The spindle assembly checkpoint

Adam D Rudner* and Andrew W Murray

The spindle assembly checkpoint monitors proper chromosome attachment to spindle microtubules and is conserved from yeast to humans. Checkpoint components reside on kinetochores of chromosomes and show changes in phosphorylation and localization as cells proceed through mitosis. Adaptation to prolonged checkpoint arrest can occur by inhibitory phosphorylation of Cdc2.

Addresses
Department of Physiology, Box 0444, University of California at San Francisco, 513 Parnassus Avenue, San Francisco, CA 94143-0444, USA
*e-mail: rudner@socrates.ucsf.edu

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Abbreviations

APC	anaphase-promoting complex
BUB	budding inhibited by benzimidazole
MAD	mitotic-arrest deficient
MPS	monopolar spindle
SPB	spindle pole body

Introduction

The survival of cells and organisms requires accurate chromosome segregation. Chromosome loss can kill cells, and chromosome loss or gain can cause birth defects or contribute to tumor progression [1]. The spindle assembly checkpoint detects defects in spindle structure or in the alignment of chromosomes on the spindle, and delays the onset of chromosome segregation (anaphase) until these defects are corrected. Disruption of the spindle with microtubule-depolymerizing drugs such as nocodazole and colchicine arrests cells in mitosis (reviewed in [2]), and this arrest depends on the integrity of the spindle assembly checkpoint. More subtle defects, such as the presence of a single kinetochore (the protein complex assembled on the centromeric DNA that attaches chromosomes to microtubules) that is not attached to spindle microtubules, can also activate the checkpoint [3]. Genetic analysis in budding yeast has identified components of the spindle assembly checkpoint [4,5], making it possible to determine which defects arrest cells in mitosis by activating the checkpoint, and which induce arrest by other means. This analysis shows that the checkpoint detects spindle depolymerization [4,5], the presence of multiple minichromosomes and dicentric chromosomes [6•,7], and defects in the spindle pole body (SPB, the budding yeast centrosome) [8•,9••], microtubules ([10]; C Mistrot, K Hardwick, A Murray, unpublished data), kinetochore proteins [11•], centromeric DNA [12], or microtubule motors ([4]; C Mistrot, K Hardwick, R Li, A Murray, unpublished data) (Table 1).

Below, we discuss: the importance of kinetochore–microtubule interactions in activating the spindle assembly checkpoint; the protein components of the checkpoint, some of which reside on the kinetochore; the potential targets of the checkpoint; and a novel pathway out of mitosis that may be used by cells to adapt to a checkpoint arrest.

The role of the kinetochore in the spindle assembly checkpoint

All of the defects that the spindle assembly checkpoint detects can affect the ability of kinetochores to attach to spindle microtubules (reviewed in [13]; see Table 1), suggesting that kinetochores that are not attached to the spindle generate an inhibitory signal that halts the cell cycle. Exit from mitosis cannot depend on a positive signal from correctly attached kinetochores, as a spindle that lacks chromosomes (and thus also kinetochores) undergoes anaphase-like movements with no delays [14••].

Direct support for the existence of an inhibitory signal from kinetochores comes from experiments on mammalian tissue culture cells. Anaphase will not begin until the last kinetochore attaches to the spindle, even if attachment takes many hours [3]. This delay can be abolished if the last unattached kinetochore is destroyed by a laser microbeam [15••]. Low doses of microtubule-polymerization inhibitors and taxol, a microtubule stabilizer, also activate the checkpoint in animal cells [15••,16,17]. In both of these situations, normal bipolar attachment of chromosomes occurs and the spindle appears normal. It is thought, however, that in the presence of drugs the number of microtubules attached per kinetochore is less than normal and that the attachment is less stable than in the absence of drugs.

In some meiotic cells, the checkpoint also monitors the tension on the kinetochore–microtubule linkage. In a fraction of insect spermatocytes, one of the sex chromosomes loses its attachment to its partners [18]. As a result, this chromosome is attached to only one spindle pole (centrosome) and the cell arrests in mitosis for many hours. Using a microneedle to apply tension to the mono-oriented chromosome induces anaphase [19••]. Tension may directly control the activity of the checkpoint, or it may stabilize the kinetochore–microtubule attachment [20], with the checkpoint responding to unoccupied microtubule-binding sites on the kinetochore. In mammalian cells and budding yeast, a single attached kinetochore that lacks a sister kinetochore does not activate the checkpoint [15••,21•,22•], suggesting that in these cells tension may be applied by forces that move the chromosome arms away from the spindle pole.

Table 1

Treatments and defects that activate the spindle assembly checkpoint.

