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Chromosome Segregation and Aneuploidy series

The spindle checkpoint: tension versus attachment

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The spindle checkpoint ensures the fidelity of chromosome segregation by preventing cell-cycle progression until all the chromosomes make proper bipolar attachments to the mitotic spindle and come under tension. Despite significant advances in our understanding of spindle checkpoint function, the primary signal that activates the spindle checkpoint remains unclear. Whereas some experiments indicate that the checkpoint recognizes the lack of microtubule attachment to the kinetochore, others indicate that the checkpoint senses the absence of tension generated on the kinetochore by microtubules. The interdependence between tension and microtubule attachment make it difficult to determine whether these signals are separable. In this article (which is part of the *Chromosome Segregation and Aneuploidy* series), we consider recent evidence that supports and opposes the hypothesis that defects in tension act as the primary checkpoint signal.

Introduction

The flawless execution of cell division is fundamental to the formation and survival of living organisms. It requires the accurate, orderly partitioning of chromosomes, and results in two daughter cells with the correct complement of genetic material. Following genome replication, the replicated chromosomes (sister chromatids) are linked physically by cohesion, which facilitates their segregation towards opposite sides of the cell. To pull the sister chromatids away from one another, the cell relies on the forces generated by the mitotic spindle. This bipolar array of microtubules is composed of dynamic tubulin polymers organized by a pair of organelles called spindle poles. The forces of the spindle are translated into chromosome movements primarily through the interaction of spindle microtubules with kinetochores, which are specialized protein complexes that assemble on centromeric DNA. In most organisms, a single kinetochore contains multiple microtubule-binding sites that interact with microtubules arising from the same spindle pole whereas its sister kinetochore binds microtubules that originate from the opposite pole. At this point, a bipolar attachment is attained and the sister chromatids are poised for proper segregation.

Although bipolar kinetochore–microtubule interactions are essential for the high fidelity of chromosome segregation, achieving the correct attachment is complicated and somewhat random (Box 1; reviewed in [1,2]). This imperfect process can result in several kinetochore–microtubule arrangements that lead to chromosome missegregation. The terminology that describes these attachments accounts for both kinetochore orientation and the pole of origin of the interacting microtubules. Bipolar, or bioriented, attachment where sister kinetochores face opposite poles and each kinetochore binds only spindle microtubules that emanate from the pole it is facing is called an amphitelic attachment (Figure 1a). Syntelic attachment indicates that both sister kinetochores face the same pole and attach to microtubules emanating from that pole (Figure 1b). By contrast, monotelic attachment describes kinetochores that face opposite poles but only one kinetochore in the pair binds to microtubules (Figure 1c). Although different, both syntelic and monotelic attachments are referred to in the literature as mono-oriented because they are bound to a single spindle pole. Finally, merotelic attachments occur when either one or both sister kinetochores bind microtubules that arise from both poles even though they orient toward opposite poles (Figure 1d). Although merotelic attachments rarely cause chromosome missegregation because the kinetochores tend to make enough bipolar attachments to pull the sister chromatids to opposite poles [3], chromosomes that make either syntelic or monotelic attachments will be segregated improperly if uncorrected. The resulting cells become aneuploid, which predisposes multicellular organisms to develop several cancers and birth defects (reviewed in [4,5]).

To ensure that segregation does not occur before all chromosomes make proper attachments, the spindle checkpoint delays the metaphase to anaphase transition to allow the cell time to correct any defects (reviewed in [6]). This conserved signal-transduction network, which consists of Mad, Bub and Mps1 proteins, prevents the premature segregation of improperly attached chromosomes by inhibiting the activity of the anaphase promoting complex (APC), a ubiquitin ligase that targets the anaphase inhibitor securin for destruction. Although it is clear that the spindle checkpoint causes metaphase arrest by inhibiting APC, the primary defect that activates the spindle checkpoint remains controversial. The simplest hypothesis is that the checkpoint monitors some aspect of kinetochore–spindle interactions. Experiments in several organisms indicate that the spindle checkpoint

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Box 1. The process of achieving bipolar attachment

Microtubules exhibit dynamic instability, a property whereby polymerizing and depolymerizing microtubules interconvert and co-exist in the same population [69]. Dynamic instability allows microtubules to probe for kinetochore attachments, stochastic events termed 'search and capture' that occur during prometaphase. The process begins with unattached sister kinetochores (Figure Ia). A spindle microtubule binds to one sister kinetochore via a side-on attachment that allows the rapid transport of the pair of sister chromatids to the pole (Figure Ib). At the spindle pole, additional microtubules bind the captured kinetochore in an end-on fashion to create a microtubule fiber (Figure Ic). A probing microtubule from the opposite pole then interacts with the remaining unattached kinetochore (Figure Id). Finally, the sister chromatids congress to the center of the spindle where the sister kinetochores achieve full microtubule occupancy. When proper bipolar attachments are formed, the pole-ward forces of the kinetochore microtubules are opposed by the cohesion between the sister chromatids, causing the sister kinetochores to come under tension (Figure Ie). When all the chromosomes are aligned properly, the cell is in metaphase. Note that there is only a single microtubule-binding site on each kinetochore in budding yeast, so the process is simplified.

