**[Frontiers in Bioscience, 5, d50-57, January 1, 2000]**

# THE CONTROL OF MITOSIS

**Mary L. Hixon [1] and Antonio Gualberto [1,2]**

[1]*Division of Cardiovascular Research, Department of Medicine, St. Elizabeth's Medical Center, Boston, MA 02135, USA,* [2]*Department of Cardiovascular and Metabolic Diseases, Pfizer Central Research Division, Eastern Point Rd., Groton, CT 06340*

## TABLE OF CONTENTS

*1. Abstract*
*2. Introduction*
*3. Discussion:*
*3.1. Chromatid cohesion*
*3.1.1. Separin, Securin and the onset of anaphase*
*3. 2 .The Anaphase Promoting Complex*
*3.2.1. The Control of APC/C activity*
*3.3. The mitotic spindle cell cycle checkpoint*
*4. Perspectives*
*5. Acknowledgements*
*6. References*

## 1. ABSTRACT

A precise coordination of multiple cell cycle events is required to ensure proper mitosis. Chromosome cohesion must be maintained until all chromosomes are attached to opposite poles of the mitotic spindle and aligned at the metaphase plate. At the onset of anaphase, the activity of separins contributes to the release of cohesins from chromosomes, allowing for the segregation of bivalents to opposite spindle poles. Separin activity is blocked by binding to a class of proteins known as securins, whose turnover at the metaphase-to-anaphase transition is triggered by the Anaphase Promoting Complex or cyclosome. The mitotic spindle cell cycle checkpoint coordinates the timing of these events and acts as input mechanism for DNA damage/stress pathways. Failure of this precise network leads to genomic instability and/or cell death.

## 2. INTRODUCTION

Mitosis is a complex process which includes nuclear envelope breakdown, chromatin condensation and chromosome segregation. Thus, multiple events must be coordinated in order to ensure the progression of mitosis. Although much is already known about the genetic and biochemical components that constitute the cell cycle machinery at mitosis, how these pathways are coordinated has been largely unknown until recently.

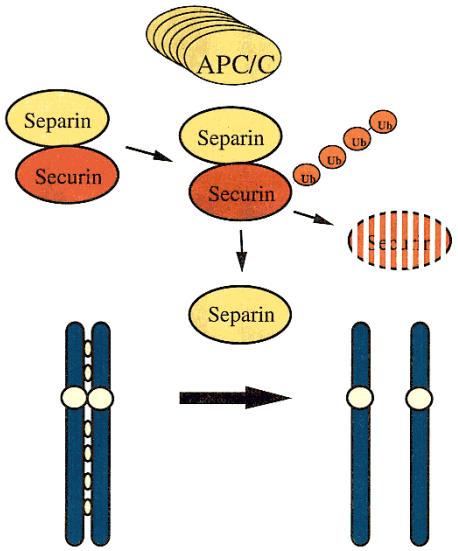
Since 1966, the existence of factors which control mitotic entry has been postulated. The first evidence for such factors came from fusion experiments in *P. polycephalum* (1). These data were later supported by experiments involving the fusion of mammalian cells arrested at $G_1$, S, or $G_2$ phase of the cell cycle with cells which arrested at mitosis, which results in chromosome condensation (2-4). Biochemical and genetic experiments established that the onset of mitosis is controlled by the activity of the mitosis promoting factor (MPF), a complex consisting of the p34 Cdc2 cyclin-dependent kinase, Cyclin B and associated proteins (5). MPF induces the formation of a mitotic spindle by phosphorylating the components of the mitotic spindle apparatus, including microtubule-based motor proteins (6). In addition, MPF activates NimA (never in mitosis), a kinase that is required for spindle formation (7). After anaphase, the destruction of the mitotic cyclins leads to MPF inactivation, originating spindle disassembly and cytokinesis (8,9).

While the activity and subunit composition of MPF have been extensively investigated, less is known about the molecular events that control the metaphase-to-anaphase transition and the exit from mitosis. However, in the last few years, extensive progress has been achieved in the understanding of these processes. Importantly, recent data indicate that the destruction of sister chromatid cohesion, rather than pulling forces, is responsible for the separation of sister chromatids at the metaphase to anaphase transition (10). Here, we will review the regulatory mechanisms that control chromatid cohesion, the onset of anaphase and the exit from mitosis.

## 3. DISCUSSION

### 3. 1. Chromatid Cohesion

Chromatid cohesion allows for sister chromatids to remain together from the time of DNA replication to




**Separins**: Esp1p, Cut1
**Securins**: Pds1p, Cut2, vSecurin (PTTG)

**Figure 1.** Model of Separin/Securin control by the APC/C.

mitosis. Chomatid cohesion also promotes proper microtubule attachment by constraining kinetochores (11,12). Multiple chromosome cohesion proteins have been identified. They include Mcd1(Scc1)p, Scc3p, Smc1p, and Smc3p in budding yeast (13-16) and Mis4 in fission yeast (17). In *Xenopus*, homologs of Mcd1p, Smc1p, and Smc3p have also been recently identified (18). Smc1p and Smc3p are members of the structural maintenance of chromosomes (SMC) gene family (19). Mcd1p requires Smc1p to associate with chromatin, plays a role in chromosome condensation (13,14), and is homologous to fission yeast Rad21 (20). The process of chromosome condensation; however, is independent from sister chromatid cohesion (18) and is mediated by two additional SMC family members, Smc2p and Smc4p (19,21-23). Importantly, chromosome cohesion varies during mitosis (24). These changes may result from the dissociation of Mcd1p, Smc1p, and Smc3p from the chromosome along the progression of mitosis (18). Another Rad21 homologue, Rec8, seems to play a central in yeast chromosome cohesion during meiosis (25).

