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Centromere tension: a divisive issue

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Abstract

It has been proposed that the spindle assembly checkpoint detects both unattached kinetochores and lack of tension between sister kinetochores when sister chromatids are not attached to opposite spindle poles. However, here we argue that there is only one signal — whether kinetochores are attached to microtubules or not — and this has implications for our understanding of both chromosome segregation and the control of genomic stability.

To segregate chromosomes properly between daughter cells, sister kinetochores on each chromosome must establish stable connections with opposite spindle poles through microtubules (amphitelic attachment; Fig. 1a). As the interaction between kinetochores and microtubules is stochastic, the time required for all chromosomes to attach is variable and sister kinetochores rarely attach to spindle microtubules simultaneously. Instead, initially chromosomes become monotelic — connected to the spindle through only one kinetochore (Fig. 1a). However, exit from mitosis before attachment of all kinetochores to spindle microtubules results in ‘lost’ chromosomes that distribute randomly and generate aneuploid progeny. Mis-segregation of chromosomes can also arise from improper microtubule attachments during spindle assembly. Two types of erroneous attachments exist: syntelic, where both sister kinetochores connect to the same spindle pole, and merotelic, where a single kinetochore simultaneously connects to both spindle poles (Fig. 1a). Fortunately, an error correction mechanism discriminates between amphitelic and erroneous attachments and actively destabilizes the latter.

The spindle assembly checkpoint (SAC) prevents cells from separating their sister chromatids and exiting mitosis until all kinetochores are connected to the spindle. A single unattached kinetochore can delay cells in mitosis for hours. Intuitively, it might be hypothesised that cells should also delay mitotic exit in the presence of erroneously attached chromosomes to provide the opportunity to correct the problem. Indeed, a common view is that the SAC also monitors tension between sister kinetochores, which increases significantly when amphitelic attachment is achieved. But, as we discuss here, significant data support the alternative view that the SAC allows exit from mitosis whether there are correct or erroneous chromosome attachments, which suggests that the checkpoint machinery primarily detects whether kinetochores are attached or not. To differentiate between proper and erroneous kinetochore attachments, cells instead rely on a correction mechanism that detects tension between sister kinetochores.

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Much discussion has centred on whether error correction and the SAC are independent or constitute two parts of the same pathway. This issue is important because mitotic exit in the presence of erroneously attached chromosomes is a hallmark of cancer cells. Thus, the question arises of whether cancer cells have a deficient checkpoint, or whether an inefficient error-correction mechanism causes chromosome mis-segregation despite a proficient SAC¹, which has implications for anti-cancer therapies.

Numerous excellent reviews on the molecular mechanisms of the SAC and error correction are available and we do not intend to repeat them here. Instead, we attempt to clarify the issue of what is detected by the SAC by reconciling some classic and more recent observations that link mitotic progression and error correction with the forces acting on the kinetochores and the centromere.

History of the spindle assembly checkpoint

It has long been established that normal animal cells do not initiate anaphase until all chromosomes congress in the middle of the spindle². However, the question of whether the cell directly monitors chromosome positioning, the type of kinetochore attachment, or something else, remains contentious. In part, this stems from the assays used, and it should be stressed that kinetochore attachment and chromosome orientation are not interchangeable because chromosome positioning cannot discriminate between proper and erroneous attachments. Mono-oriented chromosomes can be monotelic, a normal intermediate before proper attachment, or syntelic, which is an erroneous condition. Similarly, bi-oriented chromosomes can comprise both proper amphitelic and erroneous merotelic attachments. That improper chromosome positioning is not detected by the checkpoint was revealed in the seminal laser-ablation experiments that established that unattached kinetochores are the source of the 'wait anaphase' signal during mitosis, but unfortunately this important ramification is often overlooked. Using a highly focused laser beam, Rieder and colleagues demonstrated that ablation of the unattached kinetochore on monotelic chromosomes rendered these chromosomes invisible to the SAC: although the chromosome remained mono-oriented, it had no unattached kinetochores and cells consistently exited mitosis with normal dynamics³. Thus, the SAC did not prevent mitotic exit, despite the unstretched centromere (Fig. 1b).

