Balancing the kinetochore ledger

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Reduction of polo-like kinase-1 (Plk1) at kinetochores as cells progress from prometaphase to metaphase is surprising given that the kinase is thought to stabilize kinetochore–microtubule (kt–MT) attachments. In this issue, Liu et al. (2012. J. Cell Biol. doi:10.1083/jcb.201205090) demonstrate that kinetochore-associated Plk1 is a potent suppressor of microtubule plus-end dynamics. The authors propose that Plk1 activity facilitates the establishment of kt–MT attachments in prometaphase by stabilizing microtubules and that reduction of the kinase in metaphase promotes force generation by dynamic microtubules.

What do the kinetochore and Goldman Sachs have in common? This may sound like the beginning of a bad joke told over cocktails at an ASCB meeting, but the truth is, they have more in common than you might think. In fact, emerging research has revealed that the kinetochore carries out a balancing act rivaling that of a Wall Street banker dealing with a ledger full of complex financial derivatives.

The kinetochore ensures that the genome is accurately segregated during cell division by accomplishing two major tasks. First, it mediates the attachment of chromosomes to dynamic spindle microtubules. Second, when erroneous kt–MT attachments arise, the kinetochore fulfills its spindle assembly checkpoint (SAC) responsibilities by catalyzing production of a wait-anaphase signal that delays cell division to provide time to correct the errors. As it performs these functions, the kinetochore must manage a shifting balance of microtubule stabilizing and destabilizing activities as well as checkpoint regulators to establish and maintain kt–MT attachments and to coordinate SAC signaling.

Establishing and maintaining correct kt–MT attachments requires a precise balance between two antagonistic inputs: one favoring microtubule plus-end dynamics and the other favoring plus-end stability (reviewed by Bakhoum and Compton, 2012). Stabilizing inputs gain the upper hand over those favoring microtubule plus-end dynamics as chromosomes align during the progression from prometaphase to metaphase. Mounting evidence suggests that the transition in kt–MT attachment stability is mediated by changes in the molecular composition and phosphorylation profile of the kinetochore.

Plk1 has been implicated in both stabilizing and destabilizing kt–MT attachments. In support of a stabilizing role for Plk1, it has been shown that disrupting Plk1 activity by either RNAi or chemical inhibition prevents the formation of stable kt–MT attachments (Sumara et al., 2004; Hanisch et al., 2006; Peters et al., 2006; Lénárt et al., 2007) and that phosphorylation of the checkpoint kinase BubR1 by Plk1 is required for normal kt–MT attachment stability (Elowe et al., 2007). On the other hand, Plk1 activity is required for the recruitment and activation of two known destabilizing factors at prometaphase kinetochores: the microtubule depolymerizing motor Kif2b (Hood et al., 2012) and Aurora B kinase (Chu et al., 2011; Moutinho-Santos et al., 2012). Depletion of Plk1 in Drosophila melanogaster cells leads to hyper-stable syntelic attachments (Moutinho-Santos et al., 2012)—erroneous attachments in which both members of a kinetochore pair attach to microtubules from the same spindle pole—and this finding also cuts in favor of an attachment-destabilizing function for Plk1. Moreover, inhibiting Plk1 eliminates the attachment instability otherwise generated by depletion of the phosphatase B56-PP2A (Foley et al., 2011), suggesting, again, that Plk1 activity contributes to kt–MT attachment destabilization. In this issue, Liu et al. (2012) advance our understanding of the attachment-stabilizing role of Plk1 by providing convincing evidence that Plk1 activity at the kinetochore contributes to the establishment of kt–MT attachments by suppressing microtubule plus-end dynamics.