In fission yeasts, Mis4, initially identified by its requirement for stable maintenance of minichromosomes (26), was found to be also required for sister chromatid cohesion (17,27), although its function seems to be quite different from cohesin. Temperature-sensitive *mis4* mutants are lethal at restrictive temperature and grow at the permissive temperature with loss of minichromosomes. To date, twelve *mis* genes have been identified. The *mis5* gene encodes one of the MCM subunits essential for replication (26,28); whereas, *mis6* encodes for a centromere protein essential for the formation of centromere-specific chromatin in the $G_1$/S phase (29). The phenotype of *mis6* mutants is similar to that of *mis4*, however, not only minichromosomes, but also normal chromosomes are missegregated (26). Thus, *mis* genes appear to play a variety of important roles in maintaining chromosomal dynamics and integrity.

In *Drosophila*, Mei-S332 is required for the maintenance of sister chromatid cohesion at meiosis (30,31). This protein localizes to centromeres until sister chromatids separate and dissociate from chromosomes at anaphase, suggesting that its release is involved in sister chromatid separation (30). Mutations in the *mei-S332* gene lead to early sister chromatid separation and chromosome loss or mis-segregation (32).

In *Xenopus*, recent work has identified homologs of budding yeast Smc1p,Smc3p, Mcd1p/Scc1p and Rad21p (18). In contrast to yeast, the cohesin complex of frog extracts does not persist throughout mitosis. It dissociates at the onset of mitosis when it is replaced by the condensin complex (18).

### 3.1.1. Separin, Securin and the onset of anaphase

In addition to structural proteins, the cohesion complex includes also proteins with regulatory function. The dissociation of cohesins from chromatids results in sister chromatid separation and seems to be triggered by proteolytic cleavage of Scc1p. This cleavage depends upon the activity of a separin, Esp1p (figure 1) (33,34). Esp1p is essential for the dissociation of Scc1p from all regions of the chromosome. Furthermore, Scc1p dissociation from chromosomes is accompanied by its cleavage at two specific sites and requires Esp1p (35-37). However, it is not known whether Esp1p is a Scc1p protease or if it activates a protease that cleaves Scc1p.

Importantly, Esp1p is tighly associated to Pds1p (securin) and this association inhibits Esp1p separin function and the onset of anaphase (36,38,39). The carboxy terminal region of Esp1p has high sequence similarity with the fission yeast *cut1* and *A. nidulans bimB* gene products, which are required for chromosome segregation (40,41). In *cut1* mutants, centromeres and most of the chromosome separate normally, but telomeric cohesion remains (41,42). Cut1 also associates with an inhibitor protein, Cut2 (36,41-43).

A vertebrate securin (vSecurin) has recently been identified on the basis of its biochemical analogy to Pds1p and Cut2. The vSecurin protein binds to a vertebrate homolog of Esp1p and Cut1, and is degraded by proteolysis mediated by the cyclosome complex (44). Human securin is identical to the product of the gene called pituitary tumor-transforming gene (PTTG), which is overexpressed in some tumors and exhibits transforming activity in NIH 3T3 cells (44). Furthermore, the addition of a non-degradable form of PTTG to Xenopus egg extracts prevented sister chromatid separation but not exit from mitosis. These parallels demonstrate that both securin and separin functions are well conserved.

### 3.2. The Anaphase Promoting Complex

Both, the metaphase-to-anaphase transition and the exit from mitosis, are regulated by protein degradation

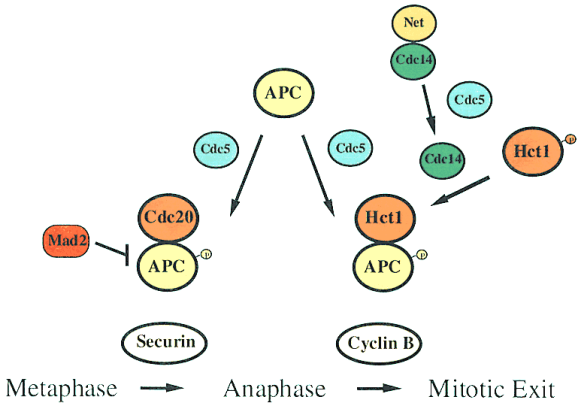



**Figure 2.** Sequential activation of the APC/C by Cdc20p and Hct1p allows metaphase-to-anaphase transition and mitotic exit.

mediated by a specific ubiquitin ligase complex known as the Anaphase-Promoting Complex or Cyclosome (APC/C) (figure 2) (45-49). APC/C targets contain a conserved nine amino acid motif called the destruction box that is required for APC/C-mediated ubiquitination (46, 50-55). During this process, ubiquitin is activated forming a thioester bond with a cysteine residue at the E1 ubiquitin-activating enzyme. Ubiquitin is then transferred to an ubiquitin-conjugating enzyme, called UBC or E2, forming a second thioester. Finally, an isopeptide bond is formed between ubiquitin and a lysine residue at the substrate. More ubiquitin molecules can then be conjugated to those already attached creating a polyubiquitin chain that is targeted for degradation by the 26S proteasome (56). The transfer of ubiquitin from E2 enzymes to substrates requires a third activity, called ubiquitin-protein ligase or E3. Unlike E1 and E2, ubiquitin ligase activity is cell cycle regulated, active from anaphase up to late $G_1$ phase. Fractionation experiments using extracts from clam and *Xenopus* eggs demonstrated that this ligase activity sedimented as a large particle of 20S. The clam ubiquitin ligase particle was called the cyclosome, the yeast and *Xenopus* particles were called the anaphase-promoting complex. The composition of APC/C was further investigated by immunopurification. The *Xenopus* and human particles were found to contain 10 subunits, whereas the budding yeast particle contains 12 subunits. The core APC/C subunits in mammals are Tgs24, Apc2, Cdc16, Cdc27, Apc7, Apc4, Apc5, Cdc23, Apc10, Apc11 and Cdc26 (56).

