CELL CYCLE CONTROL PART I
cdc related kinases

M. AKIF ÖZTÜRK*
MUSTURAY KARÇAALTINCABA*
WAYNE E. CRISS*

SUMMARY : The central and rate-limiting function in the cell cycle control is performed by $p34^{cdc2}$ kinase in lower organisms, and by $p33^{cdk2}$ and $p34^{cdc2}$ in higher organisms. This control is highly conserved throughout evolution. The activities of these kinases are regulated by phosphorylation and dephosphorylation as well as interaction with the cyclins during different phases of the cell cycle. The product $p34^{cdc2}$ regulates both G1/S and G2/M transitions in yeast. While it is generally accepted that cdk2 plays a critical direct role at G1/S transition in mammalian cells. However, the functions of human cdc2-related kinases, other than cdk2 and cdk3, remain obscure. Certainly the active kinase forms phosphorylate a large number of proteins and enzymes involved in nuclear and nucleolar structures, cellular cytoskeletal systems, and DNA spindle fiber associated relations during mitosis. All of these are logical targets for cell cycle control, or at least its last stage, mitosis.

Key Words : Cell cycle, $p34^{cdc2}$, cdks, mitosis, protein kinases.

INTRODUCTION

Over the past few years there has been a resurgence of interest in the cell cycle. The excitement has mainly been due to the fact that scientists all over the world who have been working on seemingly different processes in yeast, fruit flies (drosophila), marine invertebrates, frogs and man have found that many of the cell cycle processes and involved proteins have been highly conserved. In order to provide a broader perspective to this field, this review covers not only cell cycle regulation in mammalian cells but also what is known about similar mechanisms in other organisms.

The cell cycle is composed of four phases and G-0 (non-cycling); G1 (gap 1), S (DNA synthesis), G2 (gap 2), M (mitosis). During the gap phases, although not necessarily throughout them, information is integrated in order to determine the readiness of a cell to enter either the S or M phase. The molecular events that underlie these crucial cell-cycle decisions have only recently begun to become apparent. At G-0 cells do not divide; they remain quiescent. However it is understood that there are no clear cut boundaries between the phases of cell cycle. G1 activities, which are preparatory for the S phase, may begin during the previous cycle, and occur concurrently with G2 and mitotic events. Similarly, early molecular alterations for mitosis may overlap with S (1,2).

G1 CONTROL

G1 events appear to occur sequentially in a cause-effect relation. Temperature sensitive mutant cells are arrested at different positions in G1. These cells are specifically blocked at various points during progression through G1, and thereby provide a means to sequence metabolic events (2). Probably these points include such targets as oncogenes, suppressor genes and anti-oncogenes. Thus G1 has been divided into sub-phases depending upon the effects of limiting growth factors, nutrients or inhibitors as measured by time to reach the S phase after the block is

*From Department of Biochemistry and Institute of Oncology, Hacettepe University, Medical School, Ankara, Türkiye.

removed (3). These sub-phases are referred as competence, entry, progression and assembly (Table 1). A restriction point between progression and assembly allows cells to become independent of growth factors. In yeast, there is a similar phenomenon called START (4). $p34^{cdc2}$ (cdc stands for cell division cycle) regulates START in yeast, but not in mammalian cells. However a homologue of this protein kinase may be present in mammalian cells. In recent years, cyclins have gained importance in control of G1.

Table 1: Subphases of G1 for fibroblasts.

Subphases competence	Required GFs PDGF	Important Events chromatin changes, increased nutrient transport, novel mRNA production.
entry	EGF and insulin	macromolecule synthesis, polysomes, glycolytic enzymes increased
progression	IGF-1	DNA synthesis enzymes are activated and induced.
assembly	-	replicative enzymes are transported into the nucleous and organized into a complex that catalyze DNA synthesis.

M PHASE CONTROL

Because the protein products of the cdc2 gene probably play a direct role in the M phase of the cell cycle, we will first describe the current data in this phase.

The onset of M phase is regulated by a mechanism common to all eukaryotic cells. Entry into the M phase is determined by activation of $p34^{cdc2}$ (serine/threonine) kinase which requires both a dephosphorylation and a physical association with a cyclin protein. Once cells enter M phase they complete mitosis in a very short time interval. Thus the critical control point is the onset of M phase. There seems to be one special protein complex that is required. It is called the M phase promoting factor (MPF) (5,7-12). It consists of cyclin B and $p34^{cdc2}$ proteins. MPF subunits are highly conserved in eukaryotic cells which implies that they play a key role in the requirement of mitosis by all cells. Microinjection of antibodies against $p34^{cdc2}$ was found to block entry into mitosis in fibroblasts (6). More genetic proof for the essential mitotic function of cdc2 was obtained through the rescue of the FT210 defect with a transfected wild type cdc2 gene (7).

