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Review

Cell reproduction: induction of M-phase events by cyclin-dependent cdc2 kinase

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ABSTRACT Although a major concern in the development of multicellular organisms is cell differentiation, the construction of a multicellular system essentially depends on cell multiplication, which consists of genomic duplication and segregation. Recent progress has revealed that cyclin-dependent kinases (CDKs) are key components of a cell cycle engine that governs cell proliferation. This article focuses on how CDKs induce M-phase events characterized by nuclear membrane breakdown, chromosome condensation and mitotic spindle formation to assure genomic segregation.

KEY WORDS: *cdc2 kinase, cyclin B, M-phase events, chromosome condensation, spindle formation*

Introduction

Multicellular organisms are composed of a wide variety of differentiated cells, all of which are in general genetically identical and originate from one cell, that is a fertilized egg. This indicates that there are two major aspects to the construction of a multicellular system: how genetically identical cells are reproduced, and then how these cells are differentiated. Although cell differentiation is a major concern in development, a wide variety of cell differentiation is possible only after the multiplication of cells. Thus the existence of multicellular organisms essentially depends on cell reproduction.

Cell reproduction consists of the accurate replication of chromosomes and their segregation into two daughter cells. These events occur in S-phase and M-phase, respectively, and hence are considered to be downstream events that are induced by a cell-cycle engine (Murray, 1992). Recent exciting progress in the research of cell cycle control has revealed that the major components of the cell-cycle engine are cyclin-dependent kinases (CDKs) in all eukaryotic cells (for reviews, Pines, 1993; Sherr, 1993). The active kinase is a complex of one of the cyclin protein family with one of the cdc2-related gene product family. The cyclin family is composed of type A to G cyclins, and functions as a regulatory subunit of the kinase. The cdc2 family is composed of cdc2 gene product and its related proteins such as CDK2 to 6, and functions as a catalytic subunit. Depending on the combination between the cyclin family and the cdc2 family, cyclin-associated CDKs constitute the cell-cycle engine that regulates major key points during the cell cycle, the G1/S-phase transition which initiates the genomic duplication, and the G2/M-phase transition which initiates the genomic segregation (see Fig. 1).

How do CDKs induce their downstream events? In contrast to S-phase events, some clues are now accumulating on the molecular mechanism of the execution of M-phase events in higher eukaryotic cells (see Nigg, 1993). Here I will discuss how M-phase events such as nuclear membrane breakdown, chromosome condensation, spindle formation and cell division are performed by mitotic CDKs.

M-phase cell-cycle engine

The molecular component of the cell-cycle engine which induces downstream events of M-phase is now known to be the cyclin B•cdc2 complex or cdc2 kinase. Before this was established, there had traditionally been three separate experimental approaches; MPF (maturation-promoting factor), cdc2, and cyclin.

MPF

In primary oocytes with a germinal vesicle, the cell cycle is arrested at the prophase of the first meiosis, that is the G2/M-phase border. The meiotic arrest in immature oocytes of frog and starfish is released by a maturation-inducing hormone (for reviews, Kanatani, 1973; Masui and Clarke, 1979). At the beginning of the 1970s, MPF was first detected as a cytoplasmic activity which mediates maturation-inducing hormonal action at the oocyte surface to the germinal vesicle: the hormone causes the activation of MPF in oocyte cytoplasm, which in turn brings about germinal vesicle breakdown (GVBD) and the progression of the first meiotic cycle (Masui and Markert, 1971; Kishimoto and Kanatani, 1976). Around 1980, MPF study entered a new phase: MPF was found to function as a universal regulator of M-phase in all eukaryotic cells regard-

Abbreviations used in this paper: MPF, maturation or M-phase promoting factor; CDK, cyclin-dependent kinase.