### 3.2.1. The Control of APC/C activity

The activity of the APC/C is regulated at multiple levels. WD-repeat regulatory proteins seem to work as adapters that confer substrate specificity to the APC/C (54-57). Importantly, since mitotic cyclin degradation must not take place until cells are ready to exit mitosis, the APC/C that promotes the metabolism of the anaphase inhibitor at the metaphase-to-anaphase transition cannot simultaneously promote the destruction of mitotic cyclins. In budding yeast, at least two different forms of APC/C have been demonstrated. These forms can be distinguished by their association with the WD-repeat regulatory proteins Cdc20p (Cdc20/p55CDC/Fizzy) and Hct1(Cdh1)p (57-60). Cdc20p mediates the degradation of Pds1p (61,62) and the mitotic cyclin Clb3p (63) at the metaphase-to-anaphase transition. On the other hand, Hct1(Cdh1)p promotes the degradation at anaphase of the mitotic cyclin Clb2, the polo-like kinase Cdc5p, and the spindle-associated Ase1p (57,64,65). In addition, Hct1(Cdh1)p associates with the APC/C later in the cell cycle than Cdc20p. Cdc20p levels and association to the APC/C rise and fall at mitotic entry and exit. In contrast, the level of Hct1(Cdh1)p is constant, but its association to the APC/C is observed from anaphase to G1 (49,66). Furthermore, Hct1p/APC/C association requires dephosphorylated Hct1p (49, 67,68). In addition to Cdc20p and Hct1(Cdh1)p, other WD proteins involved in the control of mitosis have been recently identified, including the fission yeast Cdc20p homolog Slp1 and the Hct1(Cdh1)p homolog Srw1 (69-72). The function of these proteins, however, is less understood.

The activity of the APC/C is also regulated by cell cycle–specific phosphorylation (45,46,55,57,58,66,73-78). Reversible phosphorylation by MPF inactivates APC/C activity (45,73,79,80). In addition, Polo-like kinase 1 (Plk1), a mammalian homologue of Drosophila polo and budding yeast Cdc5p, phosphorylates at least three APC/C subunits, Apc1, Apc3, and Apc6, activating the APC/C (figure 2) (69-66,81-83). In contrast, cAMP-dependent protein kinase (PKA) phosphorylates Apc1 and Apc3 (82) suppressing APC/C activity (74,75,82,84). Furthermore, protein phosphatase 1 (PP1) has been found to be required for the activation of APC/C during the metaphase-to-anaphase transition (74). Hct1(Cdh1)p is also dephosphorylated by Cdc14p (49,67,68), which may be itself regulated by Plk1/Cdc5p and the Cdc15p kinase, all of which are essential for mitotic exit (67,85,86). The yeast inhibitor of mitosis, Sic1p, is regulated by similar mechanisms: Cdk1-dependent phosphorylation inhibits its synthesis and stability, and Cdc14-dependent dephosphorylation reverses these effects and leads to Sic1p accumulation (67,87-89).

Finally, the APC/C is inactivated by the mitotic spindle cell cycle checkpoint pathway (78,90-95). The checkpoint proteins Mad1, Mad2, Mad3/Bub1, and Bub3 suppress the activation of APC/C by Cdc20p. Also, Bub2p localizes to the spindle pole body and regulates Hct1(Cdh1)p -dependent APC/C activation (63,96,97). Dephosphorylation of Hct1(Cdh1)p by Cdc14p is controlled by Bub2(Byr4)p and the RENT (regulator of nucleolar silencing and telophase exit) complex (63,85,86).

### 3.3. The Mitotic Spindle Cell Cycle Checkpoint

The progression through mitosis is monitored by surveillance mechanisms (98). One of these surveillance mechanisms, known as the mitotic spindle cell cycle checkpoint, ensures the proper segregation of chromosomes by establishing the timing of anaphase onset (99) and by monitoring the attachment of microtubules to the kinetochores and the tension exerted on the kinetochores by the microtubules (100,101). Studies in yeast, *Drosophila*, and *Xenopus* have identified multiple genes involved in the activity of this checkpoint (102-104). Initially, the *MAD* (mitotic arrest deficient) and *BUB* (budding uninhibited by

benomyl) genes were identified by a screening for mutants sensitive to microtubule depolymerizing compounds (102,103). It was found that, in response to mitotic spindle depolymerizing agents, mutations in any of these genes (*BUB1*, *BUB2*, or *BUB3*, and *MAD1*, *MAD2*, or *MAD3* ) resulted in aberrant mitosis and cell death. Another gene, *MPS1*, is required for spindle pole body duplication and has been shown to be involved in cell cycle checkpoint control (105). *MPS1* encodes a protein kinase believed to function in the signalling pathway (106). Hence, the spindle checkpoint detects spindle pole body duplication and defects in microtubule polymerization, microtubule motors or kinetochore components (107).

The recent characterization of some of the *MAD* and *BUB* gene products have determined their localization within the cell. For example, Mad2 protein has been shown to localize to kinetochores that are not yet attached to the mitotic spindle (78,90,97,107-109). Similar to its human counterpart, the murine homolog of yeast Bub1p localizes to unattached kinetochores during mitosis (99). Additional genetic and biochemical studies have also shown that Bub1p, Bub3p, and Mps1p act upstream of Mad1p and Mad2p; whereas, Bub2p and Mad3p act either downstream of Mad proteins, or in a parallel pathway (98,101). Both the human and *Xenopus* homologs of the yeast Mad2p, HMAD2 and XMAD2, have been cloned and have been shown to be required for spindle checkpoint control (108,109). At the present time, however, it is not known if all the Mad and Bub proteins form a complex at the kinetochore. Generally, a normal bipolar attachment at the kinetochore results in dissociation of the Bub1 and Mad2 proteins from the kinetochore. However, if the kinetochore is unattached from the microtubules, the Bub and Mad proteins remain bound to the kinetochore and arrest the cell in metaphase, delaying the activation of the APC/C (110).

Cdc20p-dependent proteolysis appears to be the target of the mitotic spindle cell cycle checkpoint pathway (59,63,78,92,94,97,111). Importantly, some studies have recently identified a physical interaction between Mad2p and the APC/C. Mad2p binds Cdc20p, inhibiting the destruction of cyclins as well as Pds1p (59,63,78,92,94). The checkpoint-mediated inhibition of APC/C has been reconstituted in vitro with purified Mad2p and Cdc20p. Intriguingly, Mad2p exists in two different forms, a tetramer and a monomer. Only the tetramer inhibits the activation of APC/C by Cdc20p in vitro, suggesting that a structural change of Mad2p may be necessary for transducing the checkpoint signal (92). There is also evidence suggesting that Cdc20p is a target of the DNA damage response; overexpression of Cdc20p overrides metaphase arrest caused by DNA damage (94,111). However, evidence is still lacking for a direct regulation of Cdc20p to the DNA damage response pathway.

