Attachment and tension in the spindle assembly checkpoint

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Journal of Cell Science 115, 3547-3555 (2002) © The Company of Biologists Ltd
doi:10.1242/jcs.00029

Summary

Faithful transmission of chromosomes during mitosis is ensured by the spindle assembly checkpoint. This molecular safeguard examines whether prerequisites for chromosome segregation have been satisfied and thereby determines whether to execute or to delay chromosome segregation. Only when all the chromosomes are attached by kinetochore microtubules from two opposite spindle poles and proper tension is placed on the paired kinetochores does anaphase take place, allowing the physical splitting of sister chromatids. Recent studies have provided novel insights into the molecular mechanisms through which the spindle assembly checkpoint is regulated by both the attachment of chromosomes to kinetochore microtubules and the tension exerted on kinetochores.

Key words: Spindle assembly checkpoint, Mitosis, Chromosome, Kinetochore, Microtubule, Attachment, Tension

Introduction

The goal of the mitotic cell cycle is to produce two genetically identical cells from one. In order to accomplish this, the mother cell must replicate its chromosomes exactly once prior to entering mitosis, and at the end of mitosis each daughter cell must receive one and only one copy of each chromosome. To ensure this, the cell must precisely coordinate various complex events; one event must be prevented if a preceding event is not complete. This is mediated by cellular regulatory mechanisms called cell cycle checkpoints, initially conceived by Mazia and then articulated by Hartwell and Weinert, and McIntosh (Mazia, 1961; Hartwell and Weinert, 1989; McIntosh, 1991). In particular, during mitosis, cells have evolved a surveillance mechanism called the spindle assembly checkpoint (Rudner and Murray, 1996; Wells, 1996), which is also known as the mitotic checkpoint (Li and Benezra, 1996), kinetochore attachment checkpoint (Rieder et al., 1994), chromosome distribution checkpoint (Nicklas, 1997) or simply the spindle checkpoint (Waters et al., 1998), which is crucial for ensuring fidelity in chromosome segregation. The spindle assembly checkpoint examines whether prerequisites for chromosome segregation have been met and thereby determines whether to execute or to delay chromosome segregation.

The kinetochore-microtubule attachment

At the heart of the spindle assembly checkpoint is the kinetochore, a multi-layered proteinaceous complex that assembles on the centromeric DNA of each chromosome (Rieder and Salmon, 1998). During mitosis, the kinetochore mediates the interaction between the chromosome and spindle microtubules. At the very beginning of prometaphase (i.e. immediately after the nuclear envelope breaks down), kinetochores are not attached to microtubules. Subsequently, one kinetochore on a chromosome captures microtubules from one spindle pole. When its sister kinetochore captures microtubules from the other pole, the now bioriented chromosome moves to the equatorial plane (also called the metaphase plate); this process is known as chromosome congression (Rieder and Salmon, 1994). Finally, all the chromosomes are attached through both kinetochores to microtubules from two opposite spindle poles and aligned at the equatorial plane, a stage referred to as metaphase. The spindle assembly checkpoint ensures that, only when all the chromosomes are properly attached and aligned at the equatorial plane, anaphase onset is triggered, allowing the splitting of sister chromatids and their delivery to each spindle pole (Fig. 1).

The checkpoint components

The major components involved in the spindle assembly checkpoint were identified in two similar genetic screens in budding yeast for mutants that fail to arrest in mitosis in the presence of spindle-damaging agents such as microtubule-depolymerizing drugs. These checkpoint components include Mad1, Mad2, Mad3 (mitotic arrest deficient) (Li and Murray, 1991), Bub1 and Bub3 (budding uninhibited by benzimidazole) (Hoyt et al., 1991). Subsequently, Mps1 (monopolar spindle 1), initially identified as a kinase functioning in duplication of the spindle pole body (the yeast equivalent of mammalian centrosome), was also found to play a role in the spindle assembly checkpoint (Weiss and Winey, 1996). Over the past several years, homologs of many of these proteins have been identified in *Schizosaccharomyces pombe* (He et al., 1997; He et al., 1998; Bernard et al., 1998), *Xenopus laevis* (Chen et al., 1996; Chen et al., 1998), *Drosophila melanogaster* (Basu et al., 1998; Basu et al., 1999), *Caenorhabditis elegans* (Kitagawa and Rose, 1999), *Mus musculus* (Taylor and McKeon, 1997; Matinez-Exposito et al., 1999) and *Homo sapiens* (Li and Benezra, 1996; Jin et al., 1998; Cahill et al., 1998; Taylor et al., 1998; Chan et al., 1998; Chan et al., 1999).

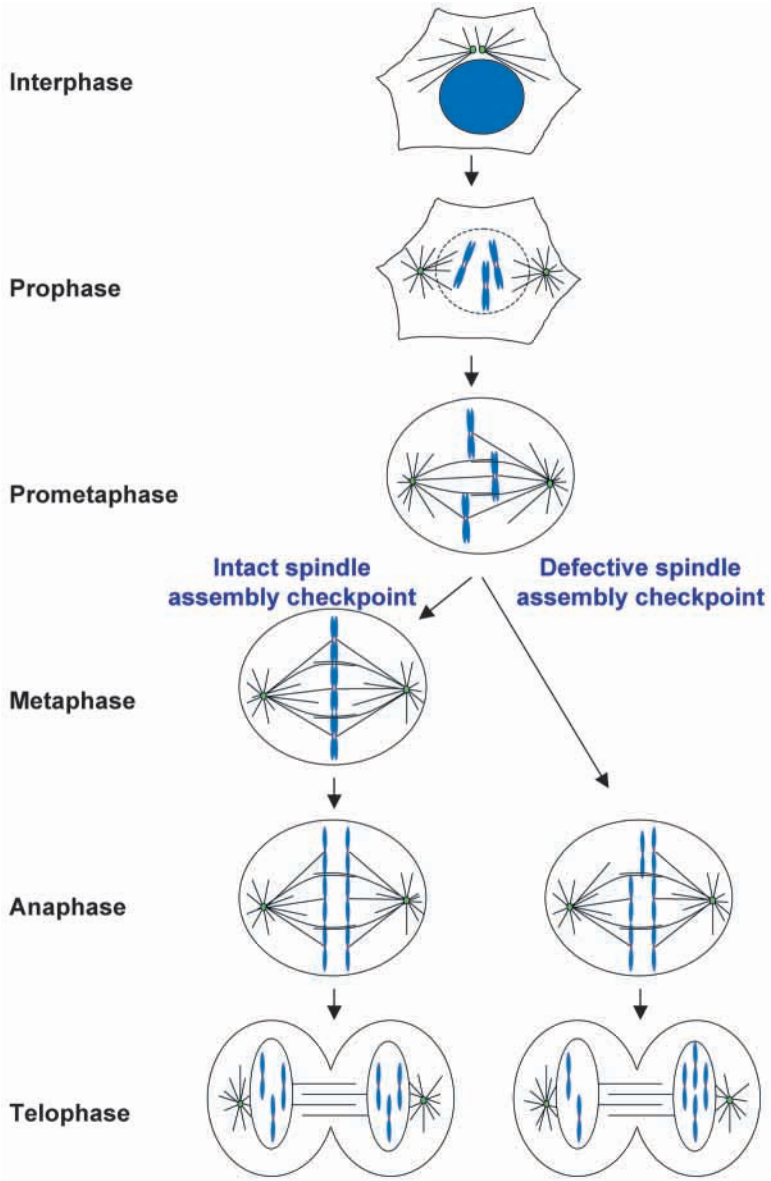

Fig. 1. The spindle assembly checkpoint acts as a molecular safeguard in ensuring faithful chromosome transmission during mitosis. During prophase, the duplicated interphase chromatin condenses into chromosomes (blue) within the nucleus. Simultaneously, the radial array of microtubules (black lines) disappears, and a bipolar array (the mitotic spindle) forms by microtubules emanating from two opposite poles (green) defined by the previously duplicated and now separated pair of centrosomes. Subsequently, the nuclear envelope breaks downs marking the initiation of prometaphase. During this stage, the kinetochores (red) on chromosomes encounter and capture spindle microtubules. When a chromosome becomes attached by microtubules from two opposite spindle poles, it congresses to the equatorial plane (the metaphase plate). In the presence of an intact spindle assembly checkpoint, anaphase onset is triggered when and only when all the chromosomes are attached via kinetochores by microtubules from two spindle poles and correctly aligned at the equatorial plane, a stage referred to as metaphase. However, in cells that have defective spindle assembly checkpoint, anaphase onset is triggered prematurely despite the presence of unattached or improperly attached chromosomes, resulting in missing or extra chromosomes (aneuploidy) in the daughter cells.