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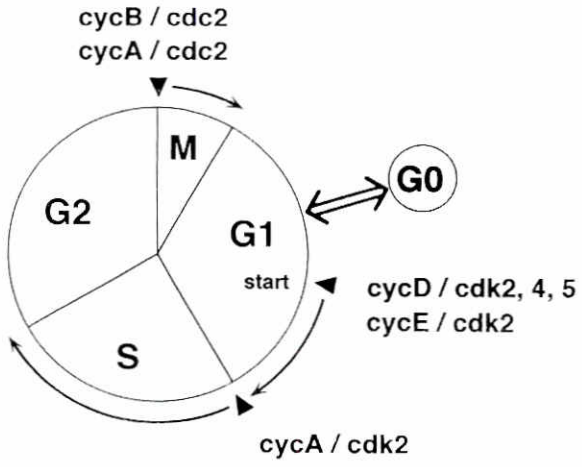

Fig. 1. Cell cycle control by cyclin-dependent kinases (CDKs). *Each of the CDKs (the cdc2-related proteins) forms a complex with one of the cyclins. Different complexes regulate different key points for the cell cycle control; "start" or "restriction point" when cells become committed to the G1/S-phase transition, the beginning or traverse of S-phase, and the G2/M-phase transition.*

less of oocyte meiosis or somatic cell mitosis (Kishimoto *et al.*, 1982; for review, Kishimoto, 1988). Activation of MPF triggers entry into M-phase and its subsequent inactivation, exit from M-phase. As a result of this discovery, MPF was renamed the M-phase promoting factor.

cdc2

Genetic analysis of cell-division cycle (cdc) mutants started around 1970 in yeast (for review, Hartwell and Weinert, 1989). Among these, the product of the cdc2 gene in the fission yeast, *Schizosaccharomyces pombe* and its homolog, the CDC28 gene in the budding yeast, *Saccharomyces cerevisiae* are crucial in controlling the timing of cell division (for review, Nurse, 1990). Some temperature-sensitive mutants of cdc2 cause cells to advance into mitosis precociously with a reduced cell size. In 1987 (Lee and Nurse), complementation analysis in fission yeast succeeded in the cloning of a human homolog of cdc2 from HeLa cell cDNA library. This supports the idea that the conserved role of cdc2 gene product is an essential regulator of the G2/M-phase transition in all eukaryotic cells. The cdc2 gene encodes a 34 kD serine/threonine protein kinase, which prefers to phosphorylate histone H1 *in vitro*. All cdc2 homologs contain an identical 16-amino-acid (42-57 in human cdc2) sequence EGVPSTAIREISLLKE, commonly called the PSTAIRE region.

Cyclin

In the first half of the 1980's, cyclins A and B were first identified in the early cleavage cycles of marine invertebrates, sea urchin and bivalve as two 50-60 kD proteins whose abundance fluctuates dramatically during the cell cycle (for review, Hunt, 1989). While cyclin is synthesized throughout the cell cycle, it is degraded abruptly at the end of each mitosis, and hence its abundance peaks at each M-phase. Thereafter, cyclins are found to be present from yeast to human. While removal of cyclin B mRNA from *Xenopus* egg extracts caused cell cycle arrest, its addition was sufficient to induce M-phase both in immature oocytes and in the endogenous mRNA-depleted *Xenopus* egg extracts, indicating a direct involvement of cyclin B in the G2/M-phase transition (for review, Murray and Kirschner, 1989; for example, Tachibana *et al.*, 1990).

Linking of MPF, cdc2 and cyclin

In 1988, Lohka *et al.* were the first to purify MPF from *Xenopus* eggs. The purified MPF preparation, which had a histone H1 kinase activity, consisted of two components after gel electrophoresis. The first 34 kD polypeptide was identified as a *Xenopus* homolog of the cdc2 gene product (Gautier *et al.*, 1988), and the second 45 kD polypeptide as *Xenopus* cyclin B (Gautier *et al.*, 1990), indicating that MPF is a complex of cdc2 gene product and cyclin B. Thus, three separate approaches — biochemical study of MPF in oocytes, genetic study of cdc mutants in yeasts, and cyclin study in early embryos — converged on an astonishing linking. Concurrently, similar convergence occurred from yeast to human, indicating that the linking is universal in all eukaryotic cells (for reviews, Hunt, 1989; Murray and Kirschner, 1989; Doree, 1990; Nurse, 1990; Maller, 1991).