## 4. PERSPECTIVE

Mitosis is a complex process which comprises multiple and simultaneous events that require precise regulatory mechanisms. Protein-protein association, protein phosphorylation and proteolysis play a key role in the regulation of the mitotic regulatory machinery. APC/C-dependent proteolysis drives both metaphase-to-anaphase transition and exit from mitosis and is regulated by at least three mechanisms: Activation by Cdc20p and Cdh1p; phosphorylation and dephosphorylation; and inhibition by the mitotic spindle cell cycle checkpoint.

Destruction of sister chromatid cohesion is responsible for sister chromatid separation. Dissociation of cohesin complexes from chromatids is promoted by separins whose activity is blocked by binding to securins. Securin turnover, triggered by the APC/C, determines chromatid separation and anaphase onset. Yet many unanswered questions remain as to the regulation of separin/securins by the APC/C and the localization of the cohesion complex components on the chromosome. Future work should determine the additional players of sister chromatid cohesion during both mitosis and interphase, the localization of these proteins along the chromosome and how their function is regulated by mitotic signals.

It is established that the APC/C is a multisubunit complex that promotes the proteosome-mediated proteolysis of key regulators of mitosis. However, very little is known about the functions of the individual APC/C components and how the function of these components is regulated. Is the activity of the APC/C an all-or-nothing mechanism ? It is also anticipated that new members of this complex will be soon identified in vertebrates.

Superimposed upon this, the mitotic spindle cell cycle checkpoint pathway ensures the order of the entire mitotic process and responds also to DNA damage signals, detecting spindle pole body duplications and defects in microtubule polymerization, microtubule motors or kinetochore components. Mitotic spindle sensors localize at the kinetochore and dissociate from this structure following normal bipolar attachment. In contrast, Bub and Mad proteins remain bound to unattached kinetochores determining growth arrest in metaphase. It is possible that the event sensed by these proteins is the tension generated on the kinetochore by the microtubular attachment to a bipolar spindle and some evidence suggest that both attachment and tension are required for metaphase-to-anaphase progression (112). In addition, is the APC/C the only target of the mitotic spindle checkpoint pathway? Interestingly, recent data indicate that mitotic checkpoint signals downregulate the expression of hCks1 (113), a Cdc2-associated protein that has been implicated in the control of both APC/C and proteosome functions (114-116).

In conclusion, it is evident that the control of mitosis involves genes involved in the regulation of metaphase-anaphase transition, exit from mitosis and cell cycle checkpoint function. These genes interact in intricate pathways to ensure the accurate segregation of the genome. The study of these pathways constitutes an

exciting area of research in molecular and cellular biology.

## 5. ACKNOWLEDGEMENTS

We apologize to colleagues whose work was not cited due to space limitations. We thank the American Heart Association and the Ohio Cancer Research Associates for grant support.