Treatment or defect	Organism	Description
High doses of microtubule-depolymerizing drugs	Many	Causes complete spindle depolymerization. Strength of mitotic arrest and speed of recovery varies [2,4,5,23].
Low doses of microtubule-depolymerizing drugs	Budding yeast, sea urchin embryos, HeLa cells	Bipolar spindle forms, but anaphase is delayed [16]. In budding yeast, the *BUB2* gene is not required for this delay [11•].
Taxol	Mammalian tissue culture cells	Stabilization of spindle microtubules delays anaphase [15••,17].
Defects in microtubules	Budding yeast	Some *tub2* (β-tubulin) mutants cause *MAD*-dependent arrest in mitosis[a] and some *tub1* (α-tubulin) mutants are suppressed by additional copies of *BUB* genes [10].
Excess or defective centromeres	Budding yeast	Excess circular or linear centromeric plasmids [6•], dicentric chromosomes [7] and centromeric DNA mutations [12] all cause *MAD*- or *BUB*-dependent delays in mitosis.
Defects in centromere-binding proteins	Budding yeast	Some *ctf13* and *cbf1* mutants cause *MAD*- and *BUB*-dependent delays in mitosis [11•][a]. Some *cep3*, *ndc10* and *skp1* mutants also delay in mitosis [61–63].
Injection of antibodies to kinetochore components	Mammalian tissue culture cells	Injection of 3F3/2 antibodies causes delays in mitosis [27]. Injection of antibodies to CENP-C and CENP-E also causes delays in mitosis [64,65] that may depend on the spindle assembly checkpoint.
Monopolar attachment of chromosome homologs	Insect spermatocytes	Causes 5–7 hour delay in mitosis unless tension is applied to kinetochore [19••].
Single unattached kinetochore	Mammalian tissue culture cells	Delays the initiation of anaphase [3].
Defects in SPB	Budding yeast	*kar1*, *ndc2*, *mps2* and *cdc31* mutants arrest in mitosis with monopolar spindles [35]. The *cdc31* and *mps2* arrest is *MAD*-dependent [9••].
Defects in microtubule motors	Budding yeast, sea urchin embryos, mammalian tissue culture cells	Mutants in the budding yeast Kar3, Cin8 and Kip1 kinesins delay in mitosis [66]. The *kar3* and *cin8* delay is *MAD*-dependent[a]. Injection of antibodies to the kinesin CHO1 into sea urchin embryos or tissue culture cells causes delays in mitosis [67,68] that may depend on the spindle assembly checkpoint.
Defects in B-type cyclins	Budding yeast	Deletion of *CLB3* and *CLB4* (two budding yeast B-type cyclins) causes a *MAD2*-dependent delay in mitosis [69].
Meiotic cross-overs	*Drosophila* oocytes	Tension caused by meiotic cross-overs between chromosome homologs is required for metaphase I arrest. This arrest may require the spindle assembly checkpoint [70].

[a]C Mistrot, K Hardwick, R Li, A Murray, unpublished data. CENP, centromere protein.

Do other cells have different sources for the checkpoint signal? In sea urchin embryos, partial or complete spindle depolymerization [23], or ripping the spindle in two with a microneedle [24], delays the cell cycle. In contrast, the presence of multiple unattached kinetochores causes no delay in the cell cycle [25]. Thus, in these cells, a spindle assembly checkpoint exists, but cannot be activated by unattached kinetochores. One possibility is that passage through mitosis in these cells requires the presence of overlapping antiparallel microtubules that exist only in a spindle with more than one pole. This requirement may define a novel checkpoint in embryonic cells.

Checkpoint proteins on the kinetochore

The chemistry of the kinetochore changes when it becomes attached to the spindle. The 3F3/2 antibody recognizes a phosphoepitope at the kinetochore of animal cells that is present in prophase and prometaphase [26]. Injecting the antibody into cells delays both anaphase and the loss of the phosphoepitope [27]. These correlations suggest that the 3F3/2-labeled epitope is a component of the spindle assembly checkpoint, and that its dephosphorylation occurs when kinetochores attach to the spindle and the checkpoint is turned off. In insect spermatocytes, kinetochores that are not under tension stain brightly with

3F3/2 and prevent the onset of anaphase. Applying tension to these kinetochores with a microneedle reduces 3F3/2 staining and allows cells to enter anaphase [28••].

The vertebrate homolog of the budding yeast checkpoint gene *MAD2* (mitotic-arrest deficient) codes for a protein that behaves like the 3F3/2-labeled epitope [4,29••,30••] (Table 2). XMAD2 and hsMAD2 (the *Xenopus* and human homologs) localize to kinetochores in tissue culture cells during prophase and prometaphase. Just like 3F3/2, the anit-XMAD2 antibody recognizes an epitope that is present in high levels only on kinetochores that have not attached to the spindle. XMAD2 is unlikely to be the 3F3/2-labeled epitope, as it is not phosphorylated. Like its yeast homolog, XMAD2 is required for the spindle assembly checkpoint. When XMAD2 is depleted from checkpoint-arrested frog egg extracts [31], a rapid exit from the checkpoint arrest ensues.

The conservation of *MAD2* suggests that the spindle assembly checkpoint is conserved from yeast to vertebrates. It also suggests that the putative vertebrate homolog of the yeast protein Mad1 might be the 3F3/2-labeled epitope: Mad1 binds tightly to Mad2 (R-H Chen, K Hardwick, A Murray, unpublished data), so it is probably localized to the kinetochore, and conditions that activate the spindle assembly checkpoint induce its hyperphosphorylation ([32•]; Table 2).

Checkpoint kinases

Checkpoint-induced hyperphosphorylation of Mad1 depends on four proteins: Bub1, Bub3, Mps1 and Mad2 ([9••,32•]; Table 2). The *BUB* (budding uninhibited by benzimidazole) genes, like the *MAD* genes, were isolated in a screen for checkpoint-defective mutants [4,5]. Bub1 is a protein kinase that can bind to and phosphorylate Bub3 [33]. The *mps1* (monopolar spindle) mutation results in a defect in SPB duplication [34], but, unlike other mutations involved in SPB duplication [35], fails to arrest in mitosis [8•]. The arrest of these mutants, and that due to spindle depolymerization, requires a functional *MPS1* gene. Thus, *MPS1* functions at two points in the cell cycle, in G_1 phase to ensure proper SPB duplication and spindle assembly, and in mitosis to respond to spindle defects.

Table 2

Checkpoint components. (a) Budding yeast spindle assembly checkpoint components. (b) Other known checkpoint components.