Fission yeast genetics indicates a close interaction of cdc2 gene product with suc1 gene product (Brizuela *et al.*, 1987). Based on this, the cyclin B•cdc2 complex can be efficiently purified with suc1-affinity chromatography in which bacterially-expressed yeast suc1 protein is immobilized to Sepharose gel (Labbe *et al.*, 1991; Kusubata *et al.*, 1992). In fact, the purified cdc2 kinase is able to induce meiosis reinitiation upon injection into immature oocytes. We found recently, however, that the level of histone H1 kinase activity which is required for meiosis reinitiation is much lower in cytoplasmic MPF than purified cdc2 kinase (Okumura and Kishimoto, in preparation). This fact suggests that the cyclin B•cdc2 complex constitutes only a part of MPF which is originally identified as a cytoplasmic activity. It may be necessary to reconsider the other components of MPF.

Regulation of mitotic cyclin•cdc2 kinase activity

Molecular modification of mitotic cyclin-cdc2 complex

During the cell cycle, the amount of cdc2 protein is almost constant, while that of cyclin A or B cycles with a peak at each M-

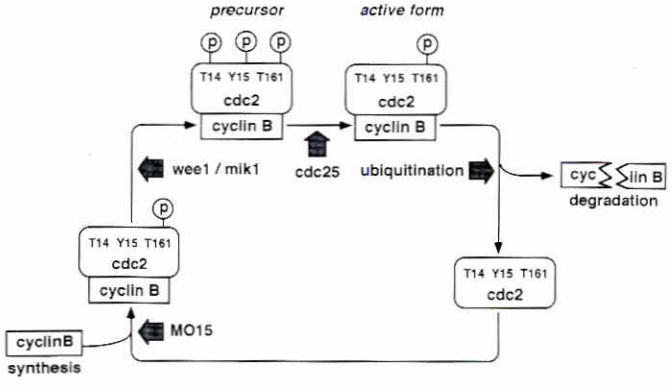

Fig. 2. Formation of the cyclin B•cdc2 complex and regulatory mechanism of cyclin B-dependent cdc2 kinase activity. *Active form of the cyclin B•cdc2 complex constitutes MPF. P indicates phosphorylated state of each amino acid residue of cdc2 protein. See text for details.*

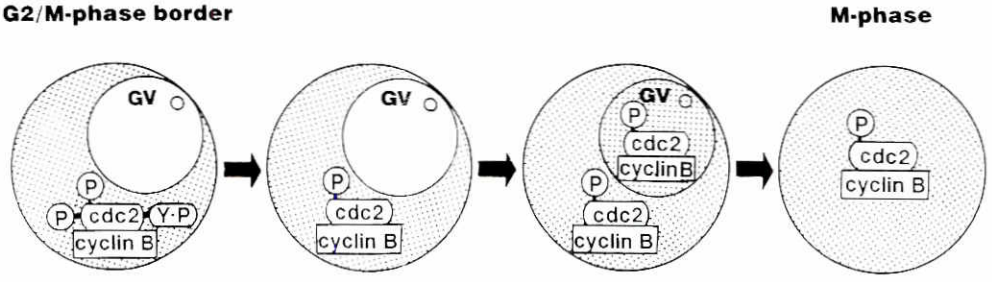

Fig. 3. Nuclear relocation of the cyclin B•cdc2 complex at the entry into M-phase. *At meiosis reinitiation in starfish oocytes, the cyclin B•cdc2 complex that is localized in the cytoplasm is transformed into active form, relocates into germinal vesicle (GV), and then induces nuclear membrane breakdown (GVBD). Ps and Y-P indicate the phosphorylated Thr14, Thr161 and Tyr15 residues of cdc2 protein.*

phase. The association of cyclin A or B with cdc2 protein is, however, not sufficient for the kinase activation at M-phase. Further modification which is necessary for the kinase activity in both cyclin A•cdc2 and cyclin B•cdc2 complexes is the phosphorylation of Thr161 residue in cdc2 protein (for review, Draetta, 1993). This phosphorylation is performed at the time of the association of cyclin A or B with cdc2 protein by a cdk-activating kinase (CAK), whose catalytic subunit is a homolog of *Xenopus* MO15 gene product (Fesquet *et al.*, 1993; Poon *et al.*, 1993; Solomon *et al.*, 1993). The phosphorylation appears to link directly to the activation of the cyclin A•cdc2 complex, but the cyclin B•cdc2 complex requires additional modification for its activity.