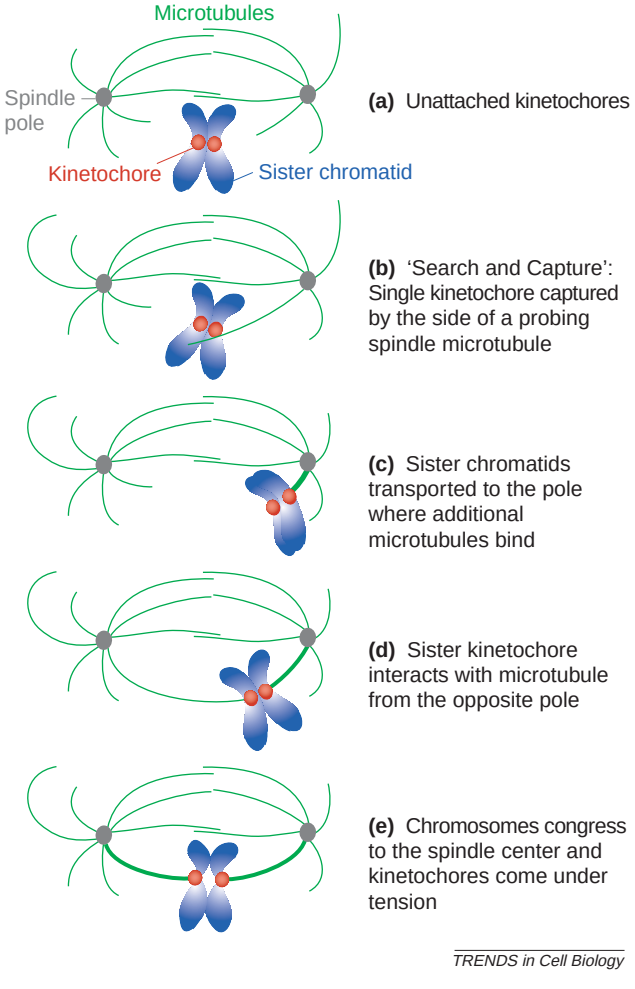

Figure I.

is activated by either lack of kinetochore–microtubule attachments or defects in the tension exerted by microtubule-generated forces on kinetochores (reviewed in [7]). However, it is unclear whether these signals are separate or interdependent. Because defects in the spindle

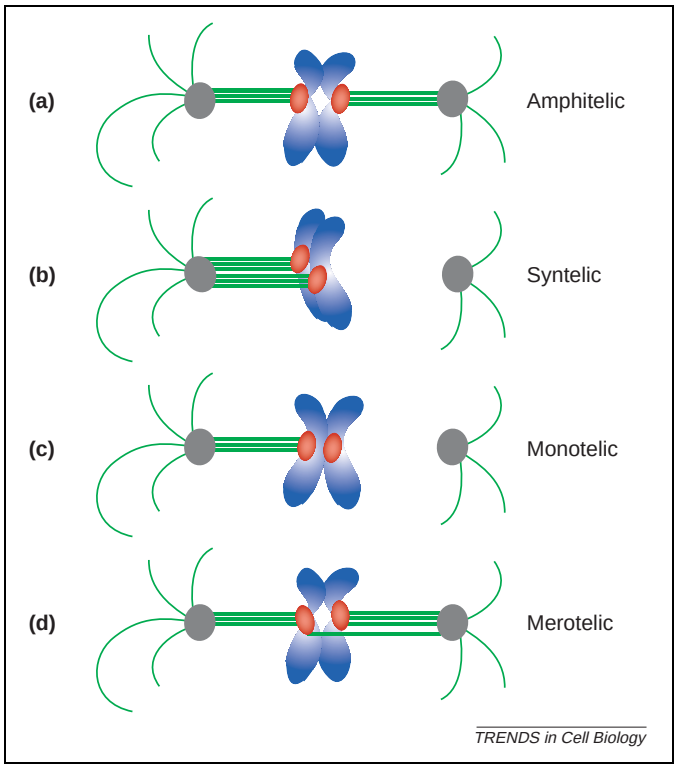

Figure 1. Types of kinetochore–microtubule attachments. **(a)** Amphitelic: either bipolar or bioriented attachment. Sister kinetochores face opposite poles and bind only microtubules arising from the adjacent pole. **(b)** Syntelic: sister kinetochores face the same pole and attach to microtubules emanating from that pole. **(c)** Monotelic: sister kinetochores face opposite poles but only one kinetochore binds microtubules, leaving an unattached kinetochore. **(d)** Merotelic: sister kinetochores face opposite poles but one (or both) kinetochore(s) interact with microtubules from both poles.

checkpoint are associated with many cancers (reviewed in [8,9]), distinguishing between these potential checkpoint activators might have implications for our understanding and treatment of human disease. Here, we examine recent developments in identifying the primary defect that is sensed by the spindle checkpoint.

The relationship between tension and attachment

Pioneering experiments in mitotic rat PtK cells and meiotic mantid spermatocytes have laid the foundation for the attachment versus tension question by providing strong evidence that the spindle checkpoint responds to both the lack of attachment and the absence of tension (Box 2). However, subsequent attempts to tease apart these signals and identify the primary defect sensed by the spindle checkpoint are complicated by the intimate relationship between attachment and tension. Because unattached kinetochores are not under tension, distinguishing between potential activators requires analysis of attached, tension-defective kinetochores. However, microtubule attachments are also affected by tension. The application of tension both stabilizes and increases the number of kinetochore–microtubule attachments [10,11]. This has been shown in an elegant set of micromanipulation experiments in which the number of microtubule attachments on an attached kinetochore was halved when tension was relieved by detaching its sister kinetochore. The subsequent reapplication of

Box 2. Origins of the tension versus attachment debate

The tension hypothesis states that the absence of mechanical tension on chromosomes activates the spindle checkpoint. If the cell was to respond only to an absolute lack of microtubule attachment, syntelic attachments (Figure 1) would fail to engage the spindle checkpoint and lead to aneuploidy. Experimental evidence for the tension hypothesis is provided by micromanipulation of chromosomes in praying mantid spermatocytes that are undergoing meiosis I [70]. In these cells, the formation of an attached, tension-defective, syntelic chromosome pair results in spindle checkpoint activation. However, when tension is applied across these kinetochores using a force-calibrated microneedle, the spindle checkpoint is satisfied and the cells enter anaphase. Although the absence of tension appears to act as a checkpoint signal in most organisms in both mitosis and meiosis, tension-micromanipulation experiments have only been performed on meiotic insect cells. Thus, there is the possibility that these results are system specific. Consistent with this, a tension signal is required for *Drosophila* oocytes to induce a metaphase arrest, but it is not known if the arrest is dependent on the spindle checkpoint [71].

