 function for cyclin-like proteins in yeast. *Cell* **59**, 1127–1133.
ROY, L. M., SINGH, B., GAUTIER, J., ARLINGHAUS, R. B., NORDEEN, S. K. AND MALLER, J. L. (1990). The cyclin B2 component of MPF is a substrate for the c-*mos*(xe) proto-oncogene product. *Cell* **61**, 825–831.
RUSSELL, P., MORENO, S. AND REED, S. I. (1989). Conservation of mitotic controls in fission and budding yeasts. *Cell* **57**, 295–303.
RUSSELL, P. AND NURSE, P. (1986). $cdc25^{+}$ functions as an inducer in the mitotic control of fission yeast. *Cell* **45**, 145–153.
RUSSELL, P. AND NURSE, P. (1987*a*). The mitotic inducer nim1+ functions in a regulatory network of protein kinase homologs controlling the initiation of mitosis. *Cell* **49**, 569–576.
RUSSELL, P. AND NURSE, P. (1987*b*). Negative regulation of mitosis by $wee1^{+}$, a gene encoding a protein kinase homolog. *Cell* **49**, 559–567.
SADHU, K., REED, S. I., RICHARDSON, H. AND RUSSELL, P. (1990). Human homolog of fission yeast cdc25 mitotic inducer is predominantly expressed in G2. *Proc. natn. Acad. Sci. U.S.A.* **87**, 5139–5143.
SANGHERA, J. S., PADDON, H. B., BADER, S. A. AND PELECH, S. L. (1990). Purification and characterization of a maturation-activated myelin basic protein kinase from sea star oocytes. *J. biol. Chem.* **265**, 52–57.
SEGER. R., AHN, N. G., BOULTON, T. G., YANCOPOULOS, G. D., PANAYOTATOS, N., RADZIEJEWSKA, E., ERICSSON, L., BRATLIEN, R. L., COBB, M. H. AND KREBS, E. G. (1991). *Proc. natn. Acad. Sci. U.S.A.* **88**, 6142–6146.
SOLOMON, M. J., GLOTZER, M., LEE, T. H., PHILIPPE, M. AND KIRSCHNER, M. W. (1990). Cyclin activation of p34cdc2. *Cell* **63**, 1013–1024.
SREULI, M., KREUGER, N. X., THAI, T., TANG, M. AND SAITO, H. (1990). Distinct functional roles of the two intracellular phophatase like domains of the receptor-linked protein tyrosine phosphatases LCA and LAR. *EMBO J.* **9**, 2399–2407.
STAUSFELD, U., LABBÉ, J. C., FESQUET, D., CAVADORE, J. C., PICARD, A., SADHU, K., RUSSELL, P. AND DORÉE, M. (1991). Dephosphorylation and activation of a p34cdc2/cyclin B complex *in vitro* by human CDC25 protein. *Nature* **351**, 242–245.
STERN, D. F., ZHENG, P., BEIDLER, D. R. AND ZERILLO, C. (1991). Spk1, a new protein kinae from *Saccharomyces cerevisiae*, phosphorylates proteins on serine, threonine and tyrosine. *Molec. cell. Biol.* **11**, 987–1001.
STURGILL, T. W. AND WU, J. (1991). Recent characterization of protein kinase cascades for phosphorylation of ribosomal protein S6. *Biochim. biophys. Acta* **1092**, 350–357.
TONKS, N. K. (1990). Protein phosphatases: key players in the regulation of cell function. *Current Opinion in Cell Biology* **2**, 1114–1124.
TONKS, N. K. AND CHARBONNEAU, H. (1989). Protein tyrosine dephosphorylation and signal transduction. *Trends Biochem. Sci.* **14**, 497–500.
WITTENBERG, C., SUGIMOTO, K. AND REED, S. I. (1990). G1-specific cyclins of *S. cerevisiae*. Cell cycle periodicity, regulation by mating pheromone and association with the $p34^{CDC28}$ protein kinase. *Cell* **62**, 225–237.

(Received 15 July 1991 – Accepted 13 August 1991)