Releasing the spindle assembly checkpoint without tension

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Eukaryotic cells have evolved a spindle assembly checkpoint (SAC) that facilitates accurate genomic segregation during mitosis by delaying anaphase onset in response to errors in kinetochore microtubule attachment. In contrast to the well-studied molecular mechanism by which the SAC blocks anaphase onset, the events triggering SAC release are poorly understood. Papers in this issue by Uchida et al. (Uchida, K.S.K., K. Takagaki, K. Kumada, Y. Hirayama, T. Noda, and T. Hirota. 2009. *J. Cell Biol.* 184:383–390) and Maresca and Salmon (Maresca, T.J., and E.D. Salmon. 2009. *J. Cell Biol.* 184:373–381) make an important advance by demonstrating that SAC release depends on molecular rearrangements within the kinetochore rather than tension-produced stretch between sister kinetochores.

SAC monitoring of kinetochore attachment is critical for accurate genomic segregation with defects in SAC function leading to loss of cell viability and several forms of cancer (Yuen et al., 2005). The SAC is required because kinetochores are initially scattered randomly throughout the cytoplasm with the result that the time required for microtubule attachment is highly variable (Rieder and Salmon, 1998). It was clear from earlier work that the SAC monitors kinetochore attachment to spindle microtubules, but there has been a continuing debate over the role of tension in SAC release (for reviews see Pinsky and Biggins, 2005; Musacchio and Salmon, 2007).

A direct role for tension was indicated by release of the SAC after the use of micromanipulation to create tension on chromosomes in which both sister kinetochores are attached to the same spindle pole (Nicklas and Ward, 1994). Micromanipulation also decreased phosphorylation of BubR1 (3F3/2 phosphoepitope), which correlates with SAC release (Campbell and Gorbsky, 1995; Nicklas et al., 1995). Furthermore, the SAC remains activated when PtK_1 cells are treated with concentrations of taxol that reduce tension without significantly affecting the numbers of kinetochore microtubule attachments (McEwen et al., 1997; Waters et al., 1998). Contradictory evidence for the role of tension came from an early laser ablation study demonstrating that the SAC is released after destruction of the last unbound kinetochore in a mitotic spindle, even though the remaining sister of the ablated kinetochore is monooriented and therefore under low tension (Rieder et al., 1995). This result is supported by a recent study showing that the chromosome fragments formed when HeLa cells undergo mitosis with unreplicated genomes are still able to satisfy the SAC even though all of the kinetochores are monooriented (O'Connell et al., 2008).

Both Uchida et al. (see p. 383 of this issue) and Maresca and Salmon (see p. 373 of this issue) assessed the role of tension in SAC release by using two fluorescent markers located in different regions of the kinetochore to distinguish stretch between sister kinetochores (interkinetochore stretch) from stretch within kinetochores (intrakinetochore stretch; Fig. 1). Uchida et al. (2009) created a HeLa cell line stably expressing GFP–centromere protein A (CENP-A) to mark the inner kinetochore and mCherry-Mis12 to mark the outer kinetochore, whereas Maresca and Salmon (2009) created a *Drosophila melanogaster* S2 cell line stably expressing mCherry–centromere identifier (CID; *Drosophila* homologue of CENP-A) as the inner kinetochore marker and GFP-Ndc80 as the outer kinetochore marker (DeLuca et al., 2005; Cheeseman et al., 2006). Using the centroids of the mCherry and GFP peaks of sister kinetochores, interkinetochore stretch can be measured as the distance between CENP-A or CID in sister kinetochores, whereas intrakinetochore stretch is measured as the distance between CENP-A and Mis12 or CID and Ndc80 within a single kinetochore.