THE ROLE OF $p34^{cdc2}$ G2/M TRANSITION

The accumulation of cyclin B during S and G2 is a gradual process; but the onset of mitosis is an abrupt and dramatic event. Sufficient cyclin B to support M phase entry is synthesized relatively early in the cycle. A pool of inactive $p34^{cdc2}$/cyclin B heterodimers, called pre-MPF, accumulate before being rapidly activated to effect the G2/M transition (7,8). The phosphorylation state of $p34^{cdc2}$ is clearly important during pre-MPF accumulation and its subsequent activation. Fission yeast S. pompe was found to be phosphorylated at Tyr 15, which lies within the nucleotide binding domain (7-9). Whereas in yeast cells entering mitosis, Tyr 15 was found to be rapidly dephosphorylated, thus leading to activation of $p34^{cdc2}$ protein kinase activity (8). In addition, it was observed that a mutant ^{cdc2}, which cannot be phosphorylated because of a substitution of phenylalanine for tyrosine at position 15, allows a premature onset of mitosis (7-10). This suggests that Tyr 15 phosphorylation normally functions to delay the M phase entry by preventing $p34^{cdc2}$ activation. It is thought that Tyr 15 phosphorylation prevents ATP binding by stearic hindrance or an electrostatic repulsion (9).

$p34^{cdc2}$ protein kinase activity is inhibited by wee-1 kinase. In wee-1 mutants, advancement of mitosis was observed (11). In addition, it was shown that phosphorylation of Tyr 15 is catalyzed by the wee-1 and also by mik-1 kinase, which were first identified in S. pompe (7,10,12,13). The mammalian homologue of wee-1 has now been isolated (7). The product of the nim-1 gene acts as a negative regulator of wee-1, but it is not known whether it also inhibits mik-1 (10,13).

Cyclic accumulation of the cdc25 gene product, $p80^{cdc25}$ and its corresponding mRNA are thought to play a role in regulating cdc2 gene function. The cdc25 phosphatase activity oscillates in both meiotic and mitotic cell cycles, being low in interphase and high in M-phase (14). Addition of the purified bacterially expressed product of the human homologue of the fission yeast cdc25 gene (p54cdc25H) triggers $p34^{cdc2}$ dephosphorylation and activates H1 histone kinase activity in inactive $p34^{cdc2}$/cyclin B preparations, which are purified from G2 arrested starfish oocytes (15). Therefore, the activation of $p34^{cdc2}$ depends

upon the activity of the cdc25 gene product, $p80^{cdc25}$. In addition, the cdc25 gene of S. pompe codes for a kinase which may phosphorylate tyrosine and hence activate $p34^{cdc2}$, allowing cells to exit from G2/M. cdc25 mutants, which are arrested in G2, can be rescued by a Tyr-specific phosphatase (10,13). Thus it is thought that cdc25 works via a pathway which activates the phosphatase. Again, cdc25 phosphatase is conserved from yeast to man (12,13) and the higher cells contain multiple cdc25 related genes (12).

Additional phosphorylation on Thr 14 also suppresses $p34^{cdc2}$ activity in interphase in eukaryotic cells, but the kinase for Thr 14 has not been identified (7,12). So, activation of the pre-MPF in higher cells requires the dephosphorylation of both Tyr 14 and Thr 15, and this is catalyzed by the cdc25 phosphatase. In vitro, cdc25 phosphatase can remove the phosphate on both Tyr 14 and Thr 15 in the pre-MPF (12).

The mechanism of MPF amplification, rapid conversion of the pre-MPF pool to an active state without the need for further protein synthesis has yet to be fully elucidated. But it seems likely that the generation of a small amount of active MPF feeds back positively to one or more of the pathways controlling $p34^{cdc2}$ phosphorylation and dephosphorylation at Thr 14 and Tyr 15, leading to rapid loss of phosphate from these two positions (7).

However, dephosphorylation alone is not sufficient to activate cdc2 kinase. The phosphorylation of another conserved Thr residue (Thr 167 in yeast cdc2 and Thr 161 in human cdc2) is also required (12). In $p34^{cdc2}$ mutations of Thr 167 to Ala or Val result in loss of activity, as assayed by the inability of the mutated gene to rescue a temperature-sensitive mutation of the wild type gene (9). Recently, it has been confirmed that Thr 167 is phosphorylated in vivo (9). Phosphorylation of this Thr is catalyzed by a newly defined kinase, CAK (cdc2/cdk2 activating kinase). $p40^{MO15}$, the protein product of the previously identified Xenopus MO15 gene, is speculated to be the functional subunit of CAK, because $p40^{MO15}$ co-purifies with CAK. In addition, antiserum to $p40^{MO15}$ specifically depleted CAK activity (16).