Gene/ protein	Function and properties
(a)	
MAD1	Encodes a coiled-coil protein. Biochemical function of protein unknown. Nonessential. Protein hyperphosphorylated during checkpoint activation [4,32•]. Protein binds to Mad2[a].
MAD2	Biochemical function of protein unknown. Nonessential. Protein binds to Mad1[a] and required for Mad1 hyperphosphorylation [4,32•]. *Xenopus* and human homologs (XMAD2 and hsMAD2) localize to unattached kinetochores. XMAD2 is required for maintenance of checkpoint in frog egg extracts [29••,30••].
MAD3	Encodes a 60 kDa protein whose biochemical function is unknown. Nonessential. Protein has homology with amino terminus of Bub1 [4][b].
BUB1	Encodes a protein kinase which binds to and phosphorylates Bub3 [5,33]. Protein required for Mad1 hyperphosphorylation [32•]. Nonessential, but mutants have growth defects. Mouse homolog of Bub1 localizes to kinetochores[c].
BUB2	Biochemical function of protein unknown [5]. Nonessential. Fission yeast homolog, *cdc16*, is required for the spindle assembly checkpoint and has essential function in cytokinesis [71].
BUB3	Protein binds to and is phosphorylated by the Bub1 protein kinase [5,33]. Bub3 required for Mad1 hyperphosphorylation [32•], but mutants have growth defects.
MPS1	Encodes a protein kinase [8•,36]. Essential for SPB duplication [34]. Required for Mad1 hyperphosphorylation. Phosphorylates Mad1 *in vitro* [9••].
CDC55	Encodes a B-regulatory subunit of PP2A [59]. Nonessential, but mutants have growth defects. *cdc55* mutants have increased tyrosine phosphorylation on Cdc28[d].
(b)	
p42-ERK2	A MAP kinase that is required for activation of the checkpoint in *Xenopus* egg extracts [31].
p53	Tumor suppressor protein that is required for the DNA-damage checkpoint in vertebrate cells [38]. Possible role in the spindle assembly checkpoint [37•]. Mutants may have additional checkpoint defects in centrosome duplication [39] and S-phase restraint [72].

[a]R-H Chen, K Hardwick, A Murray, unpublished data. [b]K Hardwick, A Murray, unpublished data. [c]S Taylor, F McKeon, personal communication. [d]J Minshull, A Straight, A Rudner, A Dernburg, A Murray, unpublished data; see Note added in proof; J Wang, D Burke, personal communication. ERK, extracellular signal regulated kinase; MAP, mitogen-activated protein.

Mps1 is likely to be the physiological Mad1 kinase [9••,36]. *MPS1* encodes a protein kinase that can phosphorylate a fragment of Mad1 *in vitro*. Overexpression of Mps1 causes a mitotic arrest and hyperphosphorylation of Mad1. This arrest is not due to spindle defects but reflects a constitutive activation of the checkpoint: mutations in the *MAD1–3* or *BUB1–3* genes prevent this arrest, confirming that these six checkpoint proteins act in a single pathway.

A vertebrate homolog of Bub1 can also be found at the kinetochore (S Taylor, F McKeon, personal communication), raising the possibility that Bub1, Bub3, Mad1, Mad2 and Mps1 form a multiprotein signaling complex at the kinetochore. Even if this appealing speculation is true, however, we know nothing about how the complex converts information about kinetochore–microtubule interactions into changes in protein phosphorylation.

p53 and the spindle assembly checkpoint

Mammalian cells that lack a functional p53 gene increase in ploidy when cultured in nocodazole or colcemid [37•], phenotypes identical to those of mutants in the budding yeast spindle assembly checkpoint genes. This result has been interpreted as indicating a role for p53 in this checkpoint. An alternative explanation is that wild-type and p53-deficient cells leave mitosis at similar rates, but that the wild-type cells either die or arrest in G_1 phase. Such behavior might reflect a novel checkpoint that monitors cell ploidy and prevents cells that have passed through aberrant mitosis from passing from G_1 phase into S phase, a cell cycle transition that p53 is known to regulate in response to DNA damage (reviewed in [38]). Passage through this checkpoint after an abortive mitosis would also lead to the increase in centrosome number that has been observed in p53-deficient cells [39].

Targets of the checkpoint

How does the spindle assembly checkpoint prevent anaphase? The cyclin-proteolysis machinery causes the destruction both of cyclin B [40] (and thus of the kinase activity of Cdc2/28–cyclin B complexes) and of proteins that regulate the separation of sister chromatids [41]. Two candidates for these regulatory proteins are Pds1 from budding yeast and Cut2 from fission yeast. These two proteins are degraded by the cyclin-proteolysis machinery as cells exit mitosis, and their degradation is required for sister chromatid separation [42•,43•,44••]. When *mad* mutants leave mitosis in the presence of spindle damage, B cyclins are degraded and sister chromatids separate, as would happen in a normal mitosis (J Minshull, A Straight, A Rudner, A Dernburg, A Murray, unpublished data; see Note added in proof). Thus, a likely target of the spindle assembly checkpoint is the cyclosome/APC (anaphase-promoting complex), the protein complex that ubiquitinates cyclin B, Pds1 and Cut2 (Fig. 1).

The cyclosome/APC has been purified from clam and frog eggs and is a 20S complex containing eight major subunits ([45•,46•]; J-M Peters, R King, C Höög, M Kirschner, personal communication; see Note added in proof), including homologs of the yeast proteins Cdc16, Cdc23 and Cdc27, all of which are required for the degradation of yeast B cyclins [47]. Unlike other components of the destruction machinery, the activity of the cyclosome/APC is regulated during the cell cycle and four of its components show changes in protein phosphorylation during the cell cycle.

Is there any direct evidence that the cyclosome/APC is the target of the checkpoint? Yeast extracts made from nocodazole-arrested cells (in which anaphase is prevented and the checkpoint is turned 'on') have no *in vitro* ubiquitination activity against Clb2 (the major budding yeast B-type cyclin) [48•]. In addition, *cdc16*, *cdc23*, and *cdc27* mutants arrest in mitosis, with high Cdc2/28–cyclin B activity and unseparated sister chromatids even in the absence of a functional *MAD*- and *BUB*-dependent checkpoint ([47]; A Rudner, A Murray, unpublished data).