## 6. REFERENCES

1. Rusch, H. P., Sachsenmaier, W., Behrens, K., and Gruter, V.: Synchronization of mitosis by the fusion of the plasmodia of Physarum polycephalum.*J Cell Biol* 31, 204-209 (1966)
2. Rao, P. N., and Johnson, R. T.: Mammalian cell fusion: studies on the regulation of DNA synthesis and mitosis.*Nature* 225, 159-164 (1970)
3. Johnson, R. T., Rao, P. N., and Hughes, H. D.: Mammalian cell fusion. 3. A HeLa cell inducer of premature chromosome condensation active in cells from a variety of animal species.*J Cell Physiol* 76, 151-157 (1970)
4. Johnson, R. T., and Rao, P. N.: Mammalian cell fusion: induction of premature chromosome condensation in interphase nuclei.*Nature* 226, 717-722 (1970)
5. Moreno, S., and Nurse, P.: Substrates for p34cdc2: in vivo veritas?*Cell* 61, 549-551 (1990)
6. Blangy, A., Lane, H. A., d'Herin, P., Harper, M., Kress, M., and Nigg, E. A.: Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo.*Cell* 83, 1159-1169 (1995)
7. Ye, X. S., Xu, G., Pu, R. T., Fincher, R. R., McGuire, S. L., Osmani, A. H., and Osmani, S. A.: The NIMA protein kinase is hyperphosphorylated and activated downstream of p34cdc2/cyclin B: coordination of two mitosis promoting kinases.*Embo J* 14, 986-994 (1995)
8. Holloway, S. L., Glotzer, M., King, R. W., and Murray, A. W.: Anaphase is initiated by proteolysis rather than by the inactivation of maturation-promoting factor.*Cell* 73, 1393-1402 (1993)
9. Surana, U., Amon, A., Dowzer, C., McGrew, J., Byers, B., and Nasmyth, K.: Destruction of the CDC28/CLB mitotic kinase is not required for the metaphase to anaphase transition in budding yeast.*Embo J* 12, 1969-1978 (1993)
10. Miyazaki, W. Y., and Orr-Weaver, T. L.: Sister-chromatid cohesion in mitosis and meiosis.*Annu Rev Genet* 28, 167-87 (1994)
11. Rieder, C. L., and Salmon, E. D.: Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle.*J Cell Biol* 124, 223-233 (1994)
12. Hyman, A. A., and Sorger, P. K.: Structure and function of kinetochores in budding yeast.*Annu Rev Cell Dev Biol* 11, 471-495 (1995)
13. Guacci, V., Koshland, D., and Strunnikov, A.: A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in S. cerevisiae.*Cell* 91, 47-57 (1997)
14. Michaelis, C., Ciosk, R., and Nasmyth, K.: Cohesins: chromosomal proteins that prevent premature separation of sister chromatids.*Cell* 91, 35-45 (1997)
15. Toth, A., Ciosk, R., Uhlmann, F., Galova, M., Schleiffer, A., and Nasmyth, K.: Yeast cohesin complex requires a conserved protein, Eco1p(Ctf7), to establish cohesion between sister chromatids during DNA replication.*Genes Dev* 13, 320-333 (1999)
16. Klein, F., Mahr, P., Galova, M., Buonomo, S. B., Michaelis, C., Nairz, K., and Nasmyth, K.: A central role for cohesins in sister chromatid cohesion, formation of axial elements, and recombination during yeast meiosis.*Cell* 98, 91-103 (1999)
17. Furuya, K., Takahashi, K., and Yanagida, M.: Faithful anaphase is ensured by Mis4, a sister chromatid cohesion molecule required in S phase and not destroyed in G1 phase.*Genes Dev* 12, 3408-3418 (1998)
18. Losada, A., Hirano, M., and Hirano, T.: Identification of Xenopus SMC protein complexes required for sister chromatid cohesion.*Genes Dev* 12, 1986-1997 (1998)
19. Strunnikov, A. V., Larionov, V. L., and Koshland, D.: SMC1: an essential yeast gene encoding a putative head-rod-tail protein is required for nuclear division and defines a new ubiquitous protein family.*J Cell Biol* 123, 1635-1648 (1993)
20. Birkenbihl, R. P., and Subramani, S.: Cloning and characterization of rad21 an essential gene of Schizosaccharomyces pombe involved in DNA double-strand-break repair.*Nucleic Acids Res* 20, 6605-6611 (1992)
21. Jessberger, R., Riwar, B., Baechtold, H., and Akhmedov, A. T.: SMC proteins constitute two subunits of the mammalian recombination complex RC-1.*Embo J* 15, 4061-4068 (1996)
22. Strunnikov, A. V., Hogan, E., and Koshland, D.: SMC2, a Saccharomyces cerevisiae gene essential for chromosome segregation and condensation, defines a subgroup within the SMC family.*Genes Dev* 9, 587-599 (1995)
23. Sutani, T., and Yanagida, M.: DNA renaturation activity of the SMC complex implicated in chromosome condensation.*Nature* 388, 798-801 (1997)
24. Sumner, A. T.: Scanning electron microscopy of mammalian chromosomes from prophase to telophase.*Chromosoma* 100, 410-8 (1991)
25. Stoop-Myer, C., and Amon, A.: Meiosis: Rec8 is the reason for cohesion.*Nature Cell Biol* 1, E125-E126 (1999)
26. Takahashi, K., Yamada, H., and Yanagida, M.: Fission yeast minichromosome loss mutants mis cause lethal aneuploidy and replication abnormality.*Mol Biol Cell* 5, 1145-1158 (1994)
27. Goshima, G., Saitoh, S., and Yanagida, M.: Proper metaphase spindle length is determined by centromere proteins Mis12 and Mis6 required for faithful chromosome segregation.*Genes Dev* 13, 1664-1677 (1999)
28. Adachi, Y., Usukura, J., and Yanagida, M.: A globular complex formation by Nda1 and the other five members of the MCM protein family in fission yeast.*Genes Cells* 2, 467-479 (1997)
29. Saitoh, S., Takahashi, K., and Yanagida, M.: Mis6, a fission yeast inner centromere protein, acts during G1/S