The function of each of these checkpoint proteins is needed to prevent anaphase entry when the spindle has a defect or when chromosomes are not properly attached (for a review, see Amon, 1999). Cells harboring mutations in many of these checkpoint genes proceed to anaphase prematurely and split sister chromatids regardless of whether the prerequisites for chromosome segregation have been satisfied. As a consequence, the delivery of exactly one copy of each chromosome to each daughter cell cannot be guaranteed, which can result in the production of daughter cells that have gained or lost one or more chromosomes, a phenomenon termed aneuploidy (Fig. 1). Missing or extra chromosomes in germ-line cells can result in premature abortion of the fetus or generation of offspring with birth defects such as Patau syndrome, Edwards syndrome, Down syndrome and Klinefelter syndrome, which are characterized by the presence of an extra copy of chromosome 13, chromosome 18, chromosome 21 and the X chromosome, respectively (Sluder and McCollum, 2000). Unequal chromosome segregation can also have severe consequences in adults by fostering tumor malignancy (Manchester, 1995). In fact, mutations in or reduced expression of spindle assembly checkpoint components has recently been found in some types of human cancer (Li and Benezra, 1996; Cahill et al., 1998; Lee et al., 1999; Takahashi et al., 1999; Michel et al., 2001; Wang et al., 2002). For example, mutational inactivation of Bub1 has been implicated in human colorectal cancer (Cahill et al., 1998), and reduced expression of Mad2 has been implicated in human breast and ovarian cancers (Li and Benezra, 1996; Wang et al., 2002).

The checkpoint signaling pathway

Recent work in yeast, frogs and mammals has produced an outline of spindle assembly checkpoint signaling (Fig. 2). In brief, a signal is generated by the presence of unattached or improperly attached kinetochores that ultimately inhibits the activity of the anaphase-promoting complex (APC, also known as the cyclosome) (King et al., 1995), the multi-subunit E3 ubiquitin ligase required for anaphase entry (Fang et al., 1999; Zachariae and Nasmyth, 1999). Active APC catalyzes ubiquitination of an anaphase inhibitor, securin (Pds1 in budding yeast and Cut2p in fission yeast), which leads to its destruction through 26S-proteosome-mediated proteolysis (Yamamoto et al., 1996; Cohen-Fix et al., 1996; Funabiki et al., 1996; Zou et al., 1999). Degradation of securin releases separin (Esp1 in budding yeast and Cut1p in fission yeast), which cleaves the Scc1/Mcd1 subunit of the cohesin complex (Ciosk et al., 1998; Uhlmann et al., 1999; Uhlmann et al., 2000; Hauf et al., 2001). This complex is established during DNA replication and maintains the linkage between sister chromatids (Michaelis et al., 1997; Guacci et al., 1997; Zachariae and Nasmyth, 1999).

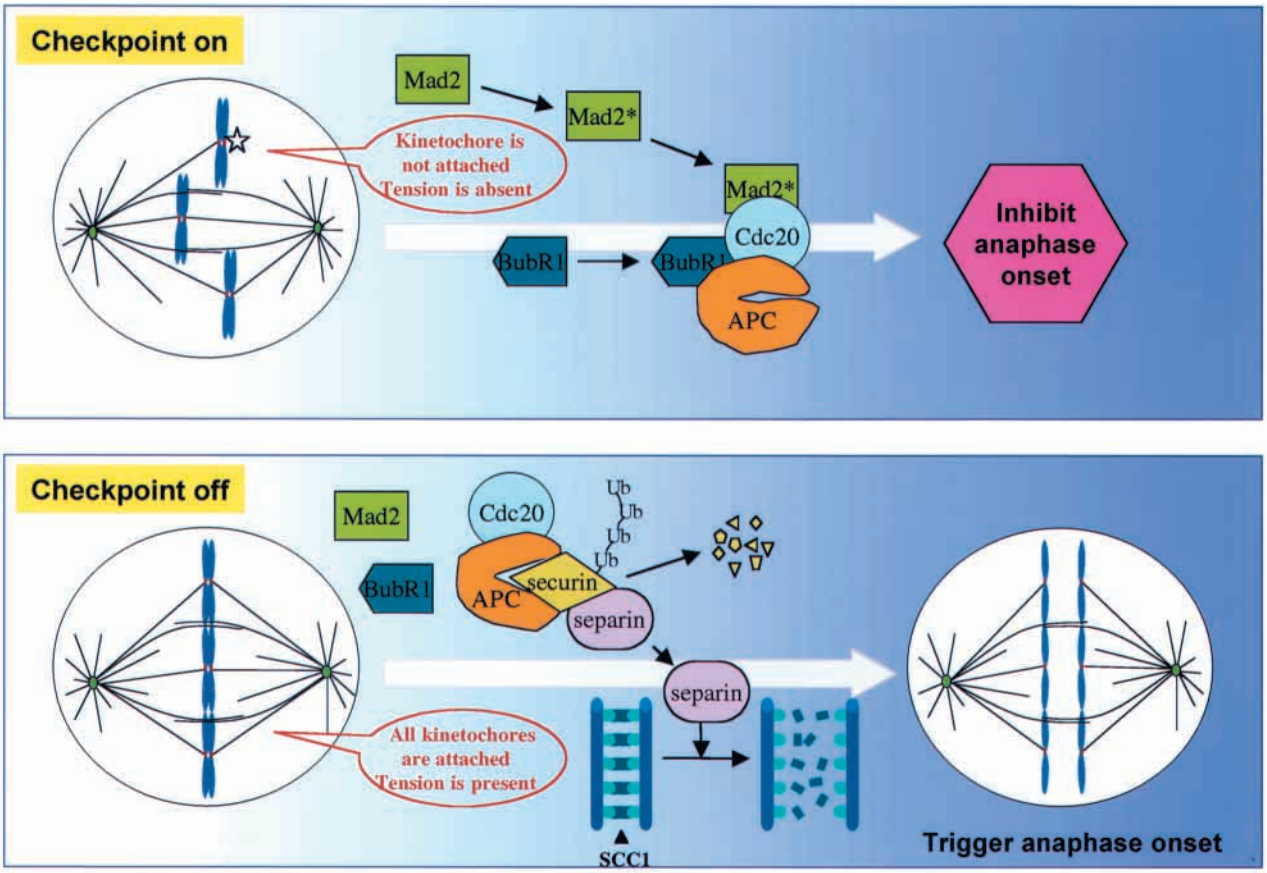

Fig. 2. Spindle assembly checkpoint signaling. In the presence of unattached or improperly attached kinetochores, the spindle assembly checkpoint is switched on (upper panel). Unattached kinenetochores act as catalytic sites for the activation of Mad2. Activated Mad2 (Mad2*) then diffuses and prevents anaphase onset by inhibiting the activity of Cdc20-APC. In addition, BubR1 functions synergistically with Mad2 in inhibiting Cdc20-APC activity. After all the chromosomes are properly attached by kinetochore microtubules and aligned at the metaphase plate, the spindle assembly checkpoint is turned off (bottom panel). Mad2* is no longer generated, and BubR1 does not interact with Cdc20-APC, resulting in the activation of Cdc20-APC. Activated Cdc20-APC catalyzes the ubiquitination of securin, leading to its degradation through proteosome-mediated proteolysis. Degradation of securin in turn causes the release of separin. The free separin is then able to cleave the SCC1 subunit of the sister-chromatid cohesion complex, triggering the separation of sister chromatids and the onset of anaphase.