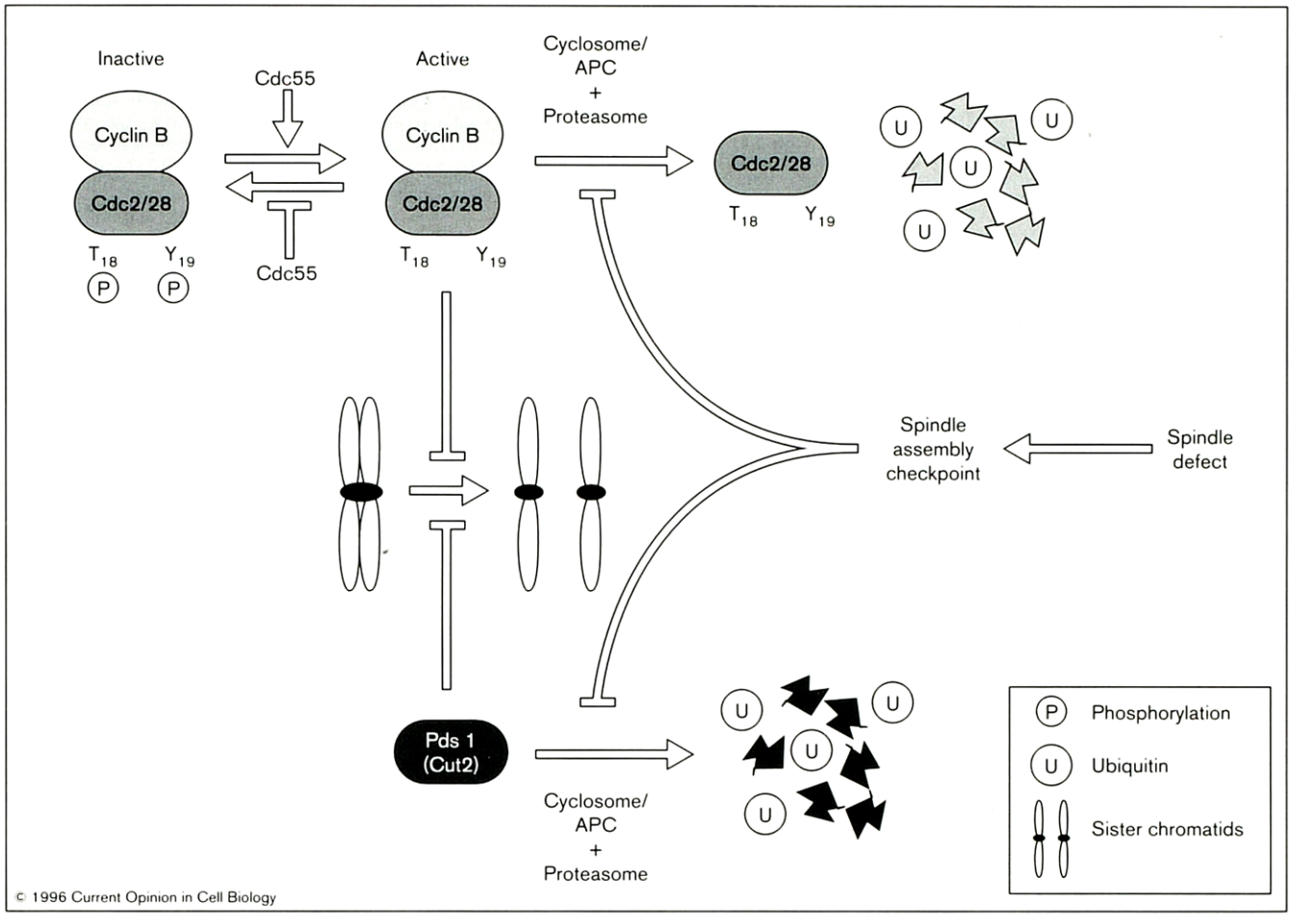
Are there other potential targets of the checkpoint? Accessibility or recognition of substrates by the ubiquitination and proteolytic machinery might be regulated. Although free cyclin B can be ubiquitinated *in vitro* [45•,46•], *Xenopus* cyclins A1, B1 and B2 require Cdc2 binding for their destruction [49]. Xe-p9 is the *Xenopus* homolog of the Cdc2-binding protein Suc1 from fission yeast [50]. Xe-p9, which binds tightly to Cdc2, is also required for the destruction of cyclin B in egg extracts [51•]. In addition, a large group of budding yeast mutants allow sister chromatid separation without cyclin B degradation [52–56], suggesting that the accessibility of different substrates to the cyclosome/APC might be differentially regulated during mitosis.

Is there regulation after cyclin ubiquitination? One report shows that the number of ubiquitin molecules on cyclin is the same in extracts in which cyclin is stable as it is in extracts in which cyclin is being rapidly degraded [57•]. Interpreting this observation is complicated by the probability that cyclin molecules that carry more than a threshold number of ubiquitins are rapidly degraded, and are thus excluded from this steady-state analysis.

Adaptation to checkpoint arrest

In many cell types, the duration of the mitotic arrest induced either by treatment with anti-microtubule drugs or by other spindle defects is not indefinite [2,58]. Cells eventually separate their sister chromatids and return to interphase despite the absence of a mitotic spindle. Continued activation of the checkpoint prevents proliferation and may be lethal. Thus, cell division in the face of the checkpoint signal produced by a persistent spindle defect (adaptation) may be beneficial because it allows a chance for survival as opposed to the certainty of death. In animals, where aneuploidy can initiate tumorigenesis [1], apoptosis may replace adaptation as the fate of cells that cannot correct their spindle defects.