The conclusion that it is primarily whether a kinetochore is attached or not that is detected by the checkpoint has been reiterated in numerous subsequent studies. For example, in cells that undergo mitosis with an unreplicated genome (MUG), the SAC does not prevent mitotic exit, despite numerous erroneous attachments. Mammalian MUG cells are generated by blocking replication and forcing cells into mitosis by inhibiting the DNA-damage checkpoint. In these cells, chromosomes have only one kinetochore and thus cannot achieve amphitelic attachment. Instead, kinetochores establish merotelic or monotelic attachments, but cells still exit mitosis with only minor delays⁴. Therefore, it seems that kinetochore attachment is the only factor necessary to satisfy the checkpoint. Importantly, this means that the checkpoint remains in this state during anaphase when sister chromatids separate, because although there is no longer tension between kinetochores, all the kinetochores remain attached. Hence, there is no need to postulate that the SAC machinery must be inactivated in anaphase.

Here we should note that budding yeast MUG cells do delay in mitosis^{5,6}. However, the authors of these studies cautioned that although chromosomes subsequently segregated correctly they could not exclude the possibility that kinetochores transiently detached during the arrest. Alternatively, there may be a difference between budding yeast and metazoan

mitosis, perhaps consequent to yeast kinetochores attaching to the spindle from S phase onwards.

The hypothesis that the checkpoint responds to tension came to prominence after the elegant micromanipulation work on praying mantid spermatocytes by Li and Nicklas⁷. It is noteworthy from the outset that these cells were in meiosis, not mitosis. In mantids, there are three sex chromosomes (two X and one Y) that are tethered together in one complex. To distribute properly, meiosis I kinetochores on both X chromosomes attach to one spindle pole, whereas the Y chromosome attaches to the opposite pole (Fig. 1c). Under these conditions, the chromosomes are under tension. Occasionally, one of the X chromosomes disconnects from the other two partners and becomes mono-oriented. This event normally delays anaphase for hours, but cells will progress rapidly into anaphase if the mono-oriented chromosome is pulled away from the spindle pole by a microneedle.

This result can be readily interpreted as evidence that tension at the attached kinetochore is necessary to satisfy the checkpoint. However, before making this conclusion (and extending it to mitosis) it is important to point out that because homologous chromosomes are paired during meiosis I, the sister kinetochores are juxtaposed and function as a single complex. Thus, an unpaired meiotic X chromosome is not analogous to a syntelic mitotic chromosome. Instead, we suggest that unpaired X chromosomes in these spermatocytes functionally resemble monotelic mitotic chromosomes that lack unattached sister kinetochores (Fig. 1b, c)⁸, although other differences between these systems might also be important. Nevertheless, there is a striking difference in the checkpoint response to monotelic chromosomes in mitosis versus meiosis. During mitosis, a monotelic chromosome delays anaphase for hours but only if it possesses an unattached kinetochore⁹. In contrast, during meiosis, the SAC can only be satisfied when tension on the monotelic kinetochore is raised, even in the absence of the unattached sister kinetochore⁷. The difference that underlies this response is potentially a consequence of the polar-ejection force that continually pushes chromosomes away from the spindle poles in mitotic cells. This provides an opposing force to the microtubules pulling on the monotelic chromosome and generates tension at the attached kinetochore. By contrast, there is no polar ejection pushing on chromosome arms in the spindle of the insect spermatocytes. As a result, there is a substantial difference in the number of microtubules attached to monotelic kinetochores in mitosis, compared with meiosis. In mitosis, monotelic attachments maintain at least 60% of the number of microtubules found in amphitelic chromosomes¹⁰, whereas during meiosis the lack of tension greatly reduces the accumulation of kinetochore microtubules (Fig. 1d). This prompted Nicklas *et al.* to conclude that the checkpoint in meiosis does not monitor tension on erroneously attached kinetochores, but instead tension stabilizes microtubule attachment, and it is this that ultimately satisfies the checkpoint¹¹.

Tension-sensors and error correction: the role of Aurora B

Although Nicklas *et al.* concluded that attachment, not tension, was detected by the checkpoint, there is still a wide perception that tension is a component of the SAC. In part, this is because Aurora B kinase seems to fulfil the role of a principal component of both the checkpoint and tension-detection machineries. However, here we emphasize that even if Aurora B is a common component this does not necessitate that the checkpoint and the error correction machineries are one and the same: Aurora B could have different activators and substrates when part of the checkpoint or error-correction machinery. Thus, as outlined below, the elegant experiments supporting the conclusion that Aurora B phosphorylation responds to different types of kinetochore attachment and consequent error correction cannot be taken as evidence that these attachments also regulate the SAC.