It is now clear that the ubiquitin ligase activity of the APC towards securin requires association of APC with Cdc20 (Slp1p in fission yeast, Fizzy in flies and p55cdc in mammals), which activates the APC by direct binding (Visintin et al., 1997; Hwang et al., 1998; Shirayama et al., 1998; Fang et al., 1998a). The checkpoint component Mad2 inhibits activation of the APC by interacting with Cdc20 (Li et al., 1997; Fang et al., 1998b; Hwang et al., 1998; Kim et al., 1998). Immunofluorescence microscopy studies with antibodies to Mad2 show that it localizes to unattached but not to fully attached kinetochores in vertebrates (Chen et al., 1996; Li and Benezra, 1996), and real-time visualization of Mad2 in living mammalian cells demonstrates that it is dynamically associated with unattached kinetochores (Howell et al., 2000). A catalytic model for the role of Mad2 in the generation of the anaphase-delaying signal has therefore been proposed (Howell et al., 2000) (Fig. 2). According to this model, unattached kinetochores on chromosomes provide sites for the activation of Mad2. Activated Mad2 (Mad2*) is then released into the cytoplasm and prevents the onset of anaphase by inhibiting the Cdc20-bound APC. After microtubules have attached to all the kinetochores, sites for Mad2 activation are no longer available, which eventually leads to APC activation by Cdc20 and triggering of anaphase onset.

More recently, BubR1, the mammalian homolog of the checkpoint protein Mad3, has been shown to be even more potent in vitro than Mad2 at inhibiting APC activity in purified preparations (Sudakin et al., 2001; Tang et al., 2001; Fang, 2002). BubR1 is a protein kinase that associates with Cdc20 and the APC (Chan et al., 1999; Wu et al., 2000; Skoufias et al., 2001). Tang et al. found that recombinant BubR1 directly inhibits the ubiquitin ligase activity of the APC and that the kinase activity of BubR1 is not required for this inhibition (Tang et al., 2001). In addition, they purified a checkpoint complex from HeLa cells that contains BubR1, Bub3 and substoichiometric amounts of Cdc20. Independently, Sudakin et al. purified a mitotic checkpoint complex (MCC) that contains nearly stoichiometric amounts of BubR1, Bub3, Mad2 and Cdc20 (Sudakin et al., 2001). They found that the isolated MCC is about 3000-fold more potent than purified Mad2 alone at inhibiting the ubiquitin ligase activity of the APC. In experiments consistent with these studies, Fang found that

BubR1 binds to Cdc20 with a high affinity and is more efficient than Mad2 as an inhibitor of Cdc20-APC in vitro (Fang, 2002). Moreover, this study demonstrated that BubR1 functions synergistically with Mad2 at physiological concentrations to inhibit APC activity. Interestingly, studies in fission yeast by Millband and Hardwick demonstrated that Mad3 also associates with Bub3, Cdc20 and Mad2 and that Mad3 is required for metaphase arrest caused by Mad2 overexpression (Millband and Hardwick, 2002). Collectively, these studies suggest that BubR1 and Mad2 cooperate in transducing the anaphase-delaying signal by inhibiting APC activity (Fig. 2).

Turning off the checkpoint signaling: attachment or tension?

How is the spindle assembly checkpoint turned off when all the chromosomes are properly attached? There has been much controversy over this issue. There are two models: (1) the 'attachment model', in which full occupation (saturation) of kinetochores by bound spindle microtubules switches off the checkpoint (Rieder et al., 1994; Reider et al., 1995); and (2) the 'tension model', in which proper tension exerted upon kinetochores due to bipolar microtubule attachment is responsible (McIntosh, 1991; Li and Nicklas, 1995).

Attachment of kinetochores to spindle microtubules probably involves kinesin- or dynein-like microtubule motors, although neither CENP-E (centromere protein E, a kinesin-like motor protein) nor dynein, the two known motor proteins present at kinetochores (Pfarr et al., 1990; Steuer et al., 1990; Yen et al., 1992; Lombillo et al., 1995; Cooke et al., 1997; Yao et al., 1997), is required for kinetochore microtubule formation (McEwen et al., 2001; Howell et al., 2001). When a pair of kinetochores becomes attached to microtubules from two opposite spindle poles, tension develops across the sister kinetochores (Fig. 3), even for those that are oscillating, by switching directions between poleward motion and away-from-the-pole motion (a phenomenon called kinetochore directional instability) (Mitchison and Salmon, 1992; Skibbens et al., 1993; Waters et al., 1996b). Tension is generated by the mitotic force that tends to pull the chromatids toward two opposite spindle poles against the glue (cohesin) that holds sister chromatids together (McIntosh, 1984; Nicklas, 1988a; Mitchison and Salmon, 1992; Rieder and Salmon, 1994). The tension across sister kinetochores is apparent as a visible increase in the distance between them in organisms from yeast to humans (a phenomenon termed kinetochore stretching) (Waters et al., 1996b; Shelby et al., 1996; Nicklas, 1997; Waters et al., 1998; Goshima and Yanagida, 2000; He et al., 2000; Tanaka et al., 2000; Skoufias et al., 2001; Zhou et al., 2002).

McIntosh first proposed that the mechanical tension exerted on kinetochores acts as a checkpoint for regulating anaphase entry (McIntosh, 1991). Li and Nicklas tested this proposal by ingenious experiments using praying mantid spermatocytes, which have three sex chromosomes: a Y chromosome and two genetically different X chromosomes (Li and Nicklas, 1995). Balance in the genetic information requires sperm that contain either both X chromosomes or the Y chromosome; this takes place only if the two X chromosomes attach to microtubules from the same spindle pole and the Y chromosome attaches to microtubules from the opposite pole (Nicklas, 1997). In some cells the three sex chromosomes fail to be connected trivalently, and they appear as an X-Y bivalent and a free X chromosome, whose kinetochore lacks tension (Li and Nicklas, 1995). Under these circumstances, the onset of anaphase is delayed by up to 9 hours. However, the cell proceeds to anaphase shortly after mechanical tension is applied to the lagging X chromosome by a force-calibrated microneedle (Li and Nicklas, 1995). These findings thus provided an experimental basis for the tension model.