In the fission yeast, the timing of the entry into mitosis is controlled by the ratio of the activity of two genes: cdc25, which stimulates the G2/M-phase transition, and wee1 or mik1, which prevents it (Lundgren *et al.*, 1991; for review, Nurse, 1990). These are elements of the cell cycle "clock", and the prediction from the fission yeast genetics primarily holds true on the regulation of the activity of the cyclin B•cdc2 complex in all eukaryotic cells (Fig. 2). At the time of complex formation of cyclin B with cdc2 protein, in addition to Thr161, both Thr14 and Tyr15 residues are also phosphorylated, and the complex represents inactive cdc2 kinase. Wee1 or mik1 kinase is responsible for Tyr15 phosphorylation, although a kinase responsible for Thr14 is yet unidentified (Parker and Piwnica-Worms, 1992). At the G2/M-phase transition, cdc25 phosphatase dephosphorylates Thr14 and Tyr15 residues, resulting in the activation of the cyclin B•cdc2 complex (for review, Millar and Russell, 1992). Active cyclin B•cdc2 complex, but not cyclin A•cdc2 complex, phosphorylates cdc25 protein, and hence the autoactivation of cdc2 kinase occurs (Hoffmann *et al.*, 1993).

The inactivation of cdc2 kinase triggers exit from M-phase

Most critical for the inactivation of cyclin A- or cyclin B-dependent cdc2 kinase is the ubiquitin-dependent degradation of cyclin (Glotzer *et al.*, 1991). A sequence which is necessary for cyclin degradation is found near N-terminus of cyclin, the nine residues 42-50. The partially conserved motif RXXLXXIXN, which is termed the «destruction box», is suggested to be recognized by the E2 or E3 component of the ubiquitin-conjugating system. The lysine-rich residues C-terminal to the destruction box are speculated to be the sites for poly-ubiquitin conjugation. Although cyclin B-dependent kinase, but not cyclin A-dependent kinase (Luca *et al.*, 1991), activates their degradation system, it is still unclear how cyclin is recognized by the ubiquitin conjugation system at the specific timing in M-phase.

Intracellular relocation of mitotic cyclin-cdc2 complex

For the function of the cyclin A•cdc2 or cyclin B•cdc2 complex *in vivo*, its activation by the intramolecular modification is not sufficient, but its subcellular distribution is crucial. While the cyclin A•cdc2 complex is present exclusively in the nucleus throughout the cell cycle (Pines and Hunter, 1991), the cyclin B•cdc2 complex is localized in the cytoplasm until the G2/M-phase transition. After its activation, the cyclin B•cdc2 complex translocates into the nucleus before its breakdown, independently of either microtubules or actin filaments (Ookata *et al.*, 1992) (Fig. 3). It appears that most of the cyclin B•cdc2 complex anchors the detergent-resistant cytoskeleton at the G2/M border, and then some of it is released at the time of its activation (Okumura and Kishimoto, unpublished). This may contribute to the initiation of the relocation of the cyclin B•cdc2 complex. However, the so-called nuclear location signal (NLS) has not yet been identified in either cyclin A, B or cdc2. Nuclear transport of the cyclin B•cdc2 complex may require further association with other NLS-containing carrier protein even after its release from the anchoring.

In starfish oocytes, while some part of the cyclin B•cdc2 complex relocates into the nucleus and then accumulates in condensed chromosomes, another portion of the complex accumulates on meiotic asters and meiotic spindles (Ookata *et al.*, 1992, 1993). These intracellular relocation and redistribution of the cyclin B•cdc2 complex at the entry into M-phase agree well with its function in M-phase as mentioned below.