The Aurora B pathway has recently been reviewed^{12,13}, therefore here we only briefly outline the principles of how it regulates the stability of kinetochore attachments. Aurora B localizes to the centromere between sister kinetochores¹⁴, whereas its opposing phosphatase, PP1, localizes to the outer kinetochore¹⁵. In the absence of microtubule attachments, Aurora B tethered to the centromere is able to reach its substrates within the kinetochores. Among these substrates are members of the kinesin 13 family that destabilize microtubule attachments. On establishing proper amphitelic attachment, the kinetochores are pulled away from the inner centromere and out of the reach of Aurora B, but still within the zone of PP1 phosphatase activity. This elegant mechanism can readily explain how centromere stretching results in selective stabilization of amphitelic microtubules. However, it also creates a conceptual problem for how initial capture of microtubules by the kinetochore can develop into amphitelic attachment. Chromosomes initially establish monotelic attachments, but from the standpoint of a tension-based error-correction mechanism, monotelic attachments are indistinguishable from syntelic. Thus, monotelic attachments should immediately be destabilized and detached from the spindle.

This can be resolved if Aurora B-mediated microtubule destabilization is slow or relatively inefficient, compared with microtubule attachment. Under these conditions, chromosomes will have sufficient time to establish amphitelic attachment before the first microtubule connection is severed. In this regard, the probability of microtubule capture in grasshopper spermatocytes is 0.13 min^{-1} , whereas microtubule release on syntelic chromosomes is approximately threefold less frequent (0.43 min^{-1})¹⁶, consistent with the notion that the longevity of the initial microtubule attachment to tensionless kinetochores exceeds the time needed to establish amphitelic attachment.

Unfortunately, no data are available regarding the efficacy and time scale of error-correction during normal spindle assembly in mammalian cells. Nevertheless, correction of experimentally-induced syntelic attachments takes up to 30 min, comparable to the entire duration of unperturbed mitosis¹⁷. Monotelic chromosomes can persist during mitosis for hours⁹, possibly because the microtubule attachment is stabilized by tension generated by the kinetochore-generated poleward forces opposed by the spindle-ejection force acting on chromosome arms.

The correction mechanism can easily be overwhelmed if the frequency of erroneous attachments is elevated. For example, chromosomes often mis-segregate in cells recovering from treatment with mitotic poisons such as nocodazole that increase the size of the kinetochore, thereby increasing the promiscuity of initial kinetochore attachments¹⁸. Similarly, even transient perturbations of spindle geometry, such as multipolar or monopolar intermediates during spindle assembly, severely increase chromosome mis-segregation^{19,20}. Importantly, accumulation of erroneous attachments in these cells does not prevent mitotic exit, supporting the notion that attachment errors are not directly monitored by the SAC.

Separation between the SAC and error-correction mechanisms is also supported by observations that an inability to destabilize microtubules attached to tensionless kinetochores does not prevent mitotic exit: inactivation the microtubule depolymerizing kinesin 13 (MCAK) at the kinetochore does not arrest cells in mitosis, despite the presence of numerous syntelic- and merotelic-attachments^{21,22}. Importantly, Aurora B activity and localization are not affected by the inactivation of MCAK²². Therefore, if Aurora B-mediated tension sensing was a direct signal for the checkpoint the cells should arrest.

Furthermore, recent data are inconsistent with the idea that erroneous attachments that inevitably result in lower tension at the centromere are directly detected by the SAC. An elegant study revealed that the mitotic checkpoint is more readily satisfied in cells treated

with very high, rather than moderate- or low-concentrations, of the microtubule-stabilizing drug, Taxol²³. Treatment with Taxol perturbs microtubule dynamics, decreases tension across the centromere and blocks cells in mitosis, but whether the lack of tension directly caused the mitotic arrest was unclear^{24,25}. Very high concentrations of Taxol make microtubules artificially stable and thus resistant to the action of microtubule depolymerases at the kinetochore, and this supports the idea that the checkpoint does not directly detect tensile signals mediated by Aurora B at the centromere. Additionally, even relatively minor changes in microtubule stability significantly elevate chromosome mis-segregation^{26,27}.

Nevertheless, biochemical data show that Aurora B interacts with several checkpoint proteins. Although these interactions do not intrinsically prove that Aurora B is itself a checkpoint protein (see ref. ²⁸ for discussion) the tension versus attachment debates continue to flourish (see ref. ¹² for an alternative view).