Figure 1



A model for the control of the exit from mitosis in budding yeast. For a successful mitosis, the sister chromatids must separate and Cdc2/28–cyclin B must be inactivated. Sister chromatid separation is inhibited by Pds1 and the presence of active Cdc2/28–cyclin B. Both of these inhibitory influences are required to maintain sister chromatid linkage. Activation of the cyclin-proteolysis machinery (which involves ubiquitination of the target proteins by the cyclosome/APC and proteolysis of the target proteins by the proteasome) induces the ubiquitin-dependent destruction of cyclin B and Pds1, thus removing both forms of inhibition and inducing sister chromatid separation. Cdc2/28–cyclin B can also be inactivated by inhibitory phosphorylations on threonine 18 (T18) and tyrosine 19 (Y19) of Cdc2/28. (Activating phosphorylation occurs at threonine 169 of Cdc2/28 [not shown]). Cdc55, a B-type regulatory subunit of protein phosphatase 2A, normally restrains the use of this alternative pathway leading out of mitosis by ensuring that Cdc2/28–cyclin B remains active. Prolonged arrest in mitosis inhibits Cdc55 activity (not shown) and leads to adaptation from the arrest. The *MAD*- and *BUB*-dependent part (not shown) of the spindle assembly checkpoint (center right) acts to inhibit the activity of the cyclin-proteolysis machinery in cells with spindle defects. For Pds1, the name of the fission yeast counterpart is indicated in parentheses.

Adaptation could occur by inactivating the checkpoint and allowing a normal exit from mitosis. An alternative possibility is that there is a second pathway out of mitosis that is turned on during periods of prolonged arrest (Fig. 1). The recent discovery that mutations in *CDC55* are checkpoint-defective supports the latter possibility (J Minshull, A Straight, A Rudner, A Dernburg, A Murray, unpublished data; see Note added in proof; J Wang, D Burke, personal communication). Like the *mad* mutants, *cdc55* mutant cells cannot maintain Cdc2/28-associated kinase activity and sister chromatid cohesion in the presence of spindle defects. The drop in Cdc2/28-associated kinase activity in *cdc55* cells is due to inhibitory phosphorylation of Cdc28 on the residues threonine 18 and tyrosine 19, rather than to cyclin proteolysis (see Fig. 1). *CDC55* encodes a B-type regulatory subunit of protein phosphatase 2A (PP2A) [59]. PP2A activity regulates Cdc2/28-associated kinase activity by regulating both Cdc25 and Wee1, the phosphatase and kinase responsible for removing and adding the inhibitory phosphorylations on Cdc2/Cdc28 [60].

The checkpoint defect of *cdc55* mutants is quite different from that of any of the *mad* or *bub* mutants and might reflect a role not in the initial response to spindle damage, but rather in an adaptive pathway. This theory

would propose that the absence of *CDC55* causes cells to immediately adapt to spindle damage and exit mitosis independently of proteolysis (Fig. 1).

Conclusions and future directions

Molecular details of the spindle assembly checkpoint are conserved from yeast to humans. Sensing and transducing proteins of the checkpoint reside on the kinetochores of chromosomes. Many details, though, remain to be elucidated. For example, what are the identities of the 3F3/2-labeled epitope and its kinase? Are all of the yeast checkpoint proteins conserved in larger eukaryotes and do they reside on the kinetochore? Is the cyclosome/APC the target of the checkpoint? The *cdc55* mutation serves as a starting point for the investigation of adaptation to prolonged checkpoint arrest. This novel pathway that leads out of mitosis may also allow a deeper understanding of the normal exit from mitosis.

Note added in proof

The paper referred to in the text as J Minshull, A Straight, A Rudner, A Dernburg, A Murray, unpublished data, has now been accepted for publication [73].

The paper referred to in the text as J-M Peters, R King, C Höög, M Kirschner, personal communication, has now been accepted for publication [74].

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hartwell LH, Kastan MB: **Cell cycle control and cancer.** *Science* 1994, **266**:1821–1828.

2. Rieder CL, Palazzo RE: **Colcemid and the mitotic cell cycle.** *J Cell Sci* 1992, **102**:387–392.

3. Rieder CL, Schultz A, Cole R, Sluder G: **Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle.** *J Cell Biol* 1994, **127**:1301–1310.

4. Li R, Murray AW: **Feedback control of mitosis in budding yeast.** *Cell* 1991, **66**:519–531.

5. Hoyt MA, Trotis L, Roberts BT: ***S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function.** *Cell* 1991, **66**:507–517.

6. • Wells WAE, Murray AW: **Aberrantly segregating centromeres activate the spindle assembly checkpoint in budding yeast.** *J Cell Biol* 1996, **133**:75–84.
Multiple circular-centromeric or single short-linear plasmids cause *MAD*-dependent delays in mitosis.

7. Neff MW, Burke DJ: **A delay in the *Saccharomyces cerevisiae* cell cycle that is induced by a dicentric chromosome and dependent upon mitotic checkpoints.** *Mol Cell Biol* 1992, **12**:3857–3864.

8. • Weiss E, Winey M: **The *S. cerevisiae* SPB duplication gene *MPS1* is part of a mitotic checkpoint.** *J Cell Biol* 1996, **132**:111–123.
The kinase Mps1 is identified as a component of the spindle assembly checkpoint.

9. •• Hardwick KG, Weiss E, Luca FC, Winey M, Murray AW: **Activation of the budding yeast spindle assembly checkpoint without mitotic spindle disruption.** *Science* 1996, **273**:953–956.
Overexpression of the kinase Mps1 arrests cells in mitosis by constitutive activation of the checkpoint. This arrest depends on *MAD1–3* and *BUB1–3*, placing *MPS1* as the most upstream component of the checkpoint pathway. Mps1 phosphorylates Mad1 *in vitro*, suggesting that it is the *in vivo* Mad1 kinase.

10. Guenette S, Magendantz M, Solomon F: **Suppression of a conditional mutation in alpha tubulin by overexpression of two checkpoint genes.** *J Cell Sci* 1995, **108**:1195–1204.

11. • Wang Y, Burke DJ: **Checkpoint genes required to delay cell division in response to nocodazole respond to impaired kinetochore function in the yeast *Saccharomyces cerevisiae*.** *Mol Cell Biol* 1995, **15**:6838–6844.
Low doses of benomyl, a microtubule-depolymerizing drug, or a mutation in *CTF13*, which encodes a protein component of the centromere, cause *MAD1,2* and *BUB1,3*, but not *BUB2*, dependent delays in mitosis. The authors conclude that microtubule attachment to the kinetochore is defective in both situations and that *BUB2* is not required for detecting this defect.