The role of tension in spindle assembly checkpoint signaling is not easy to distinguish from that of attachment, however, as application of tension on kinetochores can enhance both the stability of individual microtubule attachments and the overall occupancy of kinetochores (by slowing the turnover rate of

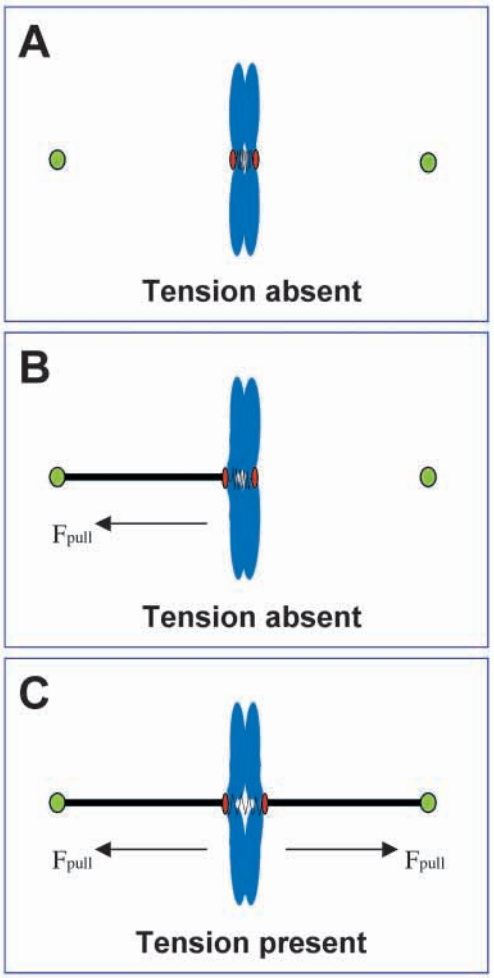

Fig. 3. Tension develops across sister kinetochores (red) upon their bipolar attachment by spindle microtubules (black line). The paired sister kinetochores are not under tension when they are not attached by spindle microtubules (A) and are under little or no tension when one or both of them are attached by microtubules from one spindle pole (green) (B). However, when microtubules from two opposite spindle poles attach to the sister kinetochores, tension develops across the paired kinetochores owing to the mitotic force that tends to pull the sister chromatids toward two opposite poles against the cohesive force that holds sister chromatids together (C). The level of tension is reflected by an increase in the distance between the paired sister kinetochores.

kinetochore microtubules) (Nicklas and Koch, 1969; Nicklas, 1988b; Nicklas and Ward, 1994; Nicklas, 1997; King and Nicklas, 2000; Nicklas et al., 2001) (see below for more discussion). It is possible that, in the experiment performed by Li and Nicklas, the spindle assembly checkpoint was switched off by tension-induced accumulation of microtubules at the kinetochore (Li and Nicklas, 1995) and not by the applied tension itself, as suggested by others (Rieder and Khodjakov, 1997). In fact, Rieder and colleagues found that, during mitosis in the rat kangaroo kidney epithelial cell line PtK1, selectively destroying (by laser-irradiation) the unattached kinetochore on the last, mono-oriented chromosome immediately triggered anaphase onset (Rieder et al., 1995). In this experiment, anaphase onset was not inhibited, despite the lack of tension between sister kinetochores on the last chromosome. Furthermore, using PtK1 cells, Waters et al. demonstrated that loss of Mad2 staining at kinetochores, a sign that the spindle assembly checkpoint has turned off, depends on microtubule attachment not tension (Waters, 1998).

Interestingly, in a study performed in maize, Yu et al. found that during mitosis, loss of Mad2 staining at kinetochores correlates with attachment of kinetochores to spindle microtubules (Yu et al., 1999). However, during meiosis in the same organism, loss of Mad2 staining at kinetochores instead correlates with the tension exerted on kinetochores by bipolar microtubule attachment. It has therefore been proposed that the controversy between the attachment model and the tension model could reflect differences between mitosis and meiosis, attachment being used in mitosis and tension being used in meiosis.

Lessons from budding yeast

Studies on the roles of attachment and tension in spindle assembly checkpoint signaling have mainly focused on multicellular organisms. In these organisms, because each kinetochore can attach to multiple spindle microtubules (e.g. up to 30 in mammals) (Rieder, 1982), it has been difficult to distinguish whether a given kinetochore is fully or partially occupied. However, studying attachment and tension in budding yeast avoids the issue of full or partial kinetochore occupancy, because the budding yeast kinetochore captures only a single microtubule during mitosis (Winey et al., 1995).

Taking advantage of this system, Murray and colleagues recently performed a series of elegant experiments in budding yeast to test the role of tension in spindle assembly checkpoint signaling (Shonn et al., 2000; Stern and Murray, 2001; Biggins and Murray, 2001). They first directly visualized chromosome segregation in budding yeast by targeting homologs of a chromosome with green fluorescence protein (GFP), a method initially developed by (Straight et al., 1996). Mad2-deficient cells had increased frequencies of chromosome missegregation in meiosis I (Shonn et al., 2000). In addition, blocking recombination between homologous chromosomes, which causes a loss of tension between them without affecting microtubule attachment (a probable consequence is attachment of homologs to the same pole), led to a remarkable delay in anaphase entry. Furthermore, forcing bipolar attachment of the unrecombined homologs restored tension between them and allowed the cell to overcome the delay in anaphase entry (Shonn et al., 2000).

In another experiment, Stern and Murray demonstrated that loss of tension at the budding yeast kinetochore, when cells enter mitosis without a prior round of DNA replication, is sufficient to cause the spindle assembly checkpoint to block anaphase entry (Stern and Murray, 2001). Similarly, Biggins and Murray found that, in budding yeast mitosis, the spindle assembly checkpoint was activated when they reduced tension by preventing DNA replication or sister chromatid cohesin (Biggins and Murray, 2001); neither of these two manipulations affects attachment of kinetochores to microtubules. These studies thus indicate that proper tension exerted upon kinetochores, resulting from bipolar microtubule attachment, is crucial for turning off the spindle assembly checkpoint in both meiosis and mitosis in budding yeast. However, since spindle disruption and microtubule detachment induced by nocodazole produces a long-term block in budding yeast, but loss of tension produces only a delay, the role of tension in the checkpoint signaling needs to be investigated further.

Taken together, the above studies in insect, yeast, maize and mammalian cells have significantly extended our knowledge of how the spindle assembly checkpoint is monitored during mitosis and meiosis. The contradictory results among these studies could reflect differences among cell types or organisms; some cell types or organisms might use both attachment and tension, whereas others might use only one of these mechanisms. However, considering the known interdependence of attachment and tension in higher eukaryotes, checkpoint signaling is most probably monitored by both attachment and tension, although the relative contributions of each mechanism may be different, depending on the cell type or organism.

It remains a riddle as to how tension enhances the stability and the number of kinetochore microtubules, which are dynamically attached at both minus ends (to spindle poles) and plus ends (to kinetochores) (Mitchison, 1989; Mitchison and Salmon, 1992; Zhai et al., 1995; Waters et al., 1996a). Nicklas and Ward speculated that tension might promote stability of kinetochore microtubules at the spindle pole, and not at the kinetochores (Nicklas and Ward, 1994). For the effect of tension on microtubule number at kinetochores, since kinetochore microtubules can turn over slowly (Zhai et al., 1995), it is possible that tension affects the kinetic balance between the capture of new microtubules, the release of the existing kinetochore microtubules and their assembly dynamics.

How do attachment and tension monitor the checkpoint?

Studies on Mad2, Cdc20 and the APC have established a general model for how the occupancy of kinetochores by spindle microtubules monitors the spindle assembly checkpoint (Fig. 2), but it remains unclear how Mad2 is recruited to unattached kinetochores for its activation and how Mad2* is released from kinetochores. The tight association of Mad1 and Mad2 and the compromised kinetochore localization of Mad2 in the absence of Mad1 suggest that Mad1 attracts Mad2 to unattached kinetochores (Chen et al., 1998; Chen et al., 1999; Sironi et al., 2001). As to releasing Mad2*, Shah and Cleveland (2000) have proposed that other checkpoint proteins such as Zw10 (Zeste-White 10) and Rod (Rough deal) might

be involved (Shah and Cleveland, 2000) as indicated by two parallel studies in human cells and flies (Chan et al., 2000; Basto et al., 2000).