Mutation of Thr 161 to an aspartic acid residue generated a protein which bound markedly reduced levels of cyclin B. This protein did not become phosphorylated on Thr 14 and Tyr 15 (8). However the double mutant Ala 14 Phe 15, though it cannot be phosphorylated in its ATP-binding region, could still bind cyclin B (8). This implies that $p34^{cdc2}$ undergoes a conformational change upon binding cyclin B, which results in an exposure of residues 14 and 15 to analogues of wee-1 and mik-1 gene products. It was also shown that $p107^{wee-1}$ was not able to phosphorylate purified $p34^{cdc2}$ in vitro (17). This suggests that if $p34^{cdc2}$ is the target in vivo, it must be presented in a special conformation, perhaps as a result of cyclin B binding. Regardless of the mechanism, phosphorylation of Thr 14 and Tyr 15, immediately after assembly of the pre-MPF heterodimer, facilitates its accumulation in an inactive form. Interestingly, the phosphorylation of Thr 167 appeared, like the phosphorylation of Thr 14 and Tyr 15, to depend upon the presence of cyclin B. Therefore phosphorylation may be important for stabilizing an otherwise transient association between $p34^{cdc2}$ and cyclin B (7).

At the G2/M transition, we must discuss the role of $p34^{cdc2}$ in the linking of the completion of DNA replication to the onset of the subsequent M phase. In most eukaryotic cells, entry into mitosis is delayed or blocked by the presence of unreplicated or damaged DNA (18-20). Using Xenopus egg extracts, it was shown that incomplete DNA replication blocks activation of pre-MPF, probably by preventing dephosphorylation of $p34^{cdc2}$ at Tyr 15 and possibly also Thr 14. This occurs because a mutant which cannot be phosphorylated at residues 14 and 15 escapes this inhibition (8). Blockage of MPF activation was also overcome by the addition of the protein product of the Drosophila cdc25 homologue (8). Brief treatment of early S-phase HeLa cells with camptothecin, an inhibitor of DNA topoisomerase 1, caused arrest at the G2 phase (18). Similarly, a brief exposure to etoposide during G2 phase, which induces DNA strand breaks in mammalian cells via interaction with topoisomerase 2, inhibits mitotic progression (19). In addition, dependence of mitosis on DNA synthesis is lost in wee-1 mutants in which cdc25 control is circumvented either by mutations in cdc2 or by overproduction of cdc25 (11). So, it is proposed that cdc25 activity requires completion of earlier cell-cycle events such as DNA synthesis, and thus links $p34^{cdc2}$ kinase activation to completion of these earlier events.

The signal transmitted from unreplicated DNA has not been identified, although it is likely to involve the chromatin associated protein, RCC1. After S phase, once RCC1

function was lost, $p34^{cdc2}$ was dephosphorylated and the M-phase specific histone H1 kinase was activated. However, in G1 phase, shifting to the non-permissive temperature did not activate $p34^{cdc2}$ histone H1 kinase. So, the normal inhibition of $p34^{cdc2}$ activation by incompletely replicated DNA is lost by the lack of RCC1 (21). Thus, the status of DNA replication may act upon other protein kinases and phosphatases to control the activity of wee-1 or cdc25, which in turn controls the activity of pre-MPF.

The pathways that link the DNA replication to pre-MPF activation should include the rad genes, because mutation in a number of rad genes allow cells with damaged or unreplicated DNA to enter mitosis (20,22). A novel fission yeast protein kinase called chk-1 (checkpoint kinase) is suggested to link the rad checkpoint pathway to cdc2, because multiple copies of chk-1 partially rescue the ultraviolet sensitivity of rad1-1, mutant deficient checkpoint control (22).

G1/S TRANSITION

In addition to arresting in G2, conditional cdc2 mutants of fission yeast also become arrested in G1 at the restrictive temperature (12). Furthermore, cloning of the human cdc2 homologue by functional complementation of a fission yeast cdc2 mutant demonstrated that this human gene function can fully substitute for the yeast counterpart in both its G1 (START) and G2/M roles (7). The cdc2 homologue in the budding yeast S. cerevisae, cdc28, is essential for progression through START. This is analogous to the G1 restriction point of animal cells (7,10,12). Hence, the product of a single gene regulates both G1/S and G2/M in yeasts.