Liu et al. (2012) tethered a fluorescence resonance energy transfer (FRET)–based Plk1 phosphorylation sensor to HeLa cell kinetochores and found, consistent with previous results showing a decline in kinetochore-associated Plk1 from prometaphase to metaphase (Lénárt et al., 2007), that phosphorylation of the FRET probe was reduced as chromosomes aligned. The reduction in Plk1 levels and in FRET probe phosphorylation is a result, in part, of recruitment of protein phosphatase 1 (PP1) to metaphase kinetochores, where PP1 likely dephosphorylates (and thereby renders unavailable) potential binding sites for Plk1’s polo box domain (PBD). To better define the effects of Plk1 activity on kt–MT attachment stability, the authors fused a constitutively active form of Plk1 (T210D mutant) to the outer kinetochore protein Hec1 to maintain constitutively high kinase activity at prometaphase kinetochores. The authors propose that Plk1 activity facilitates the establishment of kt–MT attachments in prometaphase by stabilizing microtubules and that reduction of Plk1 in metaphase promotes force generation by dynamic microtubules. This may sound like the beginning of a bad joke told over cocktails at an ASCB meeting, but the truth is, they have more in common than you might think. In fact, emerging research has revealed that the kinetochore carries out a balancing act rivaling that of a Wall Street banker dealing with a ledger full of complex financial derivatives.

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These assays are the current gold standard in the field for investigating kt–MT attachment stability, and it is difficult to imagine that they would fail to detect a sevenfold increase in unattached kinetochores. Alternatively, constitutive Plk1 activity may drive recruitment of checkpoint proteins to aligned and stably attached kinetochores. Yet, Mad2 was not detected at every Hec1-Plk1T210D kinetochore, and Plk1 activity is not required for Mad2 localization (Sumara et al., 2004; Hanisch et al., 2006; Lénárt et al., 2007). Thus, we favor the view that in Hec1-Plk1T210D cells, the wait-anaphase signal is generated in response to suppression of kt–MT plus-end dynamics and the resulting reduction in intrakinetochore stretch rather than in response to unattached kinetochores. Indeed, a direct role for suppressed kt–MT dynamics and reduced intrakinetochore stretch in generating a wait-anaphase signal independent of defects in kt–MT attachment has previously been hypothesized (Maresca and Salmon, 2009, 2010).

It is becoming increasingly evident that balancing stabilizing and destabilizing activities at the kinetochore is a complex undertaking (Fig. 1), and the list of influences of both kinds is already a long one. Centromeric Aurora B kinase represents perhaps the most widely recognized example of an attachment destabilizer. The influence of this kinase predominates early in mitosis until tension across the centromeres/kinetochores of bioriented chromosomes moves Aurora B substrates beyond the effective range of the kinase’s activity, shifting the balance of inputs toward attachment stabilization (reviewed by Maresca and...
Salmon, 2010). More recently, it has been demonstrated that Aurora B inhibits the localization of the stabilizing complexes Ska (Chan et al., 2012) and Astrin–SKAP (Schmidt et al., 2010) to prometaphase kinetochores and that both complexes later confer stability by localizing to metaphase kinetochores. In addition, kinetochore-associated phosphatase activity contributes to the formation of stable kt–MT attachments, with B56-PP2A apparently playing a key role in prometaphase (Foley et al., 2011) and PP1 in metaphase (Liu et al., 2010). Looking beyond kinase and phosphatase activity, it has also recently been shown that the outer kinetochore protein CLASP1 participates in an attachment-regulating molecular switch system. It engages with two different binding partners in succession, first forming a stabilizing CLASP1–Kif2b complex in prometaphase and then a stabilizing CLASP1–Astrin complex in metaphase (Manning et al., 2010). The results reported by Liu et al. (2012) support the hypothesis that, during prometaphase, Plk1 contributes an essential stabilizing activity to counteract the destabilizing activities that would otherwise dominate. But because Plk1 has also been implicated in kt–MT destabilization, it appears that Plk1 may sit on both sides of the kinetochore ledger. Given the recent finding that Plk1 localizes both to centromeres and, separately, to kinetochores (Carmena et al., 2012), it is possible that distinct cellular populations of Plk1 differentially regulate kt–MT attachment stability. Additional work is clearly needed to address whether and how Plk1 might play seemingly contradictory roles at the kinetochore.

So, like any successful Wall Street financier, the kinetochore is a true polo enthusiast. And, although the kinetochore may have more in common with Goldman Sachs than we thought, it is far more useful to focus on the key difference: the kinetochore actually pulls off its complex balancing act. We would benefit from learning more about exactly how.

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References


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