Compared with what is known about attachment, much less is known about how tension, a mechanical property, monitors the spindle assembly checkpoint. A possible mechanism is tension-sensitive kinetochore protein phosphorylation, which might link kinetochore mechanics to the chemical regulation of the spindle assembly checkpoint, as suggested early on by Gorbsky and Nicklas (Gorbsky, 1995; Nicklas, 1997). In particular, a phosphorylated kinetochore protein recognized by the 3F3/2 antibody seems to participate in this tension-mediated signaling pathway (Gorbsky and Ricketts, 1993; Nicklas et al., 1995; Campbell and Gorbsky, 1995; Li and Nicklas, 1997). Gorbsky and Ricketts first reported that, in mitotic PtK1 cells, this phosphorylated epitope stains brightly with the 3F3/2 antibody at unattached kinetochores but very weakly at attached kinetochores (Gorbsky and Ricketts, 1993). However, during meiosis I in grasshopper and mantid spermatocytes, phosphorylation of this 3F3/2-recognized kinetochore epitope is regulated by tension exerted upon kinetochores instead of just microtubule attachment (Nicklas et al., 1995; Li and Nicklas, 1997). Tension, whether from normal mitotic forces or from a micromanipulation needle, could cause dephosphorylation of the 3F3/2 phosphoepitope at kinetochores (Nicklas et al., 1995; Li and Nicklas, 1997) and could also trigger anaphase onset (Li and Nicklas, 1995). Furthermore, when the 3F3/2 antibody was injected into mitotic cells, the normal dephosphorylation of the 3F3/2 phosphoepitope and onset of anaphase were inhibited (Campbell and Gorbsky, 1995). It is thus very likely that tension-sensitive phosphorylation and dephosphorylation of this kinetochore epitope regulates the spindle assembly checkpoint signaling.

Recently, Biggins and Murray reported that in budding yeast, aurora/Ipl1p, a protein kinase, plays an important role in tension-dependent spindle assembly checkpoint signaling (Biggins and Murray, 2001). In their experiments, aurora/Ipl1p function was required for the spindle assembly checkpoint activity induced by kinetochores not under tension yet attached to microtubules (manipulated by preventing DNA replication or sister chromatid cohesin). However, aurora/Ipl1p was not required for the checkpoint activity induced by microtubule depolymerization. The role of aurora/Ipl1p in tension-dependent checkpoint signaling is further supported by a more recent study by Tanaka et al. in which aurora/Ipl1p was demonstrated to be critical for reorienting monopolar-attached sister chromatids whose sister kinetochores are not under tension so that they attach to microtubules from two opposite spindle poles (Tanaka et al., 2002). It will be of great interest to investigate whether aurora/Ipl1p is the kinase that phosphorylates the 3F3/2-recognized epitope at kinetochores that lack tension (Gorbsky and Ricketts, 1993; Nicklas et al., 1995; Campbell and Gorbsky, 1995; Li and Nicklas, 1997).

Why does the checkpoint need both attachment and tension?

The spindle assembly checkpoint would be most efficient if various defects in chromosome attachment and alignment were sensed by a single mechanism. Then, what is the advantage for having both attachment and tension mechanisms? One probable answer is that only the tension mechanism can distinguish a chromosome that is attached at sister kinetochores by microtubules from two opposite spindle poles (under this circumstance, kinetochores are under tension) from one that is attached at the sister kinetochores by microtubules from the same spindle pole (under this circumstance, kinetochores lack tension). The signal generated by lack of tension might allow the cell to release microtubules from the sister kinetochores and allow the re-attachment of kinetochores by microtubules from opposite poles. From this point of view, the loss of tension might be eventually sensed through the loss of occupancy (attachment) of kinetochores by microtubules.

To have both attachment and tension mechanisms might be an advantage even after bipolar attachment of kinetochores: stabilization of kinetochore attachment by proper tension might be essential for the correct alignment of kinetochores at the metaphase plate, the final event before anaphase entry. This idea is supported by three recent studies in mammalian cells (Hoffman et al., 2001; Skoufias et al., 2001; Zhou et al., 2002). Hoffman et al. reported that, in PtK1 cells, when chromosomes are bipolar-attached and aligned at the metaphase plate, Mad2 was completely gone from the kinetochores whereas BubR1 was still visible (Hoffman et al., 2001). Skoufias et al. found that, in the presence of low-dose vinblastine, which arrests HeLa cells at mitosis with normal chromosome alignment yet without tension, Bub1 and BubR1 are recruited to kinetochores but Mad2 is not (Skoufias et al., 2001). Mad2 is recruited to kinetochores at higher vinblastine doses, which disrupt attachment of kinetochores to microtubules. Zhou et al. studied noscapine-arrested mitotic HeLa cells, which have bipolar spindles but do not complete chromosome alignment; some chromosomes are aligned at the metaphase plate and others remain near spindle poles – both groups of chromosomes lack tension to a similar extent (Zhou et al., 2002). Upon chromosome alignment, Mad2 became undetectable at kinetochores (138-fold reduction); by contrast, Bub1 and BubR1 were only diminished to 3.7- and 3.9-fold, respectively (Zhou et al., 2002).

Collectively, these studies suggest that the checkpoint proteins Mad2 and Bub1/BubR1 primarily sense attachment and tension, respectively. It is worth calling attention to a previous study conducted in PtK1 cells by Waters et al., in which loss of tension was insufficient to recruit Mad2 to kinetochores although some kinetochores did exhibit Mad2 and antibodies to Mad2 disrupted this checkpoint (Waters et al., 1998). The recent finding that BubR1 is a more potent APC inhibitor in vitro (Sudakin et al., 2001; Tang et al., 2001; Fang, 2002) also indicates that Mad2 and Bub1/BubR1 have distinct roles in spindle assembly checkpoint signaling. However, current evidence for this model is not firm. By contrast, in a recent study performed in PtK1 cells by Hoffman et al., the average amount of BubR1 at metaphase kinetochores did not change with the loss of kinetochore tension induced by taxol stabilization of microtubules (Hoffman et al., 2001). In addition, Taylor et al. show that Bub1 and BubR1 respond differently to microtubule inhibitor-induced changes in kinetochore-microtubule attachment and tension (Taylor et al., 2001). Thus, whether Mad2 and Bub1/BubR1 have respective roles in sensing attachment and tension remains a challenge to be solved in the future.

We would like to thank R. Bruce Nicklas, Conly L. Rieder, Andrew W. Murray and Bodo Stern for comments and critical reading of the manuscript. Also, we are very thankful for the helpful suggestions from the executive editor, Richard Sever, and two anonymous reviewers of this manuscript. We sincerely apologize to those colleagues whose primary work could not be cited or is cited only through reviews because of space limitations. Work in the Joshi laboratory is supported by a grant from the National Institutes of Health.

References

Amon, A. (1999). The spindle checkpoint. *Curr. Opin. Cell Biol.* **9**, 69-75.

Basto, R., Gomes, R. and Karess, R. E. (2000). Rough deal and Zw10 are required for the metaphase checkpoint in *Drosophila*. *Nat. Cell Biol.* **2**, 939-943.

Basu, J., Logarinho, E., Herrmann, S., Bousbaa, H., Li, Z., Chan, G. K., Yen, T. J., Sunkel, C. E. and Goldberg, M. L. (1998). Localization of the *Drosophila* checkpoint control protein Bub3 to the kinetochore requires Bub1 but not Zw10 or Rod. *Chromosoma* **107**, 376-385.

Basu, J., Bousbaa, H., Logarinho, E., Li, Z., Williams, B. C., Lopes, C., Sunkel, C. E. and Goldberg, M. L. (1999). Mutations in the essential spindle checkpoint gene bub1 cause chromosome missegregation and fail to block apoptosis in *Drosophila*. *J. Cell Biol.* **146**, 13-28.