Evidence indicating that lack of kinetochore attachment rather than absence of tension activates the spindle checkpoint is based on the analysis of chromosome behavior in mitotic rat PtK cells [72]. Live-cell imaging coupled with electron microscopy reveals that a single, unattached kinetochore inhibits anaphase onset. Ablation of the unattached kinetochore by laser microsurgery relieves the arrest, which indicates that the unattached kinetochore is the source of the checkpoint signal. However, the unattached kinetochore is neither attached nor under tension so it does not distinguish between the two possible checkpoint activators. Rather, the conclusion that attachment is the checkpoint signal is based on the behavior of the intact, attached kinetochore in this monotelic chromosome pair. This mono-oriented kinetochore is not under tension, but the checkpoint is inactive. Although it is possible that the attached, sister kinetochore is under some tension because of antipole-ward forces on the chromosome arms, it is unlikely that this tension approaches that which occurs on bioriented sister kinetochores. Therefore, the simplest explanation for the lack of spindle checkpoint activation in this experiment is that the absence of tension on the remaining attached kinetochore is not a sufficient checkpoint signal.

Although one interpretation of these results is that defects in either tension or attachment activate the spindle checkpoint specifically, they are complicated by the observations that tension defects can affect microtubule occupancy. This problem is discussed in the section 'The relationship between tension and attachment'.

tension to the relaxed kinetochore restored attachment to its original level. Therefore, microtubule attachment and tension are coupled by an unknown mechanism. This leads to the question of whether the absence of tension activates the spindle checkpoint directly, by regulating a tension-sensitive component of the checkpoint, or indirectly, by altering kinetochore-microtubule occupancy (Figure 2).

The best evidence for the interdependence of tension and attachment signals comes from the grasshopper spermatocyte system in which kinetochores with 'weak' attachments have been created using micromanipulation [12]. These kinetochores completely lack tension and have only a few attached microtubules. In this situation, molecular markers for checkpoint activation decrease despite the lack of tension, which indicates that the weak attachments are sufficient to regulate the checkpoint. However, the checkpoint is not silenced completely until the weakly attached kinetochores obtain full occupancy and come under tension. Therefore, in this system, the checkpoint appears to monitor attachment, and the role of tension is to promote the stabilization of these microtubule attachments.

In budding yeast, the relationship between tension and attachment is simplified. Unlike metaphase kinetochores from PtK cells and grasshopper spermatocytes, which bind an average of 24 and 32 microtubules, respectively, budding yeast kinetochores attach to a single microtubule [11,13,14]. The budding yeast kinetochore is either attached or unattached and cannot generate a 'weak' attachment because of partial microtubule occupancy. Based on this, experiments performed in budding yeast provide the most compelling evidence that the spindle checkpoint recognizes the absence of tension. To test the role of tension in both mitotic and meiotic progression, tension defects have been manufactured by preventing the chromosome pairing that is necessary to generate bipolar force [15,16]. This has been achieved in mitosis by conditionally inhibiting either replication or sister chromatid cohesion, and in meiosis by preventing the recombination that holds homologous chromosomes together during meiosis I. The absence of tension in these situations causes a delay in cell-cycle progression that is dependent

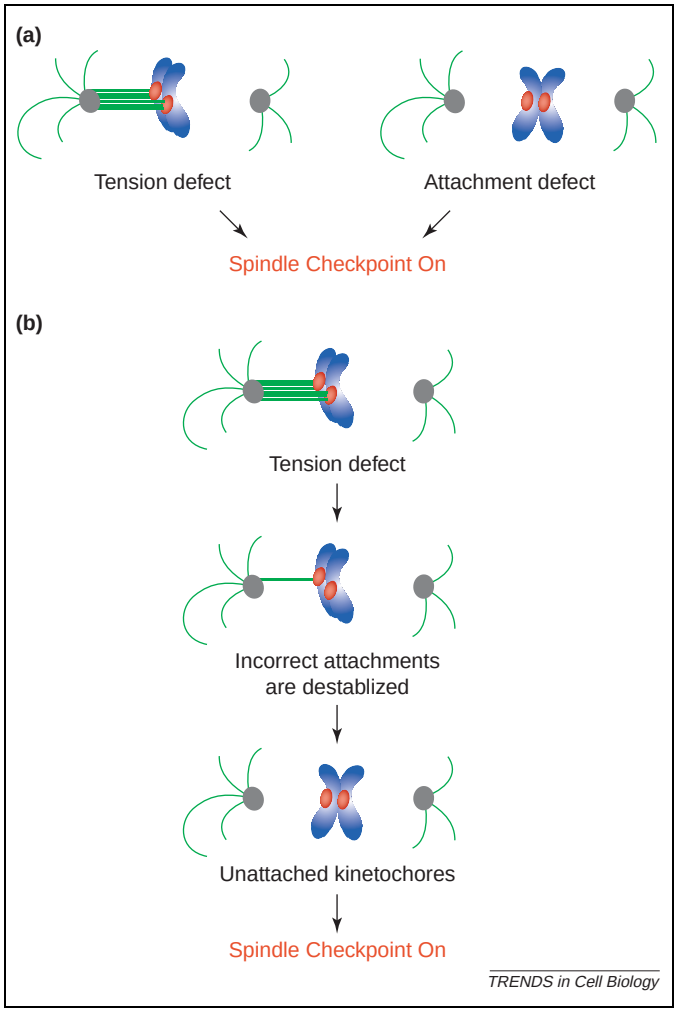

Figure 2. Two models of spindle checkpoint activation. **(a)** Defects in tension and attachment are separate signals that are sensed by the cell and lead to activation of the spindle checkpoint. **(b)** Defects in tension are sensed by the cell, which results in the destabilization of inappropriate microtubule attachments. The destabilization of these attachments creates unattached kinetochores that are monitored by the spindle checkpoint and lead to its activation.

on the spindle checkpoint. In these experiments, the chromosomes were pulled to the poles, indicating that the tensionless kinetochores make microtubule attachments, although it is possible there is an undetected delay in attachment. Because kinetochores in budding yeast cannot be visualized by electron microscopy, it is difficult to determine the precise status of attachments in this organism. Although these experiments are not complicated by questions of partial microtubule occupancy, it is not clear if these kinetochore–microtubule interactions differ from the amphitelic state. For example, the tensionless kinetochore might bind to the side of the microtubule instead of interacting properly with the microtubule end. Therefore, it is possible that the absence of tension on budding yeast kinetochores also affects microtubule attachment. It is important to determine the type of yeast attachments that occur in the absence of tension and how these budding yeast experiments translate to more complex kinetochores with multiple microtubule-binding sites.