Execution of M-phase events by cyclin•cdc2 kinase

Potential substrates

The purified cdc2 kinase phosphorylates various exogenously-added substrates including histone H1, cyclin B, lamins A, C and B, $p60^{c\text{-}src}$, $p150^{c\text{-}Abl}$, nucleolin, MAP4, caldesmon, myosin II light chain, vimentin, Rb protein, p53, SV40 large T antigen, RNA polymerase II, and neurofilament heavy chain (for reviews, Moreno and Nurse, 1990; Nigg, 1993; for example, Hisanaga *et al.*, 1991). Derived from these various *in vitro* substrates, the consensus motif for a phosphorylation site by cdc2 kinase is proposed to be *S/T*-P-X-K/R, where the phosphorylation residue is followed by proline, a polar amino acid (X) and then generally a basic amino acid (Moreno and Nurse, 1990). The N-substituted structure of proline appears to be important, since replacement of Pro by sarcosine had no effect; but the replacement of Pro by Lys had a negative effect on the phosphorylation (Ando *et al.*, 1993).

A key issue to be addressed is how mitotic cyclin•cdc2 kinase triggers the major M-phase events such as nuclear envelope breakdown, chromosome condensation and mitotic spindle forma-

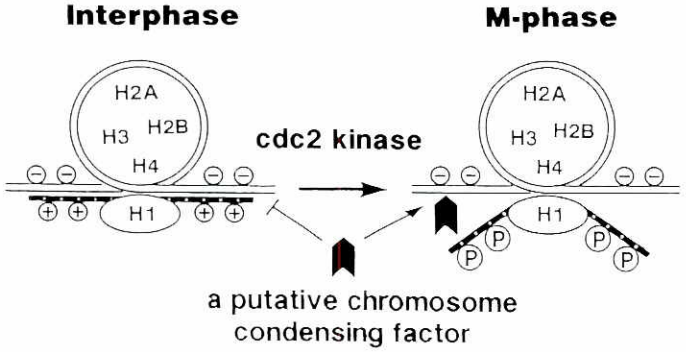

Fig. 4. Models for the role of histone H1 in mitotic chromosome condensation. *From the fact that mitotic chromosome condensation occurs even in the absence of histone H1, it is supposed that a putative factor responsible for chromosome condensation other than histone H1 is present. During interphase, histone H1 arms bind tightly to linker DNA due to their positive charge, preventing the putative chromosome condensing factor from interacting with DNA. At the entry into M-phase, histone H1 arms are highly phosphorylated by cdc2 kinase. The hyperphosphorylation weakens the association of histone H1 arms to linker DNA, allowing the chromosome condensing factor to gain access to DNA. Encircled H2A, H2B, H3 and H4 represents a nucleosome core. Adapted from Roth and Allis (1992).*

tion. In spite of the several candidate substrates for cdc2 kinase, their relationship to these multiple and profound structural reorganizations at M-phase is not yet well understood. Two possibilities appear to be mutually involved: cdc2 kinase either acts directly on the ultimate targets of mitotic phosphorylation, or it controls the activities of other enzymes.

Nuclear envelope breakdown

The nuclear envelope is composed of the outer and the inner nuclear membrane. The inner nuclear membrane is underlaid by the nuclear lamina which is a fibrillar protein network (for review, Gerace and Burke, 1988). The major components of the nuclear lamina are the lamins, classified as either A- or B-type, which are members of the intermediate filament protein family. While the nuclear lamina stabilizes the nuclear envelope during interphase, hyperphosphorylation of lamins at M-phase causes lamina disassembly, which appears to be prerequisite, although not sufficient, for nuclear envelope breakdown (Heald and McKeon, 1990). During M-phase, A- and C-type lamins are released in a soluble state, whereas B-type lamin remains associated with vesiculized remnants of the nuclear membrane.