Both groups found that fully attached sister kinetochores in untreated cells exhibit both inter- and intrakinetochore stretch when compared with sister kinetochores in the absence of microtubule binding. Uchida et al. (2009) reported that treatment with low concentrations of nocodazole (7 ng/ml) or depletion of condensin I suppressed intrakinetochore stretch, had minimal effect on interkinetochore stretch, and inhibited SAC release. In contrast, the attached kinetochores of monooriented chromosomes exhibited normal intrakinetochore stretch and no interkinetochore stretch and are known to satisfy the SAC (Rieder et al., 1995). In agreement with these data, Maresca and Salmon (2009) found that treatment of S2 cells with low concentrations

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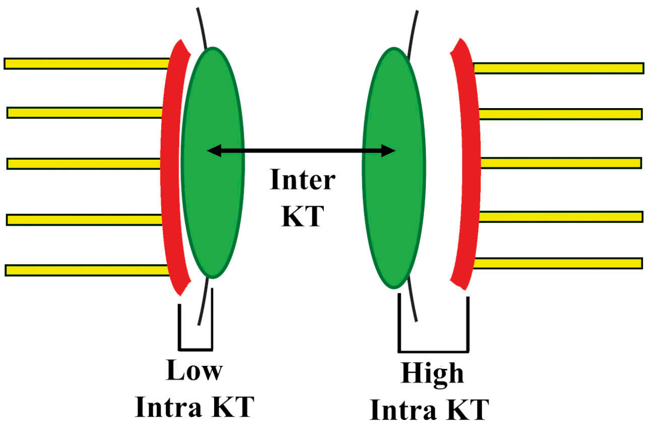

Figure 1. **Illustration of inter- and intrakinetochore stretch.** The locations of GFP–CENP-A near the surface of the centromeric heterochromatin and mCherry-Mis12 at the outer plate are indicated by the areas shaded in green and red. Kinetochore microtubules are shown in yellow. Interkinetochore stretch (Inter KT) is the distance between the centers of GFP–CENP-A location on sister kinetochores, whereas intrakinetochore stretch (Intra KT) is the distance between GFP–CENP-A and mCherry-Mis12. One possible source for intrakinetochore stretch is movement of the outer plate relative to the surface of the heterochromatin, as indicated. Microtubule-attached kinetochores in control cells undergo cycles of intrakinetochore stretch that are necessary to silence the SAC.

of taxol (20 nM) had minimal effect on intrakinetochore stretch, reduced interkinetochore stretch to near baseline levels, and satisfied the SAC. Treatment of S2 cells with higher levels of taxol (1.0 µM) reduced both intra- and interkinetochore stretch, and the SAC remained on despite relatively robust kinetochore microtubule attachments.

Collectively, the data from Uchida et al. (2009) and Maresca and Salmon (2009) demonstrate that intrakinetochore stretch is both necessary and sufficient for release of the SAC, whereas interkinetochore stretch has no effect. This implies that the SAC monitors structural rearrangements within the kinetochore rather than tension. Uchida et al. (2009) suggested that these rearrangements could be caused by tension, but this is unlikely because intrakinetochore stretch and SAC release were observed under conditions in which tension was too low to produce interkinetochore stretch and were not observed under other conditions in which tension was high enough to produce full interkinetochore stretch. By extension, it is likely that micromanipulation releases the SAC by stimulating intrakinetochore stretch rather than creating tension. This conclusion does not appear to apply to *Saccharomyces cerevisiae* because several genetic studies have implicated a direct role for tension in SAC release (for review see Pinsky and Biggins, 2005). Tension could have a greater role in budding yeast mitosis because attachment and dynamics might not be a sensitive enough indicator when kinetochores are bound to a single microtubule throughout the cell cycle.

Intrakinetochore stretch appears to detect structural rearrangements within the kinetochore such as those that occur upon microtubule attachment (Dong et al., 2007). However, Maresca and Salmon (2009) suggest that only 30–40% (10–15 nm) of intrakinetochore stretch comes from microtubule attachment, with the rest requiring microtubule dynamics and possibly other factors. These other factors could cause transient movements of the kinetochore outer plate relative to the underlying heterochromatin (i.e., outer kinetochore relative to the inner kinetochore) as illustrated in Fig. 1. Such a movement would be optimally situated to directly affect phosphorylation of BubR1, which is located between the heterochromatin and the outer plate (Campbell and Gorbsky, 1995; Wong and Fang, 2007). Further studies are required to determine the exact source of kinetochore rearrangements and how structural changes are translated into SAC release, but thanks to the work of Uchida et al. (2009) and Maresca and Salmon (2009), we know where to look for the answer.