However, the role of mammalian cdc2 for the G1/S transition has not been firmly established. There are experiments which support that $p34^{cdc2}$ is also responsible for G1/S transition in higher eukaryotes. Inhibition of the phosphorylation of $p34^{cdc2}$ by interferon-alpha caused down regulation of cdc2 mRNA in the human B-cell line Daudi, and blocked the growth of the cell in the G1 (G0) phase of the cell cycle; cells did not enter S phase (23). If $p34^{cdc2}$ synthesis is blocked with antisense oligonucleotides, then quiescent human T lymphocytes stimulated with mitogen do not enter S phase (7,10,12). However, an identical antisense oligonucleotide cannot block the proliferation of primary fibroblasts or established lines (12). The FT210 cells do not become arrested at G1/S at 39°C, while they do become arrested in G2 at this temperature. This indicates that the mammalian cdc2 is dispensable for G1 progression (7,12).

Higher eukaryotic cells do have a large number of cdc-related kinases. A prominent cdc-related kinase has been cloned from Xenopus and man. This protein of 33 kDa is called the cdk2 kinase (cdk stands for cyclin-dependant kinase;cdc2=cdk1). It is generally accepted that cdk2 plays a critical role at G1/S, largely based on the circumstantial evidence that cdk2 becomes activated around the G1/S transition in mammalian cells (12).

The cdk2 from HeLa cells contains three major tryptic phosphopeptides. The two major phosphorylation sites are Tyr 15 and Thr 160. Additional phosphorylation probably occurs on Thr 14. Replacement of Thr 160 with alanine abolishes the kinase activity of cdk2, indicating that phosphorylation at this site, as in cdc2, is required for kinase activity (24). This phosphorylation is also catalyzed by $p40^{MO15}$, similar to $p34^{cdc2}$ (25). Mutations at Tyr 15 or Thr 14 stimulate kinase activity. This implies that phosphorylation at this sites, as in cdc2, is inhibitory. Similarly, cdk2 is activated in vitro by dephosphorylation of Thr 14 and Tyr 15 by the phosphatase cdc25 (24).

The cdk2 kinase in Xenopus egg extracts has also been shown to have an essential S-function. Immunodepletion of cdk2 causes a dramatic decrease in the rate of DNA synthesis, but the depletion of cdc2 has little effect under the experimental conditions (7,12).

$p33^{cdk2}$ is a component of the E2F transcription factor-cyclin A complex. This complex accumulates during the S phase of the cell cycle and possesses H1 kinase activity. This suggests that one role of the E2F factor may be to localize the cdk2 kinase to DNA, and cdk2 kinase may then phosphorylate other DNA-bound substrates (26).

OTHER $p34^{cdc2}$ RELATED KINASES

There are newly identified 9 human protein kinases other than cdk2. This identification is based on their structural relationship to $p34^{cdc2}$: cdk3, PSSALRE (also called cdk5), PCTAIRE-1, PCTAIRE-2, PCTAIRE-3, PLSTIRE, PSK-J3 (also called cdk4), p58-GTA and KKIALRE. The identification of these kinases is not unique to human cells, as homologues have now been described in several other species (27).

The cdk3 gene, like cdc2 and cdk2, can complement cdc28 mutants of S. cerevisae, suggesting that it can play a role, in the regulation of the mammalian cell cycle (27).

Complexes of cdk4 and each of three different D-type cyclins were assembled in the insect Sf9 cells. They phosphorylated a pRb fusion protein at sites identical to those phosphorylated in human T cells. The product of the retinoblastoma gene prevents S-phase entry during the cell cycle, and inactivation of this growth-suppressive function is presumed to result from pRb hyper-phosphorylation during late G1 phase (28). cdk4 is a cyclin D-regulated catalytic subunit that acts as a Rb kinase, but does not have H1 kinase activity (29). At present, there is no evidence that any of the human cdc2-related kinases, other than cdc2, cdk2 and cdk3, are involved in cell cycle regulation (27).

PHOSPHATASES AS REGULATORS OF MPF

When BHK21 cells, which were synchronized in early S phase, were exposed to okadaic acid (an inhibitor of protein phosphatases 1 and 2A) mitosis specific events were induced. These events then disappeared upon further incubation. Within 1 hour of exposure to okadaic acid, cdc2/histone H1 kinase activity rose 10-fold, but returned to the control level upon further incubation. Dephosphorylation of the $p34^{cdc2}$/cyclin B complex, after okadaic acid treatment, was concomitant with the activation of cdc2 kinase; and subsequent degradation of cyclin B was concomitant with a decrease in cdc2 kinase activity (30).

A crude fractionation of oocyte extracts identified a protein fraction, termed INH, that inhibited activation of pre-MPF (7,13). It was demonstrated that INH was a form of protein phosphatase 2A (13,31). Both can directly inactivate an isolated $p34^{cdc2}$/cyclin B complex (31), and furthermore both are inhibited by incubation with okadaic acid (7).