Molecular markers for the lack of attachment and the absence of tension

The continuing confusion over the primary defect that activates the spindle checkpoint is fueled, in part, by the development of molecular markers that are thought to recognize either unattached or tensionless kinetochores. However, rather than clarifying the question, recent analysis of these markers highlights how difficult it is to distinguish between attachment and tension defects. When evaluating these markers, it is important to keep in mind the techniques used to create defects in tension and attachment (Box 3).

The Mad2 checkpoint protein

The conserved checkpoint protein Mad2 has been proposed to be a marker for unattached kinetochores. Localization studies show that Mad2 binds to unattached kinetochores in prometaphase of the unperturbed cell cycle and is lost from kinetochores in metaphase when amphitelic attachments are made [17,18]. In addition, Mad2 is recruited to kinetochores with reduced microtubule occupancy. Because Mad2 does not accumulate on tension-defective kinetochores that are generated by either treatment with taxol or micromanipulation (Box 3), it has been proposed that Mad2 specifically marks unattached kinetochores [19]. However, cells treated with taxol always contain at least one kinetochore that stains with Mad2, which is consistent with the possibility that taxol alters microtubule occupancy. In addition, cells treated with the kinesin inhibitor monastrol, which blocks spindle-pole separation and, therefore, results in the formation of syntelic attachments, accumulate Mad2 on the majority of attached but tension-defective kinetochores [20]. This indicates that either syntelic attachments generated by monastrol are not at their full microtubule occupancy or that Mad2 localization to kinetochores is not specific to the lack of attachment.

Another observation that indicates that the role of Mad2 in the checkpoint might not be specific to unattached kinetochores comes from the finding that Mad2 function is required for all known attachment and tension defects to activate the spindle checkpoint. In addition, it is important to realize that, although the localization of Mad2 to the kinetochore correlates with activity of the spindle checkpoint, it has not been demonstrated to be required for the checkpoint. Recent cell-culture work indicates that unattached kinetochores with either low or undetectable levels of Mad2 are capable of spindle checkpoint arrest [21,22]. In addition, in budding yeast, Mad2 is not localized to the kinetochore during the checkpoint delay induced by mutations that create tension defects [23]. Therefore, either localization of Mad2 to the kinetochore might not represent its role in the checkpoint or we might have reached the experimental limits of Mad2 detection.

Box 3. Experimental approaches to creating defects in tension and attachment

Several experimental approaches are used to activate the spindle checkpoint by creating either unattached or tension-defective kinetochores. Although the use of chromosome micromanipulation to control the status of kinetochore attachment and tension is powerful, it is challenging technically and is not amenable to most cell types. More commonly, attachment and tension are manipulated in cells in culture by chemical inhibition of spindle function. To generate unattached kinetochores, cells are exposed to either nocodazole or benomyl, which cause microtubule depolymerization and, therefore, deprive kinetochores of their attachment partners. By contrast, tension defects are produced typically by treatment with the microtubule-stabilizing drug taxol. The sister kinetochores in taxol-treated cells are closer together, indicating loss of kinetochore tension, and electron microscopy confirms that these tensionless kinetochores remain bound to microtubules [13]. Although the average number of kinetochore-bound microtubules is no different from control cells, the variation in the number of microtubules bound to each kinetochore is significantly greater, which indicates that taxol treatment alters microtubule occupancy. This might be caused by the instability of microtubule attachments that are not under proper tension [10]. Because a single unattached kinetochore is sufficient to engage the checkpoint (Box 2), one interpretation of these experiments is that taxol activates the checkpoint because it creates unoccupied microtubule-binding sites, not because the checkpoint responds to the absence of tension. It is important to keep this caveat in mind when considering the specificity of molecular markers to either unattached or tension-defective kinetochores given the standard use of taxol to create tension defects.

The 3F3/2 phosphoepitope

The 3F3/2 antibody was developed originally against thiophosphorylated substrates in *Xenopus* egg extracts [24] and shown subsequently to recognize phosphoepitopes on kinetochores and spindle poles in many cell types [25]. Micromanipulation experiments, such as those described above, have demonstrated that the intensity of the 3F3/2 kinetochore signal is sensitive to the application of tension. When one kinetochore in a syntelic attachment is put under tension, its 3F3/2 signal is reduced significantly compared with the unmanipulated control [26]. Conversely, the tension defects induced by taxol treatment result in strong 3F3/2 kinetochore staining that appear to be proportionally more intense than Mad2 staining [19]. Of course, these experiments are subject to the important caveat that tension affects the stability and number of microtubule attachments. Nevertheless, the

3F3/2 labeling of kinetochores in the absence of tension has led to speculation that the phosphorylation recognized by 3F3/2 is important for cells to signal defects in tension to the spindle checkpoint. In support of this idea, microinjection of 3F3/2 antibody into mitotic cells protects the phosphoepitope and results in a metaphase delay [27]. However, it is possible that this phenotype is caused by indirect effects of the injected antibody on kinetochore function.

The potential role of 3F3/2 phosphorylation in signaling tension defects has led to interest in identifying the 3F3/2 phospho-substrate(s), and the kinases and phosphatases that regulate kinetochore phosphorylation. Recent work shows that the Polo-like kinase 1 (Plk1) is required to generate the 3F3/2 epitope on vertebrate kinetochores [28]. Plk1 regulates numerous mitotic functions including mitotic entry, spindle assembly, cohesin dissociation and cytokinesis, but had not previously been implicated in spindle checkpoint signaling (reviewed in [29]). Plk1 can create 3F3/2 reactivity on kinetochores *in vitro* and depletion of Plk1 by siRNA leads to a decrease in 3F3/2 kinetochore staining *in vivo* [28]. In addition, cells in which Plk1 is depleted have a reduction in the kinetochore levels of many proteins involved in the spindle checkpoint, including Mad2, Cenp-E, Ndc80 and Cdc20. However, rather than the expected defect in checkpoint function, Plk1 depletion leads to a spindle checkpoint-dependent metaphase delay. Although it is possible that Plk1-mediated generation of the 3F3/2 phosphoepitope is not required for tension-dependent checkpoint signaling, it might be that either complete depletion of Plk1 has not been achieved or that additional proteins are involved in the checkpoint response to tension defects. Alternatively, Plk1 depletion might interfere with another mitotic function of Plk1 and activate the checkpoint via unrelated attachment defects.