Cyclin B-dependent cdc2 kinase phosphorylates chicken lamin B *in vitro* on the same site, Ser16 that is specifically phosphorylated during mitosis *in vivo*. This phosphorylation depolymerizes *in vitro* assembled lamin B head-to-tail polymers into the dimers (Peter *et al.*, 1991). In isolated nuclei, the same phosphorylation solubilizes lamin B from the nuclear envelope and causes nuclear lamina disassembly (Peter *et al.*, 1990). It is proposed that phosphorylation of an analogous site in lamins A and C (Ser22) has the same effect on lamina disassembly (Heald and McKeon, 1990; Ward and Kirschner, 1990). Thus, the N-terminal head domain of lamin proteins appears to be a major determinant in the longitudinal assembly of lamin dimers. Interference with this assembly by phosphorylation with cdc2 kinase is responsible for mitotic disassembly of the nuclear lamina.

Although the nuclear lamins are the best understood mitotic substrates of the cyclin B•cdc2 kinase, no nuclear envelope breakdown occurs after lamin B is disassembled by the addition of cdc2 kinase into isolated nuclei (Peter *et al.*, 1990). In addition to lamins and cdc2 kinase, multiple kinases and substrates of nuclear membrane proteins may participate in the complex process of nuclear membrane disassembly (Ward and Kirschner, 1990; Luscher *et al.*, 1991). Further, the inhibition of cAMP-dependent protein kinase appears to be involved in generating the mitotic phosphorylation pattern which is necessary for nuclear envelope breakdown (Lamb *et al.*, 1991).

Chromosome condensation

Although a causal role for topoisomerase II is strongly implicated in mitotic chromosome condensation (Adachi *et al.*, 1991), it has long been proposed that histone H1 phosphorylation is a major factor involved in physical process of chromosome condensation: during mitosis, phosphorylation of histone H1 and H3 becomes maximal *in vivo*, and the excellent *in vitro* substrate for cdc2 kinase is histone H1 (Langan *et al.*, 1989; for review, Bradbury, 1992). In spite of these correlations, there is little direct evidence for the proposal that hyperphosphorylation of histone H1 is causally related to mitotic chromosome condensation. To approach this gap, we developed the chromatins lacking histone H1 by combining a *Xenopus* egg extract immunodepleted of histone H1 and sperm nuclei lacking histone H1 (Ohsumi *et al.*, 1993). The results demonstrate that in spite of the absence of histone H1, nucleosomes are properly spaced along DNA and chromatins can be packaged into condensed metaphase chromosomes. Thus, histone H1 is not required for any level of structural reorganization that leads to condensed metaphase chromosomes.

The above facts do not necessarily mean that histone H1 is not involved in mitotic chromosome condensation. So, what is the role of metaphase-specific hyperphosphorylation of histone H1? Histone H1 is composed of three domains: a central hydrophobic globular domain that interacts with the core histones, and two basic flexible arms at the N- and C-terminal regions, that are thought to embrace linker DNA. The K/R-S/T-P-X-K/R sequences in histone H1 termini that are responsible for the tight interaction with the minor grove of linker DNA (for review, Churchill and Travers, 1991) are the same motif for the phosphorylation by cdc2 kinase. Since phosphorylation of these sites is thought to weaken the association of histone H1 arms to linker DNA, it seems very likely that the decrease in histone H1-DNA interaction allows the subsequent access to chromatin of putative factors responsible for chromosome condensation (for review, Roth and Allis, 1992) (Fig. 4). Thus, histone H1 might control chromosome condensation by modulating accessibility of putative chromosome condensing factors to DNA.

The non-essential role of histone H1 in mitotic chromosome condensation indicates that other substrates than histone H1 are a real target of cdc2 kinase in inducing chromosome condensation. In fact, the depletion of cdc2 kinase abolishes the activity of chromosome condensation in *Xenopus* mitotic extracts, confirming the dependency of chromosome condensation on cdc2 kinase. We found recently, however, that when phosphorylated states induced by cdc2 kinase are maintained in cytoplasmic extract by ATP-γ-S, chromosome condensation occurs successfully even when chromatin is added after depriving the extact of cdc2 kinase activity (Shimada *et al.*, unpublished). Further, chromosome condensation in this condition requires the activity of other kinases.