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References

Cheeseman, I.M., J.S. Chappie, E.M. Wilson-Kubalek, and A. Desai. 2006. The conserved KMN network constitutes the core microtubule-binding site of the kinetochore. *Cell*. 127:983–997.

DeLuca, J.G., Y. Dong, P. Hergert, J. Strauss, J.M. Hickey, E.D. Salmon, and B.F. McEwen. 2005. Hec1 and Nuf2 are core components of the kinetochore outer plate essential for organizing microtubule attachment sites. *Mol. Biol. Cell*. 16:519–531.

Dong, Y., K. VandenBeldt, X. Meng, A. Khodjakov, and B.F. McEwen. 2007. The outer plate in vertebrate kinetochores is a flexible network with multiple microtubule interactions. *Nat. Cell Biol.* 9:516–522.

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

Maresca, T.J., and E.D. Salmon. 2009. Intrakinetochore stretch is associated with changes in kinetochore phosphorylation and spindle assembly checkpoint activity. *J. Cell Biol.* 184:373–381.

McEwen, B.F., A.B. Heagle, G.O. Cassels, K.F. Buttle, and C.L. Rieder. 1997. Kinetochore fiber maturation in PtK_1 cells and its implications for the mechanisms of chromosome congression and anaphase onset. *J. Cell Biol.* 137:1567–1580.

Musacchio, A., and E.D. Salmon. 2007. The spindle-assembly checkpoint in space and time. *Nat. Rev. Mol. Cell Biol.* 8:379–393.

Nicklas, R.B., and S.C. Ward. 1994. Elements of error correction in mitosis: microtubule capture, release, and tension. *J. Cell Biol.* 126:1241–1253.

Nicklas, R.B., S.C. Ward, and G.J. Gorbsky. 1995. Kinetochore chemistry is sensitive to tension and may link mitotic forces to a cell cycle checkpoint. *J. Cell Biol.* 130:929–939.

Pinsky, B.A., and S. Biggins. 2005. The spindle checkpoint: tension versus attachment. *Trends Cell Biol.* 15:486–493.

O'Connell, C.B., J. Loncarek, P. Hergert, A. Kourtidis, D.S. Conklin, and A. Khodjakov. 2008. The spindle assembly checkpoint is satisfied in the absence of interkinetochore tension during mitosis with unreplicated genomes. *J. Cell Biol.* 183:29–36.

Rieder, C.L., and E.D. Salmon. 1998. The vertebrate cell kinetochore and its roles during mitosis. *Trends Cell Biol.* 8:310–318.

Rieder, C.L., R.W. Cole, A. Khodjakov, and G. Sluder. 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.

Uchida, K.S.K., K. Takagaki, K. Kumada, Y. Hirayama, T. Noda, and T. Hirota. 2009. Kinetochore stretching inactivates the spindle assembly checkpoint. *J. Cell Biol.* 184:383–390.

Waters, J.C., R.H. Chen, A.W. Murry, and E.D. Salmon. 1998. Localization of Mad2 to kinetochore depends on microtubule attachment, not tension. *J. Cell Biol.* 141:1181–1191.

Wong, O.K., and G. Fang. 2007. Cdk1 phosphorylation of BubR1 controls spindle checkpoint arrest and Plk1-mediated formation of the 3F3/2 epitope. *J. Cell Biol.* 179:611–617.

Yuen, K.W., B. Montpetit, and P. Hieter. 2005. The kinetochore and cancer: what's the connection? *Curr. Opin. Cell Biol.* 17:576–582.