The observation of premature mitotic features upon inhibition of phosphatase 2A indicates that there is a positive requirement for this activity to suppress MPF during interphase. This could be an indirect effect that promotes phosphorylation of $p34^{cdc2}$ on Thr 14 and Tyr 15. Treatment of cdc25 with either phosphatase 1 or phosphatase 2A removes the phosphate from cdc25, and decreases its ability to activate cdc2 kinase (14).

However, inhibition of type 1 phosphatase by the specific peptide inhibitors 1 and 2 did not cause premature MPF activation in mitotic egg extracts, but blocked maturation. Genetic experiments show that type 1 phosphatases have additional roles in late mitosis (7).

$p13^{suc-1}$ is also a conserved protein in evolution. Two homologues of the suc-1 gene have recently been cloned from human cells (13). Although genetic and biochemical evidence suggest close interactions between cyclins, $p13^{suc-1}$ and $p34^{cdc2}$ kinase, the roles of $p13^{suc-1}$ on $p34^{cdc2}$ function remains unclear. It was originally identified as a DNA sequence that, at high copy number, rescued S. pompe strains carrying certain temperature-sensitive alleles of the cdc2 cell cycle control gene (32). When the suc-1 gene of S. pompe was disrupted, cells were capable of very limited cell division. Later many cells become highly elongated. However, overproduction of $p13^{suc-1}$ also caused a delay in cell division (32). Similarly, microinjection of $p13^{suc-1}$ into mammalian cells blocked cell division (9).

In vitro, $p13^{suc-1}$ suppresses the catalytic function of $p34^{cdc2}$ (33). $p13^{suc-1}$ has not been identified as a component of MPF, but yeast p13 can directly bind frog MPF, inhibit the activation of histone H1 kinase, and also inhibit tyrosine dephosphorylation of $p34^{cdc2}$. It had no effect on the $p34^{cdc2}$ histone H1 kinase once it was activated (13). These results suggest a possible role for $p13^{suc-1}$ in the maintenance of pre-MPF. Yet, the biochemical role of $p13^{suc-1}$ remains obscure.

FUNCTIONS OF $p34^{cdc2}$

$p34^{cdc2}$ phosphorylates substrates in vitro on serine and threonine residues which have the consensus sequence S/T-P-X-Z (X is polar, Z is a basic amino acid) (10). Candidate substrates for MPF are more likely to be physiologically significant if these sites become phosphorylated or hyper-phosphorylated in M phase, and if a functional consequence of this phosphorylation for an M phase process can be identified.

Using these criteria, the best qualified substrates so far described for $p34^{cdc2}$ in animal cells are nuclear laminins, vimentin and caldesmon (7).

Laminins A, B and C are intermediate filament proteins that polymerize and generate a nuclear lamina, a structure that underlines the nuclear membrane in inter-

phase. Hyper-phosphorylation of laminins coincides with the disassembly of the nuclear lamina and the nuclear envelope breakdown (7,10,13,35). In addition, mutation of two of the phosphorylation sites of laminin A to non-phosphorylatable residues generated mutant laminin protein that prevented nuclear disassembly (12,13).

Vimentin is a constituent of the intermediate filament network that extends throughout the cytoplasm in higher eukaryotic cells. Caldesmon is a non-structural component of the cytoplasmic microfilaments that is thought to inhibit the actomyosin ATPase of these filaments. Reorganization of the cytoskeleton during M phase may result in part from phosphorylation of caldesmon and vimentin. Like the laminins, these proteins are subject to mitosis-specific phosphorylation at a number of sites, a subset of which can be phosphorylated by $p34^{cdc2}$ in vitro (7). A highly purified $p34^{cdc2}$/cyclin B caused de-polymerization of vimentin intermediate filaments in vitro. Similarly, phosphorylation in vitro by $p34^{cdc2}$ reduced the affinity of caldesmon for binding with actin and calmodulin. Dissociation of caldesmon from actin is thought to promote contraction of microfilaments, vital to the contractile ring function during cytokinesis, and possibly is also involved in the 'rounding up' characteristics of mitosis in many animal cells (7).

In addition to the putative in vivo substrates described above, there are a number of less well understood candidate substrates for $p34^{cdc2}$ (Table 2).