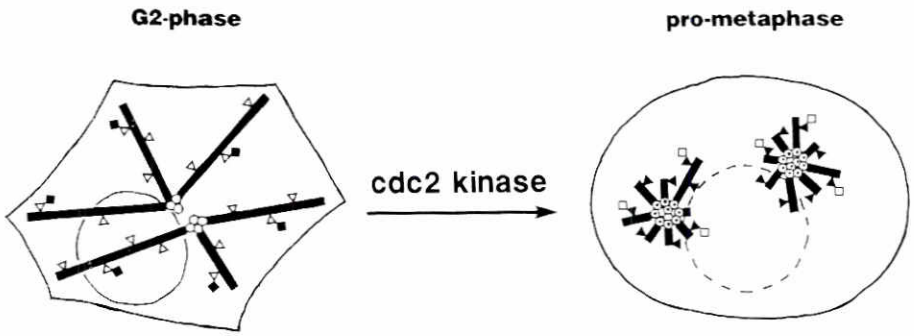

Fig. 5. Models for the dual functions of cdc2 kinase in reorganizing microtubules from interphase radial arrays to mitotic spindle. *Open and closed, triangles and circles indicate dephosphorylated and phosphorylated, MAPs and MTOCs (microtubuleorganizing centers), respectively. Closed and open squares indicate inactive and active cyclin B•cdc2 complex, respectively, both of which associate with microtubules via the binding of cyclin B with MAPs. Phosphorylation by cdc2 kinase activates MTOCs to increase the number of microtubules per a centrosome, while phosphorylation of MAPs by cdc2 kinase increases the instability of an individual microtubules.*

Thus, the major role of cdc2 kinase in inducing chromosome condensation appears to be indirect.

Spindle formation

At the G2/M-phase transition, microtubule networks undergo dramatic reorganization from interphase radial arrays to mitotic spindle. This mitotic reorganization depends on the increased nucleation activity of centrosomes and the increased instability of individual microtubules (for reviews, Karsenti, 1991; Kalt and Schliwa, 1993). Both of these seem to be controlled by phosphorylation, since the addition of cdc2 kinase into interphase extracts of *Xenopus* eggs with isolated centrosomes induces an alternation of microtubule organization into the mitotic state (Verde *et al.*, 1990).

When incubated in the extracts of *Xenopus* eggs, the isolated centrosomes exhibit microtubule-nucleating activity accompanied by an increase in the amount of pericentriolar materials around the centriole. Although the microtubule-nucleation capacity of the centrosome is observed in the interphase extracts, it is greatly increased by the addition of cdc2 kinase, but not MAP kinase, resulting in the increased number of microtubules per centrosome (Buendia *et al.*, 1992; Ohta *et al.*, 1993). These facts suggest two pathways activating centrosomal activity; accumulation of pericentriolar materials and phosphorylation mediated by cdc2 kinase. Although the effect of cdc2 kinase appears to be related to its centrosomal association (Bailly *et al.*, 1989; Ookata *et al.*, 1993), it is currently unclear whether cdc2 kinase phosphorylates centrosomal proteins directly or indirectly, and what is the real substrate in the phosphorylation of centrosomal proteins.