Bernard, P., Hardwick, K. and Javerzat, J. P. (1998). Fission yeast bub1 is a mitotic centromere protein essential for the spindle checkpoint and the preservation of correct ploidy through mitosis. *J. Cell Biol.* **143**, 1775-1787.

Biggins, S. and Murray, A. W. (2001). The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. *Genes Dev.* **15**, 3118-3129.

Cahill, D. P., Lengauer, C., Yu, J., Riggins, G. J., Willson, J. K., Markowitz, S. D., Kinzler, K. W. and Vogelstein, B. (1998). Mutations of mitotic checkpoint genes in human cancers. *Nature* **392**, 300-303.

Campbell, M. S. and Gorbsky, G. J. (1995). Microinjection of mitotic cells with the 3F3/2 anti-phosphoepitope antibody delays the onset of anaphase. *J. Cell Biol.* **129**, 1195-1204.

Chan, G. K., Jablonski, S. A., Starr, D. A., Goldberg, M. L. and Yen, T. J. (2000). Human Zw10 and ROD are mitotic checkpoint proteins that bind to kinetochores. *Nat. Cell Biol.* **2**, 944-947.

Chan, G. K., Jablonski, S. A., Sudakin, V., Hittle, J. C. and Yen, T. J. (1999). Human BUBR1 is a mitotic checkpoint kinase that monitors CENP-E functions at kinetochores and binds the cyclosome/APC. *J. Cell Biol.* **146**, 941-954.

Chan, G. K., Schaar, B. T. and Yen, T. J. (1998). Characterization of the kinetochore binding domain of CENP-E reveals interactions with the kinetochore proteins CENP-F and hBUBR1. *J. Cell Biol.* **143**, 49-63.

Chen, R. H., Brady, D. M., Smith, D., Murray, A. W. and Hardwick, K. G. (1999). The spindle checkpoint of budding yeast depends on a tight complex between the Mad1 and Mad2 proteins. *Mol. Biol. Cell* **10**, 2607-2618.

Chen, R. H., Waters, J. C., Salmon, E. D. and Murray, A. W. (1996). Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores. *Science* **274**, 242-246.

Chen, R. H., Shevchenko, A., Mann, M. and Murray, A. W. (1998). Spindle checkpoint protein Xmad1 recruits Xmad2 to unattached kinetochores. *J. Cell Biol.* **143**, 283-295.

Ciosk, R., Zachariae, W., Michaelis, C., Shevchenko, A., Mann, M. and Nasmyth, K. (1998). An ESP1/PDS1 complex regulates loss of sister chromatid cohesion at the metaphase to anaphase transition in yeast. *Cell* **93**, 1067-1076.

Cohen-Fix, O., Peters, J. M., Kirschner, M. W. and Koshland, D. (1996). Anaphase initiation in *Saccharomyces cerevisiae* is controlled by the APC-dependent degradation of the anaphase inhibitor Pds1p. *Genes Dev.* **10**, 3081-3093.

Cooke, C. A., Schaar, B., Yen, T. J. and Earnshaw, W. C. (1997). Localization of CENP-E in the fibrous corona and outer plate of mammalian kinetochores from prometaphase through anaphase. *Chromosoma* **106**, 446-455.

Fang, G. (2002). Checkpoint protein BubR1 acts synergistically with Mad2 to inhibit anaphase-promoting complex. *Mol. Biol. Cell* **13**, 755-766.

Fang, G., Yu, H. and Kirschner, M. W. (1998a). Direct binding of CDC20 protein family members activates the anaphase-promoting complex in mitosis and G1. *Mol. Cell* **2**, 163-171.

Fang, G., Yu, H. and Kirschner, M. W. (1998b). 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.

Fang, G., Yu, H. and Kirschner, M. W. (1999). Control of mitotic transitions by the anaphase-promoting complex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **354**, 1583-1590.

Funabiki, H., Yamano, H., Kumada, K., Nagao, K., Hunt, T. and Yangida, M. (1996). Cut2 proteolysis required for sister-chromatid separation in fission yeast. *Nature* **381**, 438-441.

Gorbsky, G. J. (1995). Kinetochores, microtubules and the metaphase checkpoint. *Trends Cell Biol.* **5**, 143-148.

Gorbsky, G. J. and Ricketts, W. A. (1993). Differential expression of a phosphoepitope at the kinetochores of moving chromosomes. *J. Cell Biol.* **122**, 1311-1321.

Goshima, G. and Yanagida, M. (2000). Establishing biorientation occurs with precocious separation of the sister kinetochores, but not the arms, in the early spindle of budding yeast. *Cell* **100**, 619-633.

Guacci, V., Koshland, D. and Strunnikov, A. (1997). A direct link between sister chromatid cohesion and chromosome condensation revealed through the analysis of MCD1 in *S. cerevisiae*. *Cell* **91**, 47-57.

Hartwell, L. H. and Weinert, T. A. (1989). Checkpoints: controls that ensure the order of cell cycle events. *Science* **246**, 629-634.

Hauf, S., Waizenegger, I. C. and Peters, J. M. (2001). Cohesion cleavage by separase required for anaphase and cytokinesis in human cells. *Science* **293**, 1320-1323.

He, X., Asthana, S. and Sorger, P. K. (2000). Transient sister chromatid separation and elastic deformation of chromosomes during mitosis in budding yeast. *Cell* **101**, 763-775.

He, X., Patterson, T. and Sazer, S. (1997). The *Schizosaccharomyces pombe* spindle checkpoint protein mad2p blocks anaphase and genetically interacts with the anaphase-promoting complex. *Proc. Natl. Acad. Sci. USA* **94**, 7965-7970.

He, X., Jones, M. H., Winey, M. and Sazer, S. (1998). *mph 1*, a member of the Mps1-like family of dual specificity protein kinases, is required for the spindle checkpoint in *S. pombe*. *J. Cell Sci.* **111**, 1635-1647.

Hoffman, D. B., Pearson, C. G., Yen, T. J., Howell, B. J. and Salmon, E. D. (2001). Microtubule-dependent changes in assembly of microtubule motor proteins and mitotic spindle checkpoint proteins at PtK1 kinetochores. *Mol. Biol. Cell* **12**, 1995-2009.

Hoyt, M. A., Totis, L. and Roberts, B. T. (1991). *S. cerevisiae* genes required for cell cycle arrest in response to loss of microtubule function. *Cell* **66**, 507-517.

Howell, B. J., Hoffman, D. B., Fang, G., Murray, A. W. and Salmon, E. D. (2000). Visualization of Mad2 dynamics at kinetochores, along spindle fibers, and at spindle poles in living cells. *J. Cell Biol.* **150**, 1233-1250.

Howell, B. J., McEwen, B. F., Canman, J. C., Hoffman, D. B., Farrar, E. M., Rieder, C. L. and Salmon, E. D. (2001). Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. *J. Cell Biol.* **155**, 1159-1172.

Hwang, L. H., Lau, L. F., Smith, D. L., Mistrot, C. A., Hardwick, K. G., Hwang, E. S., Amon, A. and Murray, A. W. (1998). Budding yeast Cdc20: a target of the spindle checkpoint. *Science* **279**, 1041-1044.

Jin, D. Y., Spencer, F. and Jeang, K. T. (1998). Human T cell leukemia virus type 1 oncoprotein Tax targets the human mitotic checkpoint protein MAD1. *Cell* **93**, 81-91.

Kim, S. H., Lin, D. P., Matsumoto, S., Kitazono, A. and Matsumoto, T. (1998). Fission yeast Slp1: an effector of the Mad2-dependent spindle checkpoint. *Science* **279**, 1045-1047.

King, J. M. and Nicklas, R. B. (2000). Tension on chromosomes increases the number of kinetochore microtubules but only within limits. *J. Cell Sci.* **113**, 3815-3823.