and forms specialized chromatin required for equal segregation.*Cell* 90, 131-143 (1997)
30. Moore, D. P., Page, A. W., Tang, T. T., Kerrebrock, A. W., and Orr-Weaver, T. L.: The cohesion protein MEI-S332 localizes to condensed meiotic and mitotic centromeres until sister chromatids separate. *J Cell Biol* 140, 1003-12 (1998)
31. Kerrebrock, A. W., Moore, D. P., Wu, J. S., and Orr-Weaver, T. L.: Mei-S332, a Drosophila protein required for sister-chromatid cohesion, can localize to meiotic centromere regions.*Cell* 83, 247-256 (1995)
32. Kerrebrock, A. W., Miyazaki, W. Y., Birnby, D., and Orr-Weaver, T. L.: The Drosophila mei-S332 gene promotes sister-chromatid cohesion in meiosis following kinetochore differentiation.*Genetics* 130, 827-841 (1992)
33. Uhlmann, F., Lottspeich, F., and Nasmyth, K.: Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature* 400, 37-42 (1999)
34. McGrew, J. T., Goetsch, L., Byers, B., and Baum, P.: Requirement for ESP1 in the nuclear division of Saccharomyces cerevisiae.*Mol Biol Cell* 3, 1443-1454 (1992)
35. Funabiki, H., Yamano, H., Kumada, K., Nagao, K., Hunt, T., and Yanagida, M.: Cut2 proteolysis required for sister-chromatid seperation in fission yeast.*Nature* 381, 438-441 (1996)
36. Ciosk, R., Zachariae, W., Michaelis, C., Shevchenko, A., Mann, M., and Nasmyth, K.: An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast.*Cell* 93, 1067-1076 (1998)
37. Kumada, K., Nakamura, T., Nagao, K., Funabiki, H., Nakagawa, T., and Yanagida, M.: Cut1 is loaded onto the spindle by binding to Cut2 and promotes anaphase spindle movement upon Cut2 proteolysis.*Curr Biol* 8, 633-641 (1998)
38. Cohen-Fix, O., Peters, J. M., Kirschner, M. W., and Koshland, D.: Anaphase initiation in Saccharomyces cerevisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p.*Genes & Dev* 10, 3081-3093 (1996)
39. Yamamoto, A., Guacci, V., and Koshland, D.: Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s) *J Cell Biol* 133, 99-110 (1996)
40. May, G. S., McGoldrick, C. A., Holt, C. L., and Denison, S. H.: The bimB3 mutation of Aspergillus nidulans uncouples DNA replication from the completion of mitosis.*J Biol Chem* 267, 15737-15743 (1992)
41. Uzawa, S., Samejima, I., Hirano, T., Tanaka, K., and Yanagida, M.: The fission yeast cut1+ gene regulates spindle pole body duplication and has homology to the budding yeast ESP1 gene.*Cell* 62, 913-925 (1990)
42. Funabiki, H., Hagan, I., Uzawa, S., and Yanagida, M.: Cell cycle-dependent specific positioning and clustering of centromeres and telomeres in fission yeast.*J Cell Biol* 121, 961-976 (1993)
43. Funabiki, H., Kumada, K., and Yanagida, M.: Fission yeast Cut1 and Cut2 are essential for sister chromatid separation, concentrate along the metaphase spindle and form large complexes.*Embo J* 15, 6617-6628 (1996)
44. Zou, H., McGarry, T. J., Bernal, T., and Kirschner, M. W.: Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis.*Science* 285, 418-422 (1999)
45. Sudakin, V., Ganoth, D., Dahan, A., Heller, H., Hershko, J., Luca, F. C., Ruderman, J. V., and Hershko, A.: The cyclosome, a large complex containing cyclin-selective ubiquitin ligase activity, targets cyclins for destruction at the end of mitosis.*Mol Biol Cell* 6, 185-197 (1995)
46. King, R. W., Peters, J. M., Tugendreich, S., Rolfe, M., Hieter, P., and Kirschner, M. W.: A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B.*Cell* 81, 279-288 (1995)
47. Cohen-Fix, O., Peters, J. M., Kirschner, M. W., and Koshland, D.: Anaphase initiation in Saccharomyces cerevisiae is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p.*Genes Dev* 10, 3081-3093 (1996)
48. Yu, H., Peters, J. M., King, R. W., Page, A. M., Hieter, P., and Kirschner, M. W.: Identification of a cullin homology region in a subunit of the anaphase- promoting complex.*Science* 279, 1219-1222 (1998)
49. Zachariae, W., Schwab, M., Nasmyth, K., and Seufert, W.: Control of cyclin ubiquitination by CDK-regulated binding of Hct1 to the anaphase promoting complex.*Science* 282, 1721-1724 (1998)
50. Glotzer, M., Murray, A. W., and Kirschner, M. W.: Cyclin is degraded by the ubiquitin pathway. *Nature* 349, 132-138 (1991)
51. Amon, A., Irniger, S., and Nasmyth, K.: Closing the cell cycle circle in yeast: G2 cyclin proteolysis initiated at mitosis persists until the activation of G1 cyclins in the next cycle.*Cell* 77, 1037-1050 (1994)
52. Hershko, A., Ganoth, D., Sudakin, V., Dahan, A., Cohen, L. H., Luca, F. C., Ruderman, J. V., and Eytan, E.: Components of a system that ligates cyclin to ubiquitin and their regulation by the protein kinase cdc2.*J Biol Chem* 269, 4940-4946 (1994)
53. Irniger, S., Piatti, S., Michaelis, C., and Nasmyth, K.: Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast. *Cell* 81, 269-278 (1995)
54. Brandeis, M., and Hunt, T.: The proteolysis of mitotic cyclins in mammalian cells persists from the end of mitosis until the onset of S phase.*Embo J* 15, 5280-5289 (1996)
55. Peters, J. M., King, R. W., Hoog, C., and Kirschner, M. W.: Identification of BIME as a subunit of the anaphase-promoting complex.*Science* 274, 1199-1201 (1996)
56. Zachariae, W., and Nasmyth, K.: Whose end is destruction: cell division and the anaphase-promoting complex.*Genes Dev* 13, 2039-2058 (1999)
57. Schwab, M., Lutum, A. S., and Seufert, W.: Yeast Hct1 is a regulator of Clb2 cyclin proteolysis.*Cell* 90, 683-693 (1997)
58. Sigrist, S. J., and Lehner, C. F.: Drosophila fizzy-related down-regulates mitotic cyclins and is required for cell proliferation arrest and entry into endocycles.*Cell* 90, 671-681 (1997)
59. Lorca, T., Castro, A., Martinez, A. M., Vigneron, S., Morin, N., Sigrist, S., Lehner, C., Doree, M., and Labbe, J.