In addition to the colocalization of the cyclin B•cdc2 complex, but not the cyclin A•cdc2 complex, with spindle microtubules (Pines and Hunter, 1991; Tombes *et al.*, 1991; Ookata *et al.*, 1992), microtubules prepared from cytoplasmic extracts in the presence of taxol contain distinct amounts of the cyclin B•cdc2 complex (Ookata *et al.*, 1993). The association is mediated by microtubule-associated proteins (MAPs), in particular by MAP4 in mammalian cells, and is reconstituted with purified cyclin B•cdc2 complex, MAP4 and tubulin. Cyclin B, but not cdc2 protein, associates with proline-rich region in the C-terminal half of MAP4 (Ookata *et al.*, submitted). A ternary complex of $p34^{cdc2}$, cyclin B and MAP4 exhibits intracomplex phosphorylation of MAP4. Phosphorylation of MAP4 by cdc2 kinase does not prevent its binding to microtubules, but abolishes its microtubule stabilizing activity, resulting in the increased dynamic instability and shortening of the microtubules (Ookata *et al.*, submitted). But, in the case of 220 kD MAP of *Xenopus* which is potentially related to mammalian MAP4, phosphorylation by cdc2 kinase is reported to diminish its ability to bind to and stabilize microtubules (Shiina *et al.*, 1992). In both cases, cdc2 kinase appears to be appropriately targeted for the regulation of spindle assembly and dynamics.

Taken together, the reorganization of the microtubule cytoskeleton into the formation of mitotic spindle might be, at least partly, under dual regulation by cdc2 kinase: phosphorylation of MAPs for the microtubule dynamics, i.e., shortening of individual microtubuels, and phosphorylation of centrosomal proteins for microtubule nucleation, i.e., an increased number of microtubules per centrosome (Fig. 5).

Chromosome segregation and cytokinesis

The ending of M-phase is marked by a cell division which involves both chromosome segregation and cytokinesis. It has been assumed that degradation of mitotic cyclins causes inactivation of cdc2 kinase and thereby triggers the transition from metaphase to anaphase. However, the separation of sister chromatids occurs even in the absence of cdc2 kinase inactivation, while it still depends on ubiquitin-mediated proteolysis (Holloway *et al.*, 1993; Surana *et al.*, 1993). Thus, the metaphase to anaphase transition and the destruction of mitotic cyclin-dependent cdc2 kinase activity seem to take place independently of one another. It is rather suggested that the degradation of proteins other than the known mitotic cyclins is required to dissolve the linkage between sister chromatids (Holloway *et al.*, 1993). After the onset of anaphase, microtubule-associated motor proteins may control chromosome movements.

The microfilament cytoskeleton undergoes profound reorganization at mitosis. During prophase, microfilament bundles or stress fibers are disassembled concomitant with cell rounding. Microfilaments are then transiently reorganized into contractile rings for cytokinesis, and subsequently reassembled for two spreading daughter cells (for review, Satterwhite and Pollard, 1992). Phosphorylation by cdc2 kinase of non-muscle caldesmon, an actin- and calmodulin-binding protein, causes its dissociation from microfilaments. This may release caldesmon's inhibition of actomyosin ATPase and/or gelsolin activities, contributing to cell rounding (for review, Yamashiro and Matsumura, 1991). Cdc2 kinase also phosphorylates the regulatory light chain of myosin-II

to inhibit its actin-dependent ATPase activity, thus potentially delaying cytokinesis until the inactivation of cdc2 kinase at the onset of anaphase (Satterwhite *et al.*, 1992). At present, however, we have no knowledge on the linkage between cdc2 kinase activity and the determination of the cleavage plane, in particular the so-called cleavage stimulus.

Concluding remarks

Standard yeast genetics, such as screening for extragenic suppressors, has predicted most of the regulators for CDKs. This has led to the rapid biochemical and cell biological elucidation of regulatory systems involved in controlling CDKs, in particular cyclin B-dependent cdc2 kinase. In contrast, progress towards the elucidation of molecular mechanisms involved in executing downstream events of CDKs has been rather slow. This may be due to the lack of universally appreciable methods for identifying physiological substrates of any kinase. Although in M-phase, the trigger is clearly mitotic cyclin-dependent kinases, elucidation of the induction mechanism of their downstream events awaits further biochemical and cell biological efforts.

As characterized by the usefulness of the unicellular yeast system, current CDKs research is oriented towards the generality in all kinds of eukaryotic cells. In the multicellular system, however, it is considered that the cell-cycle engine may have some aspects specific to cell types and developmental stages. Incorporation of such view points will link cell reproduction with cell differentiation.

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