12. Pangilinan F, Spencer F: **Abnormal kinetochore structure activates the spindle assembly checkpoint in budding yeast.** *Mol Biol Cell* 1996, **7**:1195–1208.

13. Wells WAE: **The spindle-assembly checkpoint: aiming for a perfect mitosis, every time.** *Trends Cell Biol* 1996, **6**:228–234.

14. •• Zhang D, Nicklas RB: **'Anaphase' and cytokinesis in the absence of chromosomes.** *Nature* 1996, **382**:466–468.
In another feat of micromanipulation, the authors are able to remove all chromosomes from insect spermatocytes. The resulting spindle is able to go through anaphase-like movements, and the cell still undergoes cytokinesis at normal rates. This experiment demonstrates that no positive signal is generated by chromosomes to promote anaphase. Note that if the chromosomes are removed too early, a spindle will not form.

15. •• Rieder CL, Cole RW, Khodjakov A, Sluder G: **The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores.** *J Cell Biol* 1995, **130**:941–948.
The delay in anaphase caused by a single unattached kinetochore [3] is abolished if that kinetochore is destroyed by a laser microbeam. The authors also show that taxol causes a similar delay in mitosis.

16. Jordan MA, Thrower D, Wilson L: **Effects of vinblastine, podophyllotoxin and nocodazole on mitotic spindles.** *J Cell Sci* 1992, **102**:401–416.

17. Jordan MA, Toso RJ, Thrower D, Wilson L: **Mechanism of mitotic block and inhibition of cell proliferation by taxol at low concentrations.** *Proc Natl Acad Sci USA* 1993, **90**:9552–9556.

18. Callan HG, Jacobs PA: **The meiotic process in *Mantis religiosa* L. males.** *J Genet* 1957, **55**:200–217.

19. •• Li X, Nicklas RB: **Mitotic forces control a cell cycle checkpoint.** *Nature* 1995, **373**:630–632.
Applying tension with a microneedle at the kinetochore of a chromosome overrides checkpoint-induced delays in anaphase. This paper and [28••] provide the most compelling evidence that lack of tension is the trait monitored by the spindle assembly checkpoint.

20. Nicklas RB, Koch CA: **Chromosome manipulation III. Induced reorientation and the experimental control of segregation in meiosis.** *J Cell Biol* 1969, **43**:40–50.

21. • Toyn JH, Johnson AL, Johnston LH: **Segregation of unreplicated chromosomes in *Saccharomyces cerevisiae* reveals a novel G1/M-phase checkpoint.** *Mol Cell Biol* 1995, **15**:5312–5321.
This paper and [22•] show that *S. cerevisiae* containing certain *cdc6* and *cdc7* mutations, which result in a failure to replicate chromosomes, still go through mitosis with no delay. This observation suggests that lack of tension at kinetochores in budding yeast does not activate the spindle assembly checkpoint.

22. • Piatti S, Lengauer C, Nasmyth K: **Cdc6 is an unstable protein whose *de novo* synthesis in G1 is important for the onset of S phase and for preventing a reductional anaphase in the budding yeast *Saccharomyces cerevisiae*.** *EMBO J* 1995, **14**:3788–3799.
See annotation [21•].

23. Sluder G, Miller FJ, Spanjian K: **The role of spindle microtubules in the timing of the cell cycle in echinoderm eggs.** *J Exp Zool* 1986, **238**:325–336.

24. Sluder G, Begg DA: **Control mechanisms of the cell cycle: role of the spatial arrangement of spindle components in the timing of mitotic events.** *J Cell Biol* 1983, **97**:877–886.

25. Sluder G, Miller FJ, Thompson EA, Wolf DE: **Feedback control of the metaphase–anaphase transition in sea urchin zygotes: role of maloriented chromosomes.** *J Cell Biol* 1994, **126**:189–198.

26. Gorbsky GJ, Ricketts WA: **Differential expression of a phosphoepitope at the kinetochores of moving chromosomes.** *J Cell Biol* 1993, **122**:1311–1321.

27. Campbell MS, Gorbsky GJ: **Microinjection of mitotic cells with the 3F3/2 anti-phosphoepitope antibody delays the onset of anaphase.** *J Cell Biol* 1994, **129**:1195–1204.

28. •• Nicklas RB, Ward SC, Gorbsky GJ: **Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint.** *J Cell Biol* 1995, **130**:929–939.
Kinetochores that are not under tension stain brightly with the antibody 3F3/2. This staining is reduced when tension is applied to these kinetochores with a microneedle. This result suggests that defects in kinetochore attachment to the spindle and a lack of tension at the kinetochore are detected by the same components, and may even reflect the same defect.

29. •• Chen R-H, Waters JC, Salmon ED, Murray AW: **Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores.** *Science* 1996, in press.
XMAD2, the *Xenopus* homolog of Mad2, is required for maintenance of the spindle assembly checkpoint in *Xenopus* egg extracts. XMAD2, like the 3F3/2-labeled epitope, localizes to kinetochores that have attached to the spindle. This paper provides the first evidence that checkpoint components are conserved from yeast to vertebrates, and also suggests that the budding yeast checkpoint components may reside at the kinetochore too.

30. •• Li Y, Benezra R: **Identification of a human mitotic checkpoint gene: hsMAD2.** *Science* 1996, in press.
This paper identifies hsMAD2, the human homolog of Mad2. hsMAD2 also localizes to the kinetochore.

31. Minshull J, Sun H, Tonks NK, Murray AW: **MAP-kinase dependent mitotic feedback arrest in *Xenopus* egg extracts.** *Cell* 1994, **79**:475–486.

32. • Hardwick K, Murray AW: **Mad1p, a phosphoprotein component of the spindle assembly checkpoint in budding yeast.** *J Cell Biol* 1995, **131**:709–720.
This paper describes the cloning of *MAD1* and its interesting localization, and shows that Mad1 is hyperphosphorylated when the checkpoint is activated. Mad1 hyperphosphorylation requires *BUB1*, *BUB3* and *MAD2*, but not *BUB2* and *MAD3*. A crude biochemical pathway can be created from these data and those found in [9••].