An intriguing possibility is that Plk1 links checkpoint signals to 3F3/2 staining through phosphorylation of the APC, a Plk1 substrate *in vitro* (reviewed in [29]). The kinetochore localization of the APC depends on the spindle checkpoint and the 3F3/2 antibody recognizes several APC components specifically from mitotic extracts [30,31]. In the future it is important to map these 3F3/2 phosphoepitopes and determine the consequences of this phosphorylation on checkpoint activity *in vivo*.

Other checkpoint proteins

In addition to 3F3/2 phosphoepitopes, reports suggest that several checkpoint proteins are recruited specifically to tension-defective but attached kinetochores. In cells in culture, the conserved checkpoint component BubR1 accumulates on kinetochores in the absence of tension produced by treatment with taxol, incubation at low temperatures, and treatment with the microtubule inhibitor vinblastine [32–34]. However, as discussed above, these treatments might also affect microtubule occupancy. In addition, BubR1 function is required to activate the checkpoint in response to the lack of attachment, which indicates that BubR1 does not play a tension-specific role in the spindle checkpoint [35–37]. By contrast, Mad3, the budding yeast homolog of BubR1, appears to be required for the checkpoint arrest that is signaled by lack of attachment because of spindle depolymerization, but is dispensable for the checkpoint delay that is generated by tension defects caused by inhibition of replication [38]. Mad3 is the only checkpoint protein that is reported to behave in this manner and additional experiments should determine if this observation is truly specific to attachment.

The conserved checkpoint component Bub1 is also reported to accumulate on kinetochores in the absence of tension [32,33,39]. However, the kinetochore localization of Bub1 also appears to be sensitive to defects in attachment [34,39,40]. Further complicating the situation is the observation that Bub1 is required for the kinetochore localization of a subset of checkpoint proteins that varies depending on the experimental system and conditions. These differences might explain why loss of Bub1 function causes varying degrees of checkpoint impairment [40–44]. Additionally, Bub1 regulates centromeric cohesion in meiosis and mitosis [43–45]. Because centromeric cohesion is important for the generation of kinetochore tension, it is tempting to speculate that this regulation might allow Bub1 to monitor tension signals.

Aurora B kinases might regulate the spindle checkpoint response to the absence of tension

In addition to proteins that might mark either unattached or tension-defective kinetochores, some appear to be able to distinguish between these two checkpoint activators. One of these proteins is Aurora B, the catalytic component of a complex that also includes the inner centromere protein (INCENP), Survivin, and the related proteins Dasra B/Borealin/Csc1 and Dasra A (reviewed in [46]). These proteins comprise the chromosomal passenger complex, which is named because of its dynamic localization pattern in which it appears first in the inner centromere region between sister kinetochores, then moves onto the elongating spindle and, finally, concentrates at the spindle midzone. Aurora B regulates numerous mitotic events including amphitelic kinetochore attachment and spindle checkpoint function, spindle assembly and disassembly, anaphase chromosome condensation, and cytokinesis.

Amphitelic kinetochore attachment

Analysis of temperature-sensitive, loss-of-function mutations in the *IPL1* gene, the homolog of Aurora B in budding yeast, reveals cells with massive chromosome missegregation caused by the formation of syntelic attachments [47–49]. Experiments in many model systems are consistent with the yeast experiments and indicate that Aurora B plays a conserved role ensuring amphitelic kinetochore attachments (reviewed in [46]). It has been proposed that Aurora B/Ipl1 promotes the turnover of kinetochore microtubule interactions that do not generate tension [49]. Consistent with this hypothesis, Ipl1 activity is required to detach microtubules from tension-defective kinetochores caused by either a mutation in the conserved Mtw1/Mis12 protein or a defect in replication [49–51]. These observations are similar to subsequent data from studies in cell culture, which indicate that Aurora B selectively disassembles kinetochore microtubules that

are attached syntelically [52]. Furthermore, impairing the function of Aurora B stabilizes kinetochore–microtubule attachments at defective kinetochores [50,53]. Together, these experiments indicate that Aurora B/Ipl1 alters kinetochore microtubules in response to the absence of tension and might be responsible for the instability of attachments at kinetochores that lack tension, as described above. These studies also indicate that Aurora B/Ipl1 is regulated by the absence of tension and, therefore, might also allow the spindle checkpoint to detect tension defects.

Spindle checkpoint

A role for Aurora B/Ipl1 in the spindle checkpoint was indicated first by the analysis of temperature-sensitive *ipl1* mutant cells, which, despite syntelic attachments, proceed through the cell cycle without detectable spindle checkpoint delay [48]. It was shown later that Ipl1 function is required to activate the checkpoint in response to the conditional inhibition of either replication or sister chromatid cohesion, conditions that prevent the chromosome pairing that is necessary to generate bipolar force and kinetochore tension [54]. By contrast, the loss of attachment that is induced by the microtubule depolymerizing drug nocodazole activates the checkpoint in an Ip1l-independent manner, which indicates a specific requirement for Ipl1 to allow the absence of tension to activate the spindle checkpoint. Similarly, in cell-culture systems, Aurora B and the chromosomal passenger complex are required for defects in tension but not attachment to activate the spindle checkpoint [55–58].