Histone H1 is a substrate of $p34^{cdc2}$ in vitro and it is widely used in assays for M phase $p34^{cdc2}$ kinase activity (13). The subunits of histone H1 kinase have been shown to be cyclins and the $p34^{cdc2}$ (34). Histone H1 is thought to play a role in chromosome condensation and gene inactivation, so it is probable that all of the processes that control chromosome structure-function relationships are also involved in the control of the cell cycle. In addition, maximal levels of histone H1 phosphorylation are clearly associated with chromosome condensation in P. polycephalum (35). But no biologic consequence of M phase phosphorylation of histone H1 has been observed (7).

It was shown in vivo that threonine phosphorylation of nucleolin was catalyzed by $p34^{cdc2}$ during mitosis. Nucleolin is associated with nucleolar organizer regions on metastatic chromosomes during mitosis and its phosphorylation by $p34^{cdc2}$ is related to mitotic reorganization of nucleolar chromatin (36).

$p34^{cdc2}$ phosphorylation of src is associated with increased src kinase activity (12,13,35,42). This kinase activity has been speculated to have an effect on events that occur during cell division, i.e. microfilament arrangement and cytoskeletal changes (10,13,43). However, mitosis specific phosphorylation does not appear to activate

*Table 2: Substrates of $p34^{cdc}$

Substrates	Possi. sites[a]	Possible Role[b]	Reference (s)
Nuclear Laminins	Yes (M)[c]	Nuclear lamina disassembly	7,10,13,35
Vimentin	Yes (M)	Intermediate filament disassembly	7
Caldesmon	Yes (M)	Microfiyalehk contraction	7,13
Histone H1	Yes (M)	(Chromosome condensation)	13,35
pp60-scr	Yes (M)	(Cytoskeletal Rearrengement)	12,13,35,43
NO38, Nucleolin	Yes (M)	(Nuclear reorganization)	7,13
SV40T Antigen	Yes	(DNA replication)	7,13
c-Abl	Yes (M)	Unknown	12,13
p105-RB	Yes	Unknown	44,45
p53	Yes	Unknown	46
RNA polymerase II	Unknown	(Transcription inhibition)	7,13
rab	Yes	(Inhibition of endocytosis)	10,38
EF-1 gamma	Unknown	(Trancslation inhibition)	7,39
Cyclin B	Unknown	(Regulation of $p34^{cdc2}$)	7-13
Myosin light chain	Unknown	(Contractile ring activation)	7
Casein Kinase II	Unknown	Unknown	7

a: Are the same sites phosphorylated in vivo as in vitro?
b: Roles proposed but as yet largely speculative are indicated in parentheses
c: Substrates which are hyperphosphorylated in vivo during mitosis are denoted (M).
* modified from reference number 7.

the c-Abl tyrosine kinase, but results in inhibition of its DNA binding which is necessary for proper condensation of the chromatin (12). Similarly, proximity of the $p34^{cdc2}$ phosphorylation sites of RNA polymerase II, histone H1, nucleolin and NO38 to their DNA binding domains suggests that phosphorylations of these sites facilitate chromosome condensation by weakening the DNA binding properties of these proteins (13).

Components of centrosomes are among the cellular proteins that are phosphorylated at the transition from interphase to mitosis. The enzyme which is associated with CHO cell mitotic spindles during mitosis was extracted. It was capable of phosphorylating many spindle components. Such extracts contain protein kinases, including $p34^{cdc2}$. This suggests that the enzyme responsible for centrosomal phosphorylation could be $p34^{cdc2}$ or other downstream mitotic kinases which may be activated by the action of $p34^{cdc2}$ (37).

It has recently been shown that $p34^{cdc2}$ also phosphorylates two GTP binding proteins of the rab family. This apparently contributes to vesiculation and membrane transport, both in vitro and in vivo, suggesting that $p34^{cdc2}$ may also inhibit endocytosis during mitosis by regulating transport between the endoplasmic reticulum and golgi (10,38).

The $p34^{cdc2}$/cyclin complex may serve several alternative functions in addition to control of cell division. It may participate in signal transduction in non-proliferating, terminally differentiated cells. With immunologic studies, $p34^{cdc2}$ was detected in the sheep platelet cytosol in a complex with cyclin A. This complex was regulated during platelet activation. Its histone H1 phosphorylating activity was stimulated two-fold in p13suc-1 sepharose extracts from platelets that had been exposed to platelet-activating factor for thrombin for one minute prior to harvesting (40).

Another interesting function of $p34^{cdc2}$, other then cell cycle control, is related to the etiology of Alzheimer's disease. It has been suggested that hyper-phosphorylation of the tau protein in neurofibrillary tangles may be relevant to the etiology of Alzheimer's disease, and that at least one of the hyper-phosphorylated sites lies within a consensus sequence for the $p34^{cdc2}$, cdc28 family of kinases. Recently it was shown that the purified $p34^{cdc28}$ kinase from S. cerevisae can phosphorylate bovine and human tau (41).