King, R. W., Peters, J. M., Tugendreich, S., Rolfe, M., Hieter, P. and Kirschner, M. W. (1995). A 20S complex containing CDC27 and CDC16 catalyzes the mitosis-specific conjugation of ubiquitin to cyclin B. *Cell* **81**, 279-288.

Kitagawa, R. and Rose, A. M. (1999). Components of the spindle-assembly checkpoint are essential in *Caenorhabditis elegans*. *Nat. Cell Biol.* **1**, 514-521.

Lee, H., Trainer, A. H., Friedman, L. S., Thistlethwaite, F. C., Evans, M. J., Ponder, B. A. J. and Venkitaraman, A. R. (1999). Mitotic checkpoint inactivation fosters transformation in cells lacking the breast cancer susceptibility gene, Brca2. *Mol. Cell* **4**, 1-10.

Li, R. and Murray, A. W. (1991). Feedback control of mitosis in budding yeast. *Cell* **66**, 519-531.
Li, X. and Nicklas, R. B. (1995). Mitotic forces control a cell-cycle checkpoint. *Nature* **373**, 630-632.
Li, X. and Nicklas, R. B. (1997). Tension-sensitive kinetochore phosphorylation and the chromosome distribution checkpoint in praying mantid spermatocytes. *J. Cell Sci.* **110**, 537-545.
Li, Y. and Benezra, R. (1996). Identification of a human mitotic checkpoint gene:hsMAD2. *Science* **274**, 246-248.
Li, Y., Gorbea, C., Mahaffey, D., Rechsteiner, M. and Benezra, R. (1997). MAD2 associates with the cyclosome/anaphase-promoting complex and inhibits its activity. *Proc. Natl. Acad. Sci. USA* **94**, 12431-12436.
Lombillo, V. A., Nislow, C., Yen, T. J., Gelfand, V. I. and McIntosh, J. R. (1995). Antibodies to the kinesin motor domain and CENP-E inhibit microtubule depolymerization-dependent motion of chromosomes in vitro. *J. Cell Biol.* **128**, 107-115.
Manchester, K. L. (1995). Boveri, Theodor and the origin of malignant-tumors. *Trends Cell Biol.* **5**, 384-387.
Martinez-Exposito, M. J., Kaplan, K. B., Copeland, J. and Sorger, P. K. (1999). Retention of the BUB3 checkpoint protein on lagging chromosomes. *Proc. Natl. Acad. Sci. USA* **96**, 8493-8498.
Mazia, D. (1961). Mitosis. In *The Cell* Vol. 3 (ed. J. Brachet and A. E. Mirsky), pp. 77-142. New York: Academic Press.
McEwen, B. F., Chan, G. K. T., Zubrowski, B., Savoian, M. S., Sauer, M. T. and Yen, T. J. (2001). CENP-E is essential for reliable bioriented spindle attachment, but chromosome alignment can be achieved via redundant mechanisms in mammalian cells. *Mol. Biol. Cell* **12**, 2776-2789.
McIntosh, J. R. (1984). Mechanisms of mitosis. *Trends Biochem. Sci.* **9**, 195-198.
McIntosh, J. R. (1991). Structural and mechanical control of mitotic progression. *Cold Spring Harbor Symp. Quant. Biol.* **56**, 613-619.
Michaelis, C., Ciosk, R. and Nasmyth, K. (1997). Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell* **91**, 35-45.
Michel, L. S., Liberal, V., Chatterjee, A., Kirchwegger, R., Pasche, B., Gerald, W., Dobles, M., Sorger, P. K., Murty, V. V. and Benezra, R. (2001). MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. *Nature* **409**, 355-359.
Millband, D. N. and Hardwick, K. G. (2002). Fission yeast mad3p is required for mad2p to inhibit the anaphase-promoting complex and localizes to kinetochores in a bub1p-, bub3p-, and mph1p-dependent manner. *Mol. Cell. Biol.* **22**, 2728-2742.
Mitchison, T. J. (1989). Polewards microtubule flux in the mitotic spindle: evidence from photoactivation of fluorescence. *J. Cell Biol.* **109**, 637-652.
Mitchison, T. J. and Salmon, E. D. (1992). Poleward kinetochore fiber movement occurs during both metaphase and anaphase-A in newt lung cell mitosis. *J. Cell Biol.* **119**, 569-582.
Nicklas, R. B. (1988a). The forces that move chromosomes in mitosis. *Annu. Rev. Biophys. Biophys. Chem.* **17**, 431-449.
Nicklas, R. B. (1988b). Chance encounters and precision in mitosis. *J. Cell Sci.* **89**, 283-285.
Nicklas, R. B. (1997). How cells get the right chromosomes. *Science* **275**, 632-637.
Nicklas, R. B. and Koch, C. A. (1969). Chromosome manipulation III. Induced reorientation and the experimental control of segregation in meiosis. *J. Cell Biol.* **43**, 40-50.
Nicklas, R. B. and Ward, S. C. (1994). Elements of error correction in mitosis: microtubule capture, release, and tension. *J. Cell Biol.* **126**, 1241-1253.
Nicklas, R. B., Ward, S. C. and Gorbsky, G. J. (1995). Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J. Cell Biol.* **130**, 929-939.
Nicklas, R. B., Waters, J. C., Salmon, E. D. and Ward, S. C. (2001). Checkpoint signals in grasshopper meiosis are sensitive to microtubule attachment, but tension is still essential. *J. Cell Sci.* **114**, 4173-4183.
Pfarr, C. M., Coue, M., Grissom, P. M., Hays, T. S., Porter, M. E. and McIntosh, J. R. (1990). Cytoplasmic dynein is localized to kinetochores during mitosis. *Nature* **345**, 263-265.
Rieder, C. L. (1982). The formation, structure, and composition of the mammalian kinetochore and kinetochore fiber. *Int. Rev. Cytol.* **79**, 1-58.
Rieder, C. L. and Khodjakov, A. (1997). Mitosis and checkpoints that control progression through mitosis in vertebrate somatic cells. *Prog. Cell Cycle Res.* **3**, 301-312.
Rieder, C. L. and Salmon, E. D. (1994). Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. *J. Cell Biol.* **124**, 223-233.
Rieder, C. L. and Salmon, E. D. (1998). The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* **8**, 310-318.
Rieder, C. L., Schultz, A., Cole, R. and Sluder, G. (1994). Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J. Cell Biol.* **127**, 1301-1310.
Rieder, C. L., Cole, R. W., Khodjakov, A. and Sluder, G. (1995). The checkpoint delaying anaphase in response to chromosome monoorientation is mediated by an inhibitory signal produced by unattached kinetochores. *J. Cell Biol.* **130**, 941-948.
Rudner, A. D. and Murray, A. W. (1996). The spindle assembly checkpoint. *Curr. Opin. Cell Biol.* **8**, 773-780.
Shan, J. V. and Cleveland, D. W. (2000). Waiting for anaphase: Mad2 and the spindle assembly checkpoint. *Cell* **103**, 997-1000.
Shelby, R. D., Hahn, K. M. and Sullivan, K. F. (1996). Dynamic elastic behavior of alpha-satellite DNA domains visualized in situ in living human cells. *J. Cell Biol.* **135**, 545-557.
Shirayama, M., Zachariae, W., Ciosk, R. and Nasmyth, K. (1998). The Polo-like kinase Cdc5p and the WD-repeat protein Cdc20/fizzy are regulators and substrates of the anaphase promoting complex in *Saccharomyces cerevisiae*. *EMBO J.* **17**, 1336-1349.
Shonn, M. A., McCarroll, R. and Murray, A. W. (2000). Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. *Science* **289**, 300-303.
Sironi, L., Melixetian, M., Faretta, M., Prosperini, E., Helin, K. and Musacchio, A. (2001). Mad2 binding to Mad1 and Cdc20, rather than oligomerization, is required for the spindle checkpoint. *EMBO J.* **20**, 6371-6382.
Skibbens, R. V., Skeen, V. P. and Salmon, E. D. (1993). Directional instability of kinetochore motility during chromosome congression and segregation in mitotic newt lung cells: a push-pull mechanism. *J. Cell Biol.* **122**, 859-875.
Skoufias, D. A., Andreassen, P. R., Lacroix, F. B., Wilson, L. and Margolis, R. L. (2001). Mammalian mad2 and bub1/bubR1 recognize distinct spindle-attachment and kinetochore-tension checkpoints. *Proc. Natl. Acad. Sci. USA* **98**, 4492-4497.
Sluder, G. and McCollum, D. (2000). The mad ways of meiosis. *Science* **289**, 254-255.
Stern, B. M. and Murray, A. W. (2001). Lack of tension at kinetochores activates the spindle checkpoint in budding yeast. *Curr. Biol.* **11**, 1462-1467.
Steuer, E. R., Wordeman, L., Schroer, T. A. and Sheetz, M. P. (1990). Localization of cytoplasmic dynein to mitotic spindles and kinetochores. *Nature* **345**, 266-268.
Straight, A. F., Belmont, A. S., Robinett, C. C. and Murray, A. W. (1996). GFP tagging of budding yeast chromosomes reveals that protein-protein interactions can mediate sister chromatid cohesion. *Curr. Biol.* **6**, 1599-1608.
Sudakin, V., Chan, G. K. T. and Yen, T. J. (2001). Checkpoint inhibition of the APC/C in HeLa cells is mediated by a complex of BUBR1, BUB3, CDC20, MAD2. *J. Cell Biol.* **154**, 925-936.
Takahashi, T., Haruki, N., Nomoto, S., Masuda, A., Saji, S. and Osada, H. (1999). Identification of frequent impairment of the mitotic checkpoint and molecular analysis of the mitotic checkpoint genes, *hsMAD2* and *p55CDC*, in human lung cancers. *Oncogene* **18**, 4295-4300.
Tanaka, T., Fuchs, J., Loidl, J. and Nasmyth, K. (2000). Cohesin ensures bipolar attachment of microtubules to sister centromeres and resists their precocious separation. *Nat. Cell Biol.* **2**, 492-499.
Tanaka, T. U., Rachidi, N., Janke, C., Pereira, G., Galova, M., Schiebel, E., Stark, M. J. and Nasmyth, K. (2002). Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell* **108**, 317-329.
Tang, Z., Bharadwaj, R., Li, B. and Yu, H. (2001). Mad2-independent inhibition of APCCdc20 by the mitotic checkpoint protein BubR1. *Dev. Cell* **1**, 227-237.
Taylor, S. S. and McKeon, F. (1997). Kinetochore localization of murine Bub1 is required for normal mitotic timing and checkpoint response to spindle damage. *Cell* **89**, 727-735.
Taylor, S. S., Ha, E. and McKeon, F. (1998). The human homologue of Bub3 is required for kinetochore localization and a Mad3/Bub1-related protein kinase. *J. Cell Biol.* **142**, 1-11.
Taylor, S. S., Hussein, D., Wang, Y., Elderkin, S. and Morrow, C. J. (2001). Kinetochore localisation and phosphorylation of the mitotic checkpoint components Bub1 and BubR1 are differentially regulated by spindle events in human cells. *J. Cell Sci.* **114**, 4385-4395.