33. Roberts RT, Farr KA, Hoyt MA: **The *Saccharomyces cerevisiae* checkpoint gene *BUB1* encodes a novel protein kinase.** *Mol Cell Biol* 1994, 14:8282–8291.

34. Winey M, Goetsch L, Baum P, Byers B: ***MPS1* and *MPS2*: novel yeast genes defining distinct steps of spindle pole body duplication.** *J Cell Biol* 1991, **114**:745–754.

35. Winey M, Byers B: **Assembly and functions of the spindle pole body in budding yeast.** *Trends Genet* 1993, 9:300–304.

36. Lauze E, Stoelcker B, Luca FC, Weiss E, Schutz AR, Winey M: **Yeast spindle pole body duplication gene *MPS1* encodes an essential dual specificity protein kinase.** *EMBO J* 1995, 14:1655–1663.

37. • Cross SM, Sanchez CA, Morgan CA, Schimke MK, Ramel S, Idzerda RL, Raskind WH, Reid BJ: **A p53-dependent mouse spindle checkpoint.** *Science* 1995, **267**:1353–1356.
This paper suggests that p53 is a component of the spindle assembly checkpoint in animal cells. Note that both wild-type and p53-mutant cells arrest poorly in nocodazole, suggesting that a different checkpoint defect may lead to the increase in ploidy of the p53-deficient cells.

38. Murray AW: **Cell cycle checkpoints.** *Curr Opin Cell Biol* 1994, 6:872–876.

39. Fukasawa K, Choi T, Kuriyama R, Rulong S, Vande Woude GF: **Abnormal centrosome amplification in the absence of p53.** *Science* 1996, **271**:1744–1747.

40. Glotzer M, Murray AW, Kirschner MW: **Cyclin is degraded by the ubiquitin pathway.** *Nature* 1991, **349**:132–138.

41. Holloway SL, Glotzer M, King RW, Murray AW: **Anaphase is initiated by proteolysis rather than by the inactivation of MPF.** *Cell* 1993, **73**:1393–1402.

42. • Yamamoto YA, Guacci V, Koshland D: **Pds1p, an inhibitor of anaphase in budding yeast, plays a critical role in the APC and checkpoint pathway(s).** *J Cell Biol* 1996, **133**:99–110.
This paper and [43•] identify Pds1 as a protein that regulates sister chromatid separation. *pds1* mutants prematurely separate their sister chromatids, and have defects in the DNA-damage checkpoint and in spindle elongation. As expected, the Pds1 protein is degraded as cells exit mitosis and contains a putative cyclin-destruction box.

43. • Yamamoto A, Guacci V, Koshland D: **Pds1p is required for faithful execution of anaphase in the yeast, *Saccharomyces cerevisiae*.** *J Cell Biol* 1996, **133**:85–97.
See annotation [42•].

44. •• Funabiki H, Yamano H, Kumada K, Nagao K, Hunt T, Yangida M: **Cut2 proteolysis required for sister-chromatid separation in fission yeast.** *Nature* 1996, **381**:438–441.
Cut2 is required for sister chromatid separation, is degraded as cells exit mitosis and contains a cyclin-destruction box. Truncating Cut2 makes it nondegradable, and replacing the amino terminus with the amino terminus of Cdc13 (fission yeast cyclin B) allows the chimera to be degraded properly and complements a *cut2* mutant. Paradoxically, *cut2* mutants and cells containing nondegradable Cut2 have the same phenotype, suggesting that Cut2 may have two roles in sister chromatid separation: its presence is required to license sisters for separation and its destruction is required to initiate separation. Pds1 and Cut2 share limited homology and may be functional homologs.

45. • King RW, Peters JM, Tugendreich S, Rolfe M, Hieter P, Kirschner MW: **A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B.** *Cell* 1995, **81**:279–288.
This paper and [46•] describe the purification of the cyclosome/APC, a 20S complex that ubiquitinates cyclin B, from *Xenopus* and clam embryos. The authors also show that homologs of yeast Cdc16 and Cdc27 are components of this complex and that antibodies to the human homolog of Cdc27 can immunoprecipitate an active complex. Note that, *in vitro*, this complex ubiquitinates free cyclin B, which may not be its physiological substrate (see [49]).

46. • Sudakin V, Ganoth D, Dahan A, Heller H, Hersko J, Luca F, Ruderman JV, Hershko A: **The cyclosome, a large complex containing cyclin-selective ubiquitination ligase activity, targets cyclins for destruction at the end of mitosis.** *Mol Biol Cell* 1995, **6**:185–198.
See annotation [45•].

47. Irniger S, Piatti S, Michaelis C, Nasmyth K: **Genes involved in sister chromatid separation are needed for B-type cyclin proteolysis in budding yeast.** *Cell* 1995, **77**:1037–1050.

48. • Zachariae W, Nasmyth K: **TPR proteins required for anaphase progression mediate ubiquitination of mitotic B type cyclins in yeast.** *Mol Biol Cell* 1996, **7**:791–801.
This paper describes an *in vitro* assay for cyclin B ubiquitination in budding yeast. *cdc16*, *cdc23*, *cdc27* and *cse1* mutants all have defects in cyclin ubiquitination. Cells arrested in nocodazole do not contain any ubiquitination activity, supporting the hypothesis that the cyclosome/APC is the target of the spindle assembly checkpoint. As in [45•,46•], the cyclin B substrate may not be complexed to Cdc2/28.

49. Stewart E, Kobayashi H, Harrison D, Hunt T: **Destruction of *Xenopus* cyclins A and B2, but not B1, requires binding to p34cdc2.** *EMBO J* 1994, 13:584–594.