Are there different kinds of tension?

There is a growing consensus that tension across the centromere is not essential for the satisfaction of the SAC (see ref. ²⁹ for a more complete review). How then is microtubule attachment detected by the kinetochore? Multicolour fluorescent-protein-imaging technology that can visualize changes in the distance between proteins within the same kinetochore has begun to yield clues. Recently, two groups addressed the issue of how the kinetochore changes on microtubule attachment^{30,31}. Both studies demonstrated that in addition to stretching the centromere, microtubule attachments stretch the kinetochore itself, manifested by a marked increase in the distance between inner- and outer-kinetochore components. Furthermore, conditions that relieve centromere stretching, but not intrakinetochore stretching (low concentrations of Taxol), satisfy the SAC, consistent with the idea that tension between kinetochores is not detected by the SAC³⁰. In contrast, conditions that collapse the intrakinetochore stretch (moderate concentrations of Taxol or nocodazole) do induce mitotic arrest³¹. These data strongly indicate that intrakinetochore stretching has a role in the satisfaction of the SAC, and a recent review presented “the revenge of tension”¹² implying that the data supports a resurrection of the hypothesis that high tension at centromere/kinetochores is necessary for both activation of the error-correction mechanisms and satisfaction of the SAC. However, we argue here that intrakinetochore stretching does not allow the cell to discriminate between proper and erroneous kinetochore attachments. Indeed, the results of multicolour analyses of kinetochore organization provide strong evidence that the only parameter monitored by the checkpoint is attachment of the kinetochores to dynamic microtubules, whereas lack of centromere tension is the principal factor in discrimination of proper, versus erroneous, kinetochore attachments.

Attachment strikes back

Our argument is based on two key findings in the work of Uchida and colleagues. First, this work demonstrates that intrakinetochore stretching occurs independently for sister kinetochores³¹ (Fig. 2a). This implies that the ensemble of sister kinetochores and the centromere is not a tandem arrangement of elastic elements. According to Hooke’s law, all elements in a tandem arrangement of springs must react to tension simultaneously with the extent of deformation in each element proportional to the tensile strength of this element (Fig. 2b). The independence of sister kinetochores proves that centromere stretching and intrakinetochore deformations arise from two different forces. The former is caused by the attempts of sister kinetochores to move simultaneously in opposite (poleward) directions. In contrast, the latter force is strictly confined within the kinetochore and is not transmitted to its sister. Consistent with this, there is no correlation between intrakinetochore deformations

and direction of chromosome movement or centromere stretching³¹. These results are conceptually important because a sensor confined strictly within the kinetochore cannot discriminate between proper amphitelic- and erroneous syntelic-attachment. Such discrimination requires information regarding the attachment states of the sister kinetochores, which would need a tensile sensor connected to both.

The second key finding is that intrakinetochore deformations occur in monotelic kinetochores with the same frequency as in amphitelic kinetochores (approximately 15% of the time)³¹, which is inconsistent with the idea that intrakinetochore stretching can discriminate between different types of kinetochore attachment. Thus, intrakinetochore stretching does not conceptually link the SAC and erroneous kinetochore attachments.

The nature of intrakinetochore deformations

The exact nature of intrakinetochore deformation remains unknown, but the data indicate that kinetochores deform only when they attach to highly dynamic microtubules^{29,31} because conditions that stabilize microtubule plus-end dynamics (for example, very low concentrations of nocodazole, or Taxol at all but very high concentrations) decrease intrakinetochore stretching. These observations, together with the notion that the force responsible for intrakinetochore stretching is confined within the kinetochore, indicate that the force responsible for intrakinetochore deformation arises from the growing tips of microtubules that periodically push against the inner parts of the kinetochore (Fig. 2c). Although clearly speculative, this model is consistent with electron-microscopy data on the structure of the mammalian kinetochore³² that show that individual microtubules interact with the kinetochore at two distinct points: the tip of the microtubule is embedded in the fibrous meshwork inside the kinetochore outer plate, whereas a set of fibres extends from the outer plates to microtubule walls 50–100 nm away from the tip. In unattached kinetochores, these latter fibres retract, thinning the outer plate, and indicate that the kinetochore outer plate is elastic. Thus, tubulin polymerization at the tip of attached microtubules should result in tension inside the kinetochore because it increases the distance between the microtubule tip and the attachment points on the microtubule wall.