Uhlmann, F., Lottspeich, F. and Nasmyth, K. (1999). Sister-chromatid separation at anaphase onset is promoted by cleavage of the cohesin subunit Scc1. *Nature* **400**, 37-42.

Uhlmann, F., Wernek, W., Poupart, M. A., Koonin, E. and Nasmyth, K. (2000). Cleavage of cohesin by the CD clan protease separin triggers anaphase in yeast. *Cell* **103**, 375-386.

Visintin, R., Prinz, S. and Amon, A. (1997). CDC20 and CDH1: A family of substrate-specific activators of APC-dependent proteolysis. *Science* **278**, 460-463.

Wang, X., Jin, D. Y., Ng, R. W., Feng, H., Wong, Y. C., Cheung, A. L. and Tsao, S. W. (2002). Significance of MAD2 expression to mitotic checkpoint control in ovarian cancer cells. *Cancer Res.* **62**, 1662-1668.

Waters, J. C., Chen, R. H., Murray, A. W. and Salmon, E. D. (1998). Localization of Mad2 to kinetochores depends on microtubule attachment, not tension. *J. Cell Biol.* **141**, 1181-1191.

Waters, J. C., Mitchison, T. J., Rieder, C. L. and Salmon, E. D. (1996a). The kinetochore microtubule minus-end disassembly associated with poleward flux produces a force that can do work. *Mol. Biol. Cell* **7**, 1547-1558.

Waters, J. C., Skibbens, R. V. and Salmon, E. D. (1996b). Oscillating mitotic newt lung cell kinetochores are, on average, under tension and rarely push. *J. Cell Sci.* **109**, 2823-2831.

Weiss, E. and Winey, M. (1996). The *S. cerevisiae* SPB duplication gene *MPS1* is part of a mitotic checkpoint. *J. Cell Biol.* **132**, 111-123.

Wells, W. A. E. (1996). The spindle-assembly checkpoint: Aiming for a perfect mitosis, every time. *Trends Cell Biol.* **6**, 228-234.

Winey, M., Mamay, C. L., O'Toole, E. T., Mastronarde, D. N., Giddings, T. H., Jr, McDonald, K. L. and McIntosh, J. R. (1995). Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle. *J. Cell Biol.* **129**, 1601-1615.

Wu, H., Lan, Z., Li, W., Wu, S., Weinstein, J., Sakamoto, K. M. and Dai, W. (2000). p55CDC/hCDC20 is associated with BUBR1 and may be a downstream target of the spindle checkpoint kinase. *Oncogene* **19**, 4557-4562.

Yamamoto, A., Guacci, V. and Koshland, D. (1996). 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.

Yao, X., Anderson, K. L. and Cleveland, D. W. (1997). The microtubule-dependent motor centromere-associated protein E (CENP-E) is an integral component of kinetochore corona fibers that link centromeres to spindle microtubules. *J. Cell Biol.* **139**, 435-447.

Yen, T. J., Li, G., Schaar, B. T., Szilak, I. and Cleveland, D. W. (1992). CENP-E is a putative kinetochore motor that accumulates just before mitosis. *Nature* **359**, 536-539.

Yu, H. G., Muszynski, M. G. and Kelly Dawe, R. (1999). The maize homologue of the cell cycle checkpoint protein MAD2 reveals kinetochore substructure and contrasting mitotic and meiotic localization patterns. *J. Cell Biol.* **145**, 425-435.

Zachariae, W. and Nasmyth, K. (1999). Whose end is destruction: cell division and the anaphase-promoting complex. *Genes Dev.* **13**, 2039-2058.

Zhai, Y., Kronebusch, P. J. and Borisy, G. G. (1995). Kinetochore microtubule dynamics and the metaphase-anaphase transition. *J. Cell Biol.* **131**, 721-734.

Zhou, J., Panda, D., Landen, J. W., Wilson, L. and Joshi, H. C. (2002). Minor alteration of microtubule dynamics causes loss of tension across kinetochore pairs and activates the spindle checkpoint. *J. Biol. Chem.* **277**, 17200-17208.

Zou, H., McGarry, T. J., Bernal, T. and Kirschner, M. W. (1999). Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis. *Science* **285**, 418-422.