The mechanism by which Aurora B/Ipl1 activates the spindle checkpoint in response to the absence of tension is not known. The simplest explanation is that Aurora B/Ipl1 facilitates both amphitelic attachment and spindle checkpoint activation by promoting the turnover of kinetochore–microtubule interactions. This hypothesis suggests that Aurora B/Ipl1 regulates checkpoint activation indirectly by creating unattached kinetochores and, essentially, amplifying a marginal checkpoint signal. Consistent with this hypothesis, impairing Ipl1 function in a kinetochore mutant in budding yeast both restores attachment and satisfies the spindle checkpoint [50]. It is also possible that Aurora B/Ipl1 has a direct role in checkpoint activation that is independent of its role in kinetochore detachment. For example, in fission yeast and extracts of *Xenopus* eggs, Aurora B is required for attachment defects to activate the spindle checkpoint, which indicates a role that is separate from creating unattached kinetochores [59,60]. The differences in spindle checkpoint regulation and the responses to spindle depolymerization that make these organisms depend completely on Aurora B/Ipl1 for checkpoint activity is not well understood. One possibility is that the kinetochore structure in these organisms is disrupted in the absence of Aurora B/Ipl1 function in a manner that prevents the spindle checkpoint from ever being activated. Consistent with this hypothesis, Aurora B is required for the kinetochore localization of all other checkpoint proteins in *Xenopus* egg extracts [61].

Additional proteins that distinguish between tension and attachment defects

Several other gene products in budding yeast are required for tension defects to activate the spindle checkpoint. One such protein is Shugoshin (Sgo1), which was identified originally as a protector of centromeric cohesion during meiosis I [62–65]. A mitotic checkpoint role for Sgo1 has been revealed in a genetic screen for mutants that do not respond to the absence of tension generated by the presence of short linear chromosomes [66]. Subsequent experiments have shown that *sgo1* mutant cells do not activate the checkpoint because of loss of cohesion but that they respond normally to spindle depolymerization, which is similar to *ipl1* mutants. It is intriguing that Sgo1 appears to have a role in both tension-specific checkpoint activation and cohesion of sister chromatids. This leads to the possibility that Sgo1 couples tension-sensing with cohesion maintenance, which itself is essential to generate tension. However, like Aurora B/Ipl1, Sgo1 also has roles in microtubule and kinetochore function that might make it difficult to distinguish whether its role in the checkpoint response to tension defects is separable.

In contrast to *sgo1* mutant budding yeast cells, siRNA-mediated depletion of human Sgo1 in cell culture results in severe mitotic defects that include premature sister separation, destabilization of kinetochore microtubules, loss of kinetochore tension and spindle checkpoint arrest that depends on Mad2 and Aurora B [43,44,67]. It is, therefore, possible that in budding yeast mutants, the role of Sgo1 in the spindle checkpoint is separate from its other mitotic functions. Alternatively, the Sgo1 defect in the checkpoint might be an indirect result of the kinetochore and microtubule defects caused by a lack of function of Sgo1.

A specific defect in engaging the spindle checkpoint in response to the absence of tension is also observed in budding yeast cells with mutations in *SKP1*, a gene that encodes a crucial component of both the kinetochore and the Skp1–Cullin–F-box (SCF), an E3 ubiquitin ligase that is important for the G1–S-phase transition. The checkpoint deficient *skp1* alleles specifically abolish the physical interaction of Skp1 with the checkpoint protein Bub1 but do not affect SCF function [68]. How Skp1–Bub1 interaction modulates tension-dependent functions of the checkpoint remains to be determined. Because Bub1 function is required for Sgo1 kinetochore localization in some organisms, and Bub1 binds to Skp1 in budding yeast, a possible link between Sgo1 and Skp1 might help to explain their roles in mediating the checkpoint response to the absence of tension [43,44,62].

In addition to genes that can distinguish between tension and attachment defects, a small-molecule inhibitor of the tension-dependent checkpoint in budding yeast has recently been identified [73]. The molecule cincreasin inhibits the kinase activity of the spindle checkpoint protein Mps1, leading to a defect in the ability of cells to activate the checkpoint in response to tension defects, but not to attachment defects. It will be interesting to determine whether this effect is due to separate roles for Mps1 in the tension- and attachment-dependent checkpoints, or whether this drug partially inhibits Mps1

function such that it cannot respond to weak tension defects but still has enough function to respond to strong attachment defects.

Concluding remarks

The primary defect that is sensed by the spindle checkpoint remains unresolved, with both the lack of attachment and the absence of tension reasonable possibilities. Distinguishing between these potential models is complicated by the clear inter-relationship between attachment and tension. In organisms with kinetochores that bind multiple microtubules, it is difficult to create tension defects that do not reduce microtubule occupancy. Because of this, the budding yeast, which has a single microtubule per kinetochore, provides the best evidence that the checkpoint directly monitors tension. However, micromanipulation experiments in grasshopper spermatocytes demonstrate that microtubule attachment is more important for regulating the spindle checkpoint and that tension facilitates occupancy. One possibility is that the difference in the number of microtubule-binding sites on kinetochores in budding yeast compared with other organisms reflects a fundamental difference in the checkpoint. In yeast, it might be more important to monitor tension because mono-oriented attachments will always lead to aneuploidy. In addition, yeast are less likely to have unattached kinetochores because microtubules remain bound to kinetochores throughout the cell cycle. However, in organisms with larger kinetochores, a single mis-attached microtubule on a kinetochore that has several, properly bound microtubules is unlikely to generate enough force to cause chromosome missegregation. Furthermore, kinetochores that have multiple microtubule-binding sites proceed through a completely unattached intermediate (Box 1), which indicates that monitoring attachment might be a more effective way for these cells to ensure proper assembly of the spindle.

It is clear that uncovering the true nature of the spindle checkpoint signal is difficult with current information. The isolation of either specific checkpoint alleles that are defective only in their response to the absence of tension or the identification of a protein whose activity *in vitro* is regulated specifically by tension might provide the evidence that tension is a direct activator of the spindle checkpoint. As we learn more, it will be interesting to determine whether the checkpoint signal differs depending on the organism and its kinetochore structure, and whether the activating signal is the same in mitosis and meiosis.