50. Hindley J, Phear GA, Stein M, Beach D: ***suc1*$^+$ encodes a 13 kilodalton protein that is essential for cell viability and directly involved in the division cycle of *Schizosaccharomyces pombe*.** *Mol Cell Biol* 1987, **7**:504–511.

51. • Patra D, Dunphy WG: **Xe-p9, a *Xenopus SUC1/CKS* homolog, has multiple essential roles in cell cycle control.** *Genes Dev* 1996, **10**:1503–1515.
The *Xenopus* homolog of Suc1, Xe-p9, is required for the degradation of B-type cyclins and for proper activation of Cdc25. The authors propose that Xe-p9 is involved in targeting Cdc2–cyclin B complexes for degradation.

52. Toyn JH, Johnston LH: **The Dbf2 and Dbf20 protein kinases of budding yeast are activated after the metaphase to anaphase cell cycle transition.** *EMBO J* 1994, 13:1103–1113.

53. Kitada K, Johnson AL, Johnston LH, Sugino A: **A multicopy suppressor gene of the *Saccharomyces cerevisiae* G1 cell cycle mutant gene *dbf4* encodes a protein kinase and is identified as *CDC5*.** *Mol Cell Biol* 1993, 13:4445–4457.

54. Shirayama M, Matsui Y, Toh-E A: **The yeast *TEM1* gene, which encodes a GTP-binding protein, is involved in termination of M phase.** *Mol Cell Biol* 1994, 14:7476–7482.

55. Morishita T, Mitsuzawa H, Nakamura S, Hattori S, Anraku Y: **Requirement of *Saccharomyces cerevisiae* Ras for completion of mitosis.** *Science* 1995, **270**:1213–1215.

56. Surana U, Amon A, Dowzer C, McGrew J, Byers B, Nasmyth K: **Destruction of the *CDC28/CLB* mitotic kinase is not required for the metaphase to anaphase transition in budding yeast.** *EMBO J* 1993, **12**:1969–1978.

57. • Klotzbücher A, Stewart E, Harrison D, Hunt T: **The destruction box of cyclin A allows B-type cyclins to be ubiquitinated, but not efficiently destroyed.** *EMBO J* 1996, **15**:3053–3064.
Chimeras of the amino terminus of cyclin A1 and the carboxyl termini of cyclin B1 and B2 bind to and activate Cdc2 normally, are ubiquitinated normally, but cannot be degraded. This same effect is observed when B-type cyclins contain a cyclin A1 destruction box.

58. Kung AL, Sherwood SW, Schimke RT: **Cell line-specific differences in the control of cell cycle progression in the absence of mitosis.** *Proc Natl Acad Sci USA* 1990, **87**:9553–9557.

59. Nickels JT, Broach JR: **A ceramide-activated protein phosphatase mediates ceramide-induced G1 arrest of *Saccharomyces cerevisiae*.** *Genes Dev* 1996, **10**:382–394.

60. Dunphy WG: **The decision to enter mitosis.** *Trends Cell Biol* 1994, **4**:202–207.

61. Bai C, Partha S, Hofmann K, Ma L, Goebl M, Harper JW, Elledge SJ: ***SKP1* connects cell cycle regulators to the ubiquitin proteolysis machinery through a novel motif, the F-box.** *Cell* 1996, **86**:263–274.

62. Connelly C, Hieter P: **Budding yeast *SKP1* encodes a evolutionarily conserved kinetochore protein required for cell cycle progression.** *Cell* 1996, **86**:275–285.

63. Hyman AA, Sorger PK: **Structure and function of kinetochores in budding yeast.** *Annu Rev Cell Dev Biol* 1996, **7**:471–495.

64. Tomkiel J, Cooke CA, Saitoh H, Bernat RL, Earnshaw WC: **CENP-C is required for maintaining proper kinetochore size and for a timely transition to anaphase.** *J Cell Biol* 1994, **125**:531–545.

65. Yen TJ, Compton DA, Wise D, Zinkowski RP, Brinkley BR, Earnshaw WC, Cleveland DW: **CENP-E a novel human centromere-associated protein required for progression from metaphase to anaphase.** *EMBO J* 1991, **10**:1245–1254.

66. Hoyt MA: **Cellular roles of kinesin and related proteins.** *Curr Opin Cell Biol* 1994, **6**:63–68.

67. Nislow C, Sellitto C, Kuriyama R, McIntosh JR: **A monoclonal antibody to a mitotic microtubule-associated protein blocks mitotic progression.** *J Cell Biol* 1990, **111**:511–522.

68. Wright BD, Terasaki M, Scholey JM: **Roles of kinesin and kinesin-like proteins in sea urchin embryonic cell division: evaluation using antibody microinjection.** *J Cell Biol* 1993, **123**:681–689.

69. Kellogg DR, Murray AW: ***NAP1* acts with Clb2 to perform mitotic functions and suppress polar bud growth in budding yeast.** *J Cell Biol* 1995, **130**:675–685.

70. Jang JK, Messina L, Erdman MB, Arbel T, Hawley RS: **Induction of metaphase arrest in *Drosophila* oocytes by chiasma-based kinetochore tension.** *Science* 1995, **268**:1917–1919.

71. Fankhauser C, Marks J, Reymond A, Simanis V: **The *S. pombe cdc16* gene is required both for maintenance of p34cdc2 kinase activity and regulation of septum formation: a link between mitosis and cytokinesis?** *EMBO J* 1993, **12**:2697–2704.

72. Waldman T, Lengauer C, Kinzler KW, Vogelstein B: **Uncoupling of S phase and mitosis induced by anticancer agents in cells lacking p21.** *Nature* 1996, **381**:713–716.

73. Minshull J, Straight A, Rudner A, Dernburg A, Murray AW: **Protein phosphatase 2A regulates MPF activity and sister chromatid cohesion in budding yeast.** *Curr Biol* 1996, in press.

74. Peters J-M, King RW, Höög C, Kirschner MW: **Identification of BIME as a subunit of the anaphase promoting complex.** *Science* 1996, in press.