C.: Fizzy is required for activation of the APC/cyclosome in Xenopus egg extracts.*Embo J* 17, 3565-3575 (1998)
60. Kramer, E. R., Gieffers, C., Holzl, G., Hengstschlager, M., and Peters, J. M.: Activation of the human anaphase-promoting complex by proteins of the CDC20/Fizzy family.*Current Biol* 8, 1207-1210 (1998)
61. Visintin, R., Prinz, S., and Amon, A.: CDC20 and CDH1: a family of substrate-specific activators of APC-dependent proteolysis.*Science* 278, 460-463 (1997)
62. Shirayama, M., Zachariae, W., Ciosk, R., and Nasmyth, K.: The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in Saccharomyces cerevisiae.*Embo J* 17, 1336-1349 (1998)
63. Alexandru, G., Zachariae, W., Schleiffer, A., and Nasmyth, K.: Sister chromatid separation and chromosome re-duplication are regulated by different mechanisms in response to spindle damage.*EMBO Journal* 18, 2707-2721 (1999)
64. Charles, J. F., Jaspersen, S. L., Tinker-Kulberg, R. L., Hwang, L., Szidon, A., and Morgan, D. O.: The Polo-related kinase Cdc5 activates and is destroyed by the mitotic cyclin destruction machinery in S. cerevisiae.*Current Biology* 8, 497-507 (1998)
65. Shirayama, M., Zachariae, W., Ciosk, R., and Nasmyth, K.: The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20p/fizzy are regulators and substrates of the anaphase promoting complex in Saccharomyces cerevisiae.*Embo J* 17, 1336-1349 (1998)
66. Fang, G., Yu, H., and Kirschner, M. W.: Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1.*Mol Cell* 2, 163-171 (1998)
67. Visintin, R., Craig, K., Hwang, E. S., Prinz, S., Tyers, M., and Amon, A.: The phosphatase Cdc14 triggers mitotic exit by reversal of Cdk-dependent phosphorylation.*Mol Cell* 2, 709-18 (1998)
68. Jaspersen, S. L., Charles, J. F., and Morgan, D. O.: Inhibitory phosphorylation of the APC regulator Hct1 is controlled by the kinase Cdc28 and the phosphatase Cdc14.*Current Biol* 9, 227-36 (1999)
69. Matsumoto, T.: A fission yeast homolog of CDC20/p55CDC/Fizzy is required for recovery from DNA damage and genetically interacts with p34cdc2.*Mol Cell Biol* 17, 742-50 (1997)
70. Yamaguchi, R., Mazaki, Y., Hirota, K., Hashimoto, S., and Sabe, H.: Mitosis specific serine phosphorylation and downregulation of one of the focal adhesion protein, paxillin.*Oncogene* 15, 1753-61 (1997)
71. Kitamura, K., Maekawa, H., and Shimoda, C.: Fission yeast Ste9, a homolog of Hct1/Cdh1 and Fizzy-related, is a novel negative regulator of cell cycle progression during G1-phase.*Mol Biol Cell* 9, 1065-80 (1998)
72. Kominami, K., Seth-Smith, H., and Toda, T.: Apc10 and Ste9/Srw1, two regulators of the APC-cyclosome, as well as the CDK inhibitor Rum1 are required for G1 cell-cycle arrest in fission yeast.*EMBO Journal* 17, 5388-99 (1998)
73. Lahav-Baratz, S., Sudakin, V., Ruderman, J. V., and Hershko, A.: Reversible phosphorylation controls the activity of cyclosome-associated cyclin-ubiquitin ligase.*Proc Nat Acad Sci USA* 92, 9303-7 (1995)
74. Ishii, K., Kumada, K., Toda, T., and Yanagida, M.: Requirement for PP1 phosphatase and 20S cyclosome/APC for the onset of anaphase is lessened by the dosage increase of a novel gene sds23+.*Embo J* 15, 6629-40 (1996)
75. Yamashita, Y. M., Nakaseko, Y., Samejima, I., Kumada, K., Yamada, H., Michaelson, D., and Yanagida, M.: 20S cyclosome complex formation and proteolytic activity inhibited by the cAMP/PKA pathway.*Nature* 384, 276-9 (1996)
76. Patra, D., and Dunphy, W. G.: Xe-p9, a Xenopus Suc1/Cks protein, is essential for the Cdc2-dependent phosphorylation of the anaphase- promoting complex at mitosis.*Genes Devt* 12, 2549-59 (1998)
77. Yu, H., Peters, J. M., King, R. W., Page, A. M., Hieter, P., and Kirschner, M. W.: Identification of a cullin homology region in a subunit of the anaphase-promoting complex.*Science* 279, 1219-22 (1998)
78. Kallio, M., Weinstein, J., Daum, J. R., Burke, D. J., and Gorbsky, G. J.: Mammalian p55CDC mediates association of the spindle checkpoint protein Mad2 with the cyclosome/anaphase-promoting complex, and is involved in regulating anaphase onset and late mitotic events.*J Cell Biol* 141, 1393-406 (1998)
79. Shteinberg, M., and Hershko, A.: Role of Suc1 in the activation of the cyclosome by protein kinase Cdk1/cyclin B.*Biochemical & Biophysical Research Communications* 257, 12-8 (1999)
80. Shteinberg, M., Protopopov, Y., Listovsky, T., Brandeis, M., and Hershko, A.: Phosphorylation of the cyclosome is required for its stimulation by Fizzy/cdc20.*Biochem Biophys Res Com* 260, 193-8 (1999)
81. Descombes, P., and Nigg, E. A.: The polo-like kinase Plx1 is required for M phase exit and destruction of mitotic regulators in Xenopus egg extracts.*Embo J* 17, 1328-35 (1998)
82. Kotani, S., Tugendreich, S., Fujii, M., Jorgensen, P. M., Watanabe, N., Hoog, C., Hieter, P., and Todokoro, K.: PKA and MPF-activated polo-like kinase regulate anaphase-promoting complex activity and mitosis progression.*Mol Cell* 1, 371-80 (1998)
83. Glover, D. M., Hagan, I. M., and Tavares, A. A.: Polo-like kinases: a team that plays throughout mitosis.*Genes Dev* 12, 3777-87 (1998)
84. Yamada, H., Kumada, K., and Yanagida, M.: Distinct subunit functions and cell cycle regulated phosphorylation of 20S APC/cyclosome required for anaphase in fission yeast.*J Cell Science*110, 1793-804 (1997)
85. Visintin, R., Hwang, E. S., and Amon, A.: Cfi1 prevents premature exit from mitosis by anchoring Cdc14 phosphatase in the nucleolus [see comments].*Nature* 398, 818-23 (1999)
86. Shou, W., Seol, J. H., Shevchenko, A., Baskerville, C., Moazed, D., Chen, Z. W., Jang, J., Charbonneau, H., and Deshaies, R. J.: Exit from mitosis is triggered by Tem1-dependent release of the protein phosphatase Cdc14 from nucleolar RENT complex.*Cell* 97, 233-44 (1999)
87. Toyn, J. H., Johnson, A. L., Donovan, J. D., Toone, W. M., and Johnston, L. H.: The Swi5 transcription factor of Saccharomyces cerevisiae has a role in exit from mitosis through induction of the cdk-inhibitor Sic1 in telophase.*Genetics* 145, 85-96 (1997)