REFERENCES

1. Müller T, Kurtz A, Grosse R, et. al. : A mammary derived growth inhibitor related 70 kDa antigen identified in nuclei of mammary epithelial cells. J Cell Physio, 138:415-423, 1989.

2. Baserga R : The biology of cell production. Harvard University Press, Cambridge, MA (First ed, 1985).

3. Pardee AB : G1 events and regulation of cell progression. Science, 246:603-608, 1989.

4. Hartwell L : Twenty-five years of cell cycle genetics. Genetics, 129:975-980, 1991.

5. Langan TA, Gautier J and Sclafani RA : Mammalian growth associated H1 histone kinase, a homologue of cdc2/cdc28 protein kinase. Mol Cell Biol, 9:3860-3868, 1989.

6. Riabowol K, Draetta G, Briuzela L, et. al. : The cdc2 kinase is a nuclear protein essential for mitosis in mammalian cells. Cell, 57:393-401, 1989.

7. Norbury C and Nurse P : Animal cell cycles and their control. Annu Rev Biochem, 61:441-470, 1992.

8. Norbury C, Blow J and Nurse P : Regulatory phosphorylation of the p34cdc2 protein kinase in vertebrates. EMBO J, 10:3321-3329, 1991.

9. Draetta G : Cell cycle control in eukaryotes : Molecular mechanisms of cdc2 activation. Trends Biochem Sci, 15:378-383, 1990.

10. Bymee A and Thomas NSB : Cell cycle regulation. Blood Rev, 5:177-192, 1991.

11. Enoch T and Nurse P : Mutation of fission yeast cell cycle control genes abolishes dependence of mitosis on DNA replication. Cell, 60:665-673, 1990.

12. Wang JYJ : Oncoprotein phosphorylation and cell cycle control. Biochimica et Biophysica Acta, 1114:179-192, 1992.

13. Freeman RS and Donoghue DJ : Protein Kinases and Protooncogenes: Biochemical Regulators of the Eukaryotic Cell Cycle. Biochemistry, 30:2293-2302, 1991.

14. Izumi T, Walker DH and Maller JL : Periodic changes in phosphorylation of the Xenopus cdc25 phosphatase regulate its activity. Mol Biol Cell, 3:927-939, 1992.

15. Strausfeld U, Labbe JC, Fesquet D, et. al. : Dephosphorylation and activation of a p34cdc2/cyclin B complex in vitro by human CDC25 protein. Nature, 351:242-245, 1991.

16. Solomon MJ, Harper JW and Shuttleworth J : CAK, the p34cdc2 activating kinase, contains a protein identical or closely related to p40MO15. EMBO J, 12:3133-3142, 1993.

17. Featherstone C and Russell P : Fission yeast p107weel-1 mitotic inhibitor is a tyrosine/serine kinase. Nature, 349:808-811, 1991.

18. Tsao YP, D'Arpa P and Liu LF : The involvement of active DNA synthesis in camptothecin-induced G2 arrest : Altered regulation of p34cdc2/cyclin B. Cancer Research, 52:1823-1829, 1992.

19. Lock RB : Inhibition of p34cdc2 kinase activation, p34cdc2 tyrosine dephosphorylation, and mitotic progression in Chinese hamster ovary cells exposed to etoposide. Cancer Research, 52:1817-1822, 1992.

20. Murray AW : Creative blocks: cell-cycle checkpoints and feedback controls. Nature, 359:599-604, 1992.

21. Nishitani H, Ohtsubo M, Yamashita K, et. al. : Loss of RCC1, a nuclear DNA binding protein, uncouples the completion of DNA replication from the activation of cdc2 protein kinase and mitosis. EMBO J, 10:1555-1564, 1991.

22. Walworth N, Davey D and Beach S : Fission yeast chk1 protein kinase links the rad checkpoint pathway to cdc2. Nature, 363:368-371, 1993.

23. Thomas NS : Regulation of the product of a possible human cell cycle control gene CDC2Hs in B-cell by alpha-interpheron and phorbol ester. J Biol Chem, 264:13697-13700, 1989.

24. Gu Y, Rosenblatt J and Morgan DO : Cell cycle regulation of CDK2 activity by phosphorylation of Thr 160 and Tyr 15. EMBO J, 11:3995-405, 1992.

25. Poon RYC, Yamashita K and Adamczewski JP : The cdc2-related protein P40MO15 is the catalytic subunit of a protein kinase that can activate p33cdk2 and p34cdc2. EMBO J, 12:3123-3132, 1993.