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References

1 Biggins, S. and Walczak, C.E. (2003) Captivating capture: how microtubules attach to kinetochores. *Curr. Biol.* 13, R449–R460
2 Hauf, S. and Watanabe, Y. (2004) Kinetochore orientation in mitosis and meiosis. *Cell* 119, 317–327
3 Cimini, D. *et al*. (2004) Anaphase spindle mechanics prevent mis-segregation of merotelically oriented chromosomes. *Curr. Biol.* 14, 2149–2155
4 Hassold, T. and Hunt, P. (2001) To err (meiotically) is human: the genesis of human aneuploidy. *Nat. Rev. Genet.* 2, 280–291
5 Rajagopalan, H. and Lengauer, C. (2004) Aneuploidy and cancer. *Nature* 432, 338–341
6 Lew, D.J. and Burke, D.J. (2003) The spindle assembly and spindle position checkpoints. *Annu. Rev. Genet.* 37, 251–282
7 Zhou, J. *et al*. (2002) Attachment and tension in the spindle assembly checkpoint. *J. Cell Sci.* 115, 3547–3555
8 Draviam, V.M. *et al*. (2004) Chromosome segregation and genomic stability. *Curr. Opin. Genet. Dev.* 14, 120–125
9 Bharadwaj, R. and Yu, H. (2004) The spindle checkpoint, aneuploidy, and cancer. *Oncogene* 23, 2016–2027
10 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
11 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
12 Nicklas, R.B. *et al*. (2001) Checkpoint signals in grasshopper meiosis are sensitive to microtubule attachment, but tension is still essential. *J. Cell Sci.* 114, 4173–4183
13 McEwen, B.F. *et al*. (1997) Kinetochore fiber maturation in PtK1 cells and its implications for the mechanisms of chromosome congression and anaphase onset. *J. Cell Biol.* 137, 1567–1580
14 Winey, M. *et al*. (1995) Three-dimensional ultrastructural analysis of the *Saccharomyces cerevisiae* mitotic spindle. *J. Cell Biol.* 129, 1601–1615
15 Shonn, M.A. *et al*. (2000) Requirement of the spindle checkpoint for proper chromosome segregation in budding yeast meiosis. *Science* 289, 300–303
16 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
17 Chen, R-H. *et al*. (1996) Association of spindle assembly checkpoint component XMAD2 with unattached kinetochores. *Science* 274, 242–246
18 Li, Y. and Benezra, R. (1996) Identification of a human mitotic checkpoint gene: hsMAD2. *Science* 274, 246–248
19 Waters, J.C. *et al*. (1998) Localization of Mad2 to kinetochores depends on microtubule attachment, not tension. *J. Cell Biol.* 141, 1181–1191
20 Kapoor, T.M. *et al*. (2000) Probing spindle assembly mechanisms with monastrol, a small molecule inhibitor of the mitotic kinesin, Eg5. *J. Cell Biol.* 150, 975–988
21 Martin-Lluesma, S. *et al*. (2002) Role of Hec1 in spindle checkpoint signaling and kinetochore recruitment of Mad1/Mad2. *Science* 297, 2267–2270
22 DeLuca, J.G. *et al*. (2002) hNuf2 inhibition blocks stable kinetochore-microtubule attachment and induces mitotic cell death in HeLa cells. *J. Cell Biol.* 159, 549–555
23 Gillett, E.S. *et al*. (2004) Spindle checkpoint proteins and chromosome-microtubule attachment in budding yeast. *J. Cell Biol.* 164, 535–546
24 Cyert, M.S. *et al*. (1988) Monoclonal antibodies specific for thiophosphorylated proteins recognize *Xenopus* MPF. *Dev. Biol.* 129, 209–216
25 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
26 Nicklas, R.B. *et al*. (1995) Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J. Cell Biol.* 130, 929–939
27 Campbell, M.S. and Gorbsky, G.J. (1994) Microinjection of mitotic cells with the 3F3/2 anti-phosphoepitope antibody delays the onset of anaphase. *J. Cell Biol.* 129, 1195–1204
28 Ahonen, L.J. *et al*. (2005) Polo-like kinase 1 creates the tension-sensing 3F3/2 phosphoepitope and modulates the association of spindle-checkpoint proteins at kinetochores. *Curr. Biol.* 15, 1078–1089
29 van Vugt, M.A. and Medema, R.H. (2005) Getting in and out of mitosis with Polo-like kinase-1. *Oncogene* 24, 2844–2859
30 Acquaviva, C. *et al*. (2004) The anaphase promoting complex/cyclosome is recruited to centromeres by the spindle assembly checkpoint. *Nat. Cell Biol.* 6, 892–898

31 Daum, J.R. *et al*. (2000) The 3F3/2 anti-phosphoepitope antibody binds the mitotically phosphorylated anaphase-promoting complex/cyclosome. *Curr. Biol.* 10, R850–R852

32 Skoufias, D.A. *et al*. (2001) Mammalian mad2 and bub1/bubR1 recognize distinct spindle-attachment and kinetochore-tension checkpoints. *Proc. Natl. Acad. Sci. U. S. A.* 98, 4492–4497

33 Shannon, K.B. *et al*. (2002) Mad2 and BubR1 function in a single checkpoint pathway that responds to a loss of tension. *Mol. Biol. Cell* 13, 3706–3719

34 Logarinho, E. *et al*. (2004) Different spindle checkpoint proteins monitor microtubule attachment and tension at kinetochores in *Drosophila* cells. *J. Cell Sci.* 117, 1757–1771

35 Chan, G.K. *et al*. (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

36 Chen, R.H. (2002) BubR1 is essential for kinetochore localization of other spindle checkpoint proteins and its phosphorylation requires Mad1. *J. Cell Biol.* 158, 487–496

37 Meraldi, P. *et al*. (2004) Timing and checkpoints in the regulation of mitotic progression. *Dev. Cell* 7, 45–60

38 Lee, M.S. and Spencer, F.A. (2004) Bipolar orientation of chromosomes in *Saccharomyces cerevisiae* is monitored by Mad1 and Mad2, but not by Mad3. *Proc. Natl. Acad. Sci. U. S. A.* 101, 10655–10660