Conclusions

The role of intrakinetochore deformation in the satisfaction of the SAC is a significant discovery. However, in contrast to interkinetochore tension, tensile elements confined within kinetochores cannot differentiate between proper amphitelic attachment and improper monotelic-, syntelic- and merotelic-attachments. Instead, they appear to monitor whether attachment is made to dynamic microtubules. It is noteworthy that even minute deviations in microtubule dynamic instability cause mitotic abnormalities and adversely affect the fidelity of chromosome distribution²⁷. Therefore, delaying mitotic progression when microtubule connections are too stable may be an important mechanism to prevent unequal chromosome segregation.

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Figure 1.

Classic experiments that revealed the link between tension, stability of attachment and control of mitotic progression. **(a)** Kinetochore attachment and chromosome orientation in mitosis. Types of kinetochore attachment are indicated. Types of chromosome orientation: M, mono-oriented; B, bi-oriented. There is no strict coordination between the type of kinetochore attachment and chromosome orientation. Bi-oriented chromosomes can be attached in a proper (amphitelic) or erroneous (merotelic) fashion. Similarly, chromosome mono-orientation can be caused by a transient monotelic state, which is a normal intermediate before proper amphitelic attachment, or erroneous syntelic attachment. For error-free chromosome distribution, incorrect microtubule connections (shown in red) must be resolved, whereas proper microtubule connections (shown in green) should be preserved. **(b)** During mitosis in vertebrate somatic cells, monotelic chromosomes delay anaphase. Laser ablation of the unattached kinetochore allows mitotic progression to resume although the chromosome remains mono-oriented and centromere tension remains low³. **(c)** During meiosis I in spermatocytes of praying mantids, a mono-oriented unpaired X chromosome delays anaphase. Sister kinetochores are fused and are a single functional unit during meiosis I. Thus, unpaired X chromosomes during meiosis I could be analogous to monotelic chromosomes that lack unattached kinetochores during mitosis. However, the effects of these chromosomes on mitotic versus meiotic progression are markedly different. Pull the unpaired X chromosome away with a microneedle relieves the block and allows the cell to initiate anaphase⁷. **(d)** Using a microneedle to pulling on a syntelic chromosome at the spindle pole leads to a marked increase in the number of microtubules in the kinetochore fibres (red lines) during meiosis I in grasshopper spermatocytes¹¹.

Figure 2.

Potential mechanisms of intrakinetochores deformations. **(a)** A kymograph depicting the dynamics of sister kinetochores in HeLa cells (reproduced with permission from ref. ³¹). Inset presents time points 9–18 s, at higher magnification. Kinetochores are double-labelled with an inner-kinetochore component CENP-A-GFP (green fluorescent protein; green) and an outer kinetochore component Mis12-mCherry (red). Notice that the red and green labels are periodically superimposed and separated, which reflects intrakinetochores deformations. Intriguingly, deformation of sister kinetochores are not coordinated. Also, intrakinetochores deformations occur several times during a single chromosome oscillation cycle and they are therefore not coordinated with the direction of the chromosome movement. **(b)** Predicted dynamics of the centromere/kinetochores if they are modelled as a tandem of Hookean springs. In the absence of attached microtubules both intrakinetochores- (red) and interkinetochores- (green) elastic elements are relaxed (top). Depending on the relative stiffness of the centromere and kinetochores, microtubule-based forces (black arrows) can primarily stretch the centromere (if the kinetochores are stiffer springs; middle) or the kinetochores (if the centromere is stiffer; bottom). However, in both models, it is impossible to stretch one kinetochore while its identical sister is relaxed. **(c)** A potential mechanism of intrakinetochores deformation. Our speculation is based on electron-microscopy reconstructions³² that reveal a fibrous connection between the kinetochore and microtubule walls approximately 50–100 nm away from the microtubule tip. In amphitelic configuration, poleward forces acting along microtubules (black arrows) are responsible for stretching the centromere (green spring). Intrakinetochores stretching occurs when growing tips of kinetochore microtubules push against the kinetochore inner layer which stretches the fibrous connection (red spring in the left kinetochore). When microtubules are shrinking or not dynamic, the fibrous connection collapses (red spring in the right kinetochore). In this model, the deformation of sister kinetochores is not coordinated.