26. Devoto SH, Mudryj M, Pines J, et. al. : A cyclin A-protein kinase complex possesses sequence-specific DNA binding activity : c33cdk2 is a component of the E2F-cyclin A complex. Cell, 68:167-176, 1992.

27. Meyerson M, Enders GH, Wu CL, et. al. : A family of human cdc2-related protein kinases. EMBO J, 11:2909-2917, 1992.

28. Kato J, Matsushime H, Hiebert SW, et. al. : Direct binding of cyclin D to the retinoblastoma gene product (pRB) and pRB phosphorylation by the cyclin D-dependant kinase cdk4. Genes Dev, 7:331-342, 1993.

29. Matsushime H, Ewen ME, Strom DK, et. al. : Identification and properties of an atypical catalytic subunit (p34PSK-J3/cdk4) for mammalian D type G1 cyclins. Cell, 71:323-334, 1992.

30. Yamashita K, Yasuda H, Pines J, et. al. : Okadaic acid, a potent inhibitor of type 1 and type 9 protein phosphatases, activates cdc2/H1 kinase and transiently induces a premature mitosis-like state in BHK21 cells. EMBO J, 9:4331-4338, 1990.

31. Lee TH, Solomon MJ, Mumby MC, et. al. : INH, a negative regulator of MPF, is a form of protein phosphatase 9. Cell, 64:415-423, 1991.

32. Hindley J, Phear G, Stein M, et. al. : Suc-1+encodes a predicted 13-kilodalton protein that is essential for cell viability and is directly involved in the division cycle of Schizosaccharomyces pombe. Mol Cell Biol, 7:504-511, 1987.

33. Kusubata M, Tokui T, Matsuoka Y, et. al. : p13suc-1 suppresses the catalytic function of p34cdc2 kinase for intermediate filament proteins, in vitro. J Biol Chem, 267:20937-20942, 1992.

34. Braudbary EM : Reversible histone modifications and the chromosome cell cycle. Bioassays, 14:9-16, 1992.

35. Norbury C and Nurse P : Control of the higher eukaryote cell cycle by p34cdc2 homologues. Biochemica et Biophysica Acta, 989:85-95, 1989.

36. Belenguer P, Caizergues-Ferrer M, Labbe JC, et. al. : Mitosis-specific phosphorylation of nucleolin by p34cdc2 protein kinase. Mol Cell Biol, 10:3607-3618, 1990.

37. Kuriyama R and Maekawa T : Phosphorylation of a 225-kDa centrosomal component in mitotic CHO cells and urchin eggs. Exp Cell Res, 202:345-354, 1992.

38. Hass R : Retrodifferention an alternative biological pathway in human leukemia cells. Eur J Cell Biol, 58:1-11, 1992.

39. Mulnerlorillon O, Cormier P, Cavadore JC, et. al. : Phosphorylation of Xenopus elongation factor-1 gamma by cdc2 protein kinase: identification of the phosphorylation site. Exp Cell Res, 202:549-551, 1992.

40. Samiei M, Daya-Makin M, Clark-Lewis I, et. al. : Platelet-activating factor -and thrombin- induced stimulation of p34cdc2-cyclin histone H1 kinase activity in platelets. J Biol Chem, 266:14889-14892, 1991.

41. Mawal Dewan M, Sen PC, Abdel-Ghany M, et. al. : Phosphorylation of tau protein by purified p34cdc28 and a related protein kinase from neurofilaments. J Biol Chem, 267:19707-19709, 1992.

42. Shenoy S, Chackalaparampil I, Bagrodia S, et. al. : Role of p34cdc2-mediated phosphorylations in two-step activation of pp60c-scr during mitosis. Proc Natl Acad Sci USA, 89:7237-7241, 1992.

43. Maller JL : Xenopus oocytes and the biochemistry of cell division. Biochemistry, 29:3157-3166, 1990.

44. Kitagawa M, Saitoh S, Ogino H, et. al. : cdc2-like kinase is associated with the retinoblastoma protein. Oncogene, 7:1067-1074, 1992.

45. Fukurawa Y, Uenoyama S, Ohta M, et. al. : Transforming growth factor-beta inhibits phosphorylation of the retinoblastoma susceptibility gene product in human monocytic leukemia cell line JOSK-I. J Biol Chem, 267:17121-17127, 1992.

46. Sturzbecher HW, Maimets T, Chumakov P, et. al. : p53 interacts with p34cdc2 in mammalian cells : Implication for cell cycle control and oncogenesis. Oncogene, 5:795-81, 1990.

Correspondence:
M. Akif Öztürk
Hacettepe Üniversitesi
Tip Fakültesi
Biyokimya Bölümü ve,
Onkoloji Enstitüsü,
Ankara, TÜRKIYE.