39 Taylor, S.S. *et al*. (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

40 Sharp-Baker, H. and Chen, R.H. (2001) Spindle checkpoint protein Bub1 is required for kinetochore localization of Mad1, Mad2, Bub3, and CENP-E, independently of its kinase activity. *J. Cell Biol.* 153, 1239–1250

41 Johnson, V.L. *et al*. (2004) Bub1 is required for kinetochore localization of BubR1, Cenp-E, Cenp-F and Mad2, and chromosome congression. *J. Cell Sci.* 117, 1577–1589

42 Tang, Z. *et al*. (2004) Phosphorylation of Cdc20 by Bub1 provides a catalytic mechanism for APC/C inhibition by the spindle checkpoint. *Mol. Cell* 16, 387–397

43 Tang, Z. *et al*. (2004) Human Bub1 protects centromeric sister-chromatid cohesion through Shugoshin during mitosis. *Proc. Natl. Acad. Sci. U. S. A.* 101, 18012–18017

44 Kitajima, T.S. *et al*. (2005) Human Bub1 defines the persistent cohesion site along the mitotic chromosome by affecting Shugoshin localization. *Curr. Biol.* 15, 353–359

45 Bernard, P. *et al*. (2001) Fission yeast Bub1 is essential in setting up the meiotic pattern of chromosome segregation. *Nat. Cell Biol.* 3, 522–526

46 Vagnarelli, P. and Earnshaw, W.C. (2004) Chromosomal passengers: the four-dimensional regulation of mitotic events. *Chromosoma* 113, 211–222

47 Chan, C.S. and Botstein, D. (1993) Isolation and characterization of chromosome-gain and increase-in-ploidy mutants in yeast. *Genetics* 135, 677–691

48 Biggins, S. *et al*. (1999) The conserved protein kinase Ipl1 regulates microtubule binding to kinetochores in budding yeast. *Genes Dev.* 13, 532–544

49 Tanaka, T.U. *et al*. (2002) Evidence that the Ipl1-Sli15 (Aurora kinase-INCENP) complex promotes chromosome bi-orientation by altering kinetochore-spindle pole connections. *Cell* 108, 317–329

50 Pinsky, B.A. *et al*. (2003) An Mtw1 complex promotes kinetochore biorientation that is monitored by the Ipl1/Aurora protein kinase. *Dev. Cell* 5, 735–745

51 Dewar, H. *et al*. (2004) Tension between two kinetochores suffices for their bi-orientation on the mitotic spindle. *Nature* 428, 93–97

52 Lampson, M.A. *et al*. (2004) Correcting improper chromosome-spindle attachments during cell division. *Nat. Cell Biol.* 6, 232–237

53 Lampson, M.A. and Kapoor, T.M. (2005) The human mitotic checkpoint protein BubR1 regulates chromosome-spindle attachments. *Nat. Cell Biol.* 7, 93–98

54 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

55 Hauf, S. *et al*. (2003) The small molecule Hesperadin reveals a role for Aurora B in correcting kinetochore-microtubule attachment and in maintaining the spindle assembly checkpoint. *J. Cell Biol.* 161, 281–294

56 Ditchfield, C. *et al*. (2003) Aurora B couples chromosome alignment with anaphase by targeting BubR1, Mad2, and Cenp-E to kinetochores. *J. Cell Biol.* 161, 267–280

57 Carvalho, A. *et al*. (2003) Survivin is required for stable checkpoint activation in taxol-treated HeLa cells. *J. Cell Sci.* 116, 2987–2998

58 Lens, S.M. *et al*. (2003) Survivin is required for a sustained spindle checkpoint arrest in response to lack of tension. *EMBO J.* 22, 2934–2947

59 Kallio, M.J. *et al*. (2002) Inhibition of aurora B kinase blocks chromosome segregation, overrides the spindle checkpoint, and perturbs microtubule dynamics in mitosis. *Curr. Biol.* 12, 900–905

60 Petersen, J. and Hagan, I.M. (2003) *S. pombe* aurora kinase/survivin is required for chromosome condensation and the spindle checkpoint attachment response. *Curr. Biol.* 13, 590–597

61 Vigneron, S. *et al*. (2004) Kinetochore localization of spindle checkpoint proteins: who controls whom? *Mol. Biol. Cell* 15, 4584–4596

62 Kitajima, T.S. *et al*. (2004) The conserved kinetochore protein shugoshin protects centromeric cohesion during meiosis. *Nature* 427, 510–517

63 Marston, A.L. *et al*. (2004) A genome-wide screen identifies genes required for centromeric cohesion. *Science* 303, 1367–1370

64 Katis, V.L. *et al*. (2004) Maintenance of cohesin at centromeres after meiosis I in budding yeast requires a kinetochore-associated protein related to MEI-S332. *Curr. Biol.* 14, 560–572

65 Rabitsch, K.P. *et al*. (2004) Two fission yeast homologs of Drosophila Mei-S332 are required for chromosome segregation during meiosis I and II. *Curr. Biol.* 14, 287–301

66 Indjeian, V.B. *et al*. (2005) The centromeric protein Sgo1 is required to sense lack of tension on mitotic chromosomes. *Science* 307, 130–133

67 Salic, A. *et al*. (2004) Vertebrate shugoshin links sister centromere cohesion and kinetochore microtubule stability in mitosis. *Cell* 118, 567–578

68 Kitagawa, K. *et al*. (2003) Requirement of skp1-bub1 interaction for kinetochore-mediated activation of the spindle checkpoint. *Mol. Cell* 11, 1201–1213

69 Mitchison, T. and Kirschner, M. (1984) Dynamic instability of microtubule growth. *Nature* 312, 237–242

70 Li, X. and Nicklas, R.B. (1995) Mitotic forces control a cell-cycle checkpoint. *Nature* 373, 630–632

71 Jang, J.K. *et al*. (1995) Induction of metaphase arrest in *Drosophila* oocytes by chiasma-based kinetochore tension. *Science* 268, 1917–1919

72 Rieder, C.L. *et al*. (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

73 Dorer, R.K. *et al*. (2005) A small-molecule inhibitor of Mps1 blocks the spindle-checkpoint response to a lack of tension on mitotic chromosomes. *Curr. Biol.* 15, 1070–1076