88. Skowyra, D., Craig, K. L., Tyers, M., Elledge, S. J., and Harper, J. W.: F-box proteins are receptors that recruit phosphorylated substrates to the SCF ubiquitin-ligase complex.*Cell* 91, 209-19 (1997)
89. Verma, R., Feldman, R. M., and Deshaies, R. J.: SIC1 is ubiquitinated in vitro by a pathway that requires CDC4, CDC34, and cyclin/CDK activities.*Mol Biol Cell* 8, 1427-37 (1997)
90. He, X., Patterson, T. E., and Sazer, S.: The Schizosaccharomyces pombe spindle checkpoint protein mad2p blocks anaphase and genetically interacts with the anaphase-promoting complex.*Proc Natl Acad Sc USA* 94, 7965-70 (1997)
91. Li, X., and Cai, M.: Inactivation of the cyclin-dependent kinase Cdc28 abrogates cell cycle arrest induced by DNA damage and disassembly of mitotic spindles in Saccharomyces cerevisiae.*Mol Cell Biol*17, 2723-2734 (1997)
92. Fang, G., Yu, H., and Kirschner, M. W.: The checkpoint protein MAD2 and the mitotic regulator CDC20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation.*Genes Dev* 12, 1871-1883 (1998)
93. Gorbsky, G. J., Chen, R. H., and Murray, A. W.: Microinjection of antibody to Mad2 protein into mammalian cells in mitosis induces premature anaphase.*J Cell Biol* 141, 1193-1205 (1998)
94. Hwang, L. H., Lau, L. F., Smith, D. L., Mistrot, C. A., Hardwick, K. G., Hwang, E. S., Amon, A., and Murray, A. W.: Budding yeast Cdc20: a target of the spindle checkpoint.*Science* 279, 1041-1044 (1998)
95. Kim, S. H., Lin, D. P., Matsumoto, S., Kitazono, A., and Matsumoto, T.: Fission yeast Slp1: an effector of the Mad2-dependent spindle checkpoint. *Science* 279, 1045-1047 (1998)
96. Fesquet, D., Fitzpatrick, P. J., Johnson, A. L., Kramer, K. M., Toyn, J. H., and Johnston, L. H.: A Bub2p-dependent spindle checkpoint pathway regulates the Dbf2p kinase in budding yeast.*Embo J* 18, 2424-2434 (1999)
97. Fraschini, R., Formenti, E., Lucchini, G., and Piatti, S.: Budding yeast Bub2 is localized at spindle pole bodies and activates the mitotic checkpoint via a different pathway from Mad2.*J Cell Biol*145, 979-991 (1999)
98. Elledge, S. J.: Cell cycle checkpoints: preventing an identity crisis.*Science* 274, 1664-1672 (1996)
99. Taylor, S. S., and McKeon, F.: Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage.*Cell* 89, 727-735 (1997)
100. Nicklas, R. B.: How cells get the right chromosomes.*Science* 275, 632-637 (1997)
101. Rudner, A. D., and Murray, A. W.: The spindle assembly checkpoint.*Current Op Cell Biol* 8, 773-780 (1996)
102. Li, R., and Murray, A. W.: Feedback control of mitosis in budding yeast .*Cell* 66, 519-531 (1991)
103. Hoyt, M. A., Totis, L., and Roberts, B. T.: S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function.*Cell* 66, 507-517 (1991)
104. Basu, J., Bousbaa, H., Logarinho, E., Li, Z., Williams, B. C., Lopes, C., Sunkel, C. E., and Goldberg, M. L.: Mutations in the essential spindle checkpoint gene bub1 cause chromosome missegregation and fail to block apoptosis in Drosophila.*J Cell Biol* 146, 13-28 (1999)
105. Hardwick, K. G., Weiss, E., Luca, F. C., Winey, M., and Murray, A. W.: Activation of the budding yeast spindle assembly checkpoint without mitotic spindle disruption.*Science* 273, 953-956 (1996)
106. Lauze, E., Stoelcker, B., Luca, F. C., Weiss, E., Schutz, A. R., and Winey, M.: Yeast spindle pole body duplication gene MPS1 encodes an essential dual specificity protein kinase.*Embo J* 14, 1655-1663 (1995)
107. Hardwick, K. G., Li, R., Mistrot, C., Chen, R. H., Dann, P., Rudner, A., and Murray, A. W.: Lesions in many different spindle components activate the spindle checkpoint in the budding yeast Saccharomyces cerevisiae.*Genetics* 152, 509-518 (1999)
108. Li, Y., and Benezra, R.: Identification of a human mitotic checkpoint gene: hsMAD2.*Science* 274, 246-248 (1996)
109. Chen, R. H., Waters, J. C., Salmon, E. D., and Murray, A. W.: Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores.*Science* 274, 242-246 (1996)
110. Orr-Weaver, T. L., and Weinberg, R. A.: A checkpoint on the road to cancer. *Nature* 392, 223-224 (1998)
111. Lim, H. H., and Surana, U.: Cdc20, a beta-transducin homologue, links RAD9-mediated G2/M checkpoint control to mitosis in Saccharomyces cerevisiae.*Mol Gen Genet* 253, 138-148 (1996)
112. Sherr, C. J.: Cancer cell cycles.*Science* 274, 1672-1677 (1996)
113. Hixon, M. L., Flores, A. I., Wagner, M. W., and Gualberto, A.: Ectopic expression of cdc2/cdc28 kinase subunit homo sapiens 1 uncouples cyclin B metabolism from the mitotic spindle cell cycle checkpoint.*Mol Cell Biol* 18, 6224-6237 (1998)
114. Kaiser, P., Moncollin, V., Clarke, D. J., Watson, M. H., Bertolaet, B. L., Reed, S. I., and Bailly, E.: Cyclin-dependent kinase and Cks/Suc1 interact with the proteasome in yeast to control proteolysis of M-phase targets.*Genes Dev* 13, 1190-1202 (1999)
115. Patra, D., and Dunphy, W. G.: Xe-p9, a Xenopus Suc1/Cks protein, is essential for the Cdc2-dependent phosphorylation of the anaphase- promoting complex at mitosis.*Genes Dev* 12, 2549-2559 (1998)
116. Richardson, H. E., Stueland, C. S., Thomas, J., Russell, P., and Reed, S. I.: Human cDNAs encoding homologs of the small p34Cdc28/Cdc2-associated protein of Saccharomyces cerevisiae and Schizosaccharomyces pombe.*Genes Dev* 4, 1332-1344 (1990)

**Key Words:** Cohesin, Separin, Securin, Anaphase Promoting Complex, Mitotic Spindle Cell Cycle Checkpoint, Review

**Send correspondence to:** Mary L. Hixon, Ph.D., Department of Cardiovascular Research, St. Elizabeth's Medical Center, 736 Cambridge St., Boston, MA 02135, Tel: 617-562-7513, Fax:617-562-7506, E-mail: mlhixon@opal.tufts.edu

This manuscript is available on line at:

http://www.bioscience.org/2000/v5/d/hixon/fulltext.htm