A TOGgle for Tension at Kinetochores

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Differential stability of kinetochore-microtubule attachments at low versus high tension is critical for accurate chromosome segregation. Miller et al. find that a TOG domain microtubule-binding protein imparts intrinsic tension selectivity to kinetochore-microtubule attachments.

During cell division, the replicated genome is distributed to daughter cells by dynamic interactions between the kinetochore, a multiprotein complex assembled on the centromeric region of chromosomes, and the microtubules of the mitotic spindle. Kinetochores couple chromosomes to the plus ends of microtubules, whose guanosine triphosphate (GTP) hydrolysis-fueled dynamic instability provides the major motive force in chromosome segregation. To ensure accurate segregation, replicated chromosomes must biorient, a configuration where sister kinetochores attach to microtubules from opposite spindle poles. Biorientation is necessary for disjunction of sister chromatids and also generates tension, which is monitored by the cell to ensure that sisters are correctly attached prior to segregation.

Tension-sensitive regulation of kinetochore-microtubule attachments by Aurora family kinases and opposing phosphatases is the primary mechanism implicated in differential stabilization of correct versus incorrect attachments (Lampson and Cheeseman, 2011). However, in vitro experiments by Biggins, Asbury, and colleagues using purified kinetochore particles from budding yeast (isolated by immunoprecipitation of a structural inner kinetochore protein) showed that tension-dependent stabilization is also an intrinsic property of kinetochore-microtubule attachments (Akiyoshi et al., 2010). In essence, purified kinetochore particles hold on longer to dynamic microtubules when under moderate tension, detaching more frequently under low tension. In this issue, Miller, Asbury, and Biggins (Miller et al. 2016) employ the same in vitro biophysical assays to show that Stu2, a TOG (tumor overexpressed gene) domain microtubule-binding protein of the XMAP215 family, strengthens kinetochore particle-microtubule attachments under tension. More surprisingly, they find that Stu2 promotes detachment under low tension, providing unexpected insight into how kinetochores intrinsically exhibit differential attachment stability at low versus high tension.

Miller et al. (2016) begin their analysis by determining how Stu2 acts in conjunction with the 4-subunit Ndc80 complex, the conserved mediator of kinetochore-microtubule attachments. In vitro, the Ndc80 complex alone exhibits direct microtubule-binding activity and species-dependent intrinsic properties under force. Multiple cooperators interact with the Ndc80 complex to strengthen microtubule attachments and modulate the dynamics of kinetochore-attached microtubule ends. In 2011, Toda and colleagues showed that Dis1, the fission yeast ortholog of Stu2, interacts with the Ndc80 complex and that mutations engineered in Ndc80 to disrupt this interaction resulted in chromosome missegregation (Hsu and Toda, 2011). In budding yeast, detailed analysis of kinetochore behavior has implicated Stu2 in promoting rescue of depolymerizing microtubule ends to ensure persistent kinetochore-microtubule attachments, and high-resolution imaging has placed Stu2 in close proximity and in near-stoichiometric levels with the Ndc80 complex (Aravamudhan et al., 2014; Gandhi et al., 2011). These prior studies suggested a potential direct role for XMAP215 family proteins at the kinetochore-microtubule interface, but did not define their mechanistic contribution. Miller et al. (2016) address this gap in understanding by directly characterizing the role of Stu2 through employing in vitro biophysical assays with purified kinetochore particles from budding yeast.

Miller et al. (2016) first show that the presence of Stu2 in kinetochore particles depends on Ndc80 and that purified Stu2 directly associates with the Ndc80 complex. Next, they link kinetochore particles with and without Stu2 to beads, bind the bead-bound particles to assembling microtubule ends polymerized from a seed anchored to a coverslip surface, and use an optical trap to place the kinetochore particle-microtubule end attachments under tension. By increasing the force applied to the beads, they measure the force at which the bead-bound kinetochore particles detached from the assembling microtubule end. Kinetochore particles without Stu2 detached from microtubule ends on average at ~4 pN applied force, significantly lower than the ~10 pN observed with control particles: this reduction was fully complemented by addition of purified Stu2. In addition, purified Stu2 strengthened attachments made by purified bead-linked Ndc80 complexes. A more modest strengthening was observed with the human XMAP215 family member Ch-TOG and the human Ndc80 complex, potentially because the human Ndc80 complex exhibits significantly stronger attachments on its own relative to the yeast Ndc80 complex. Thus, XMAP215 family proteins strengthen kinetochore particle and Ndc80 complex attachments to assembling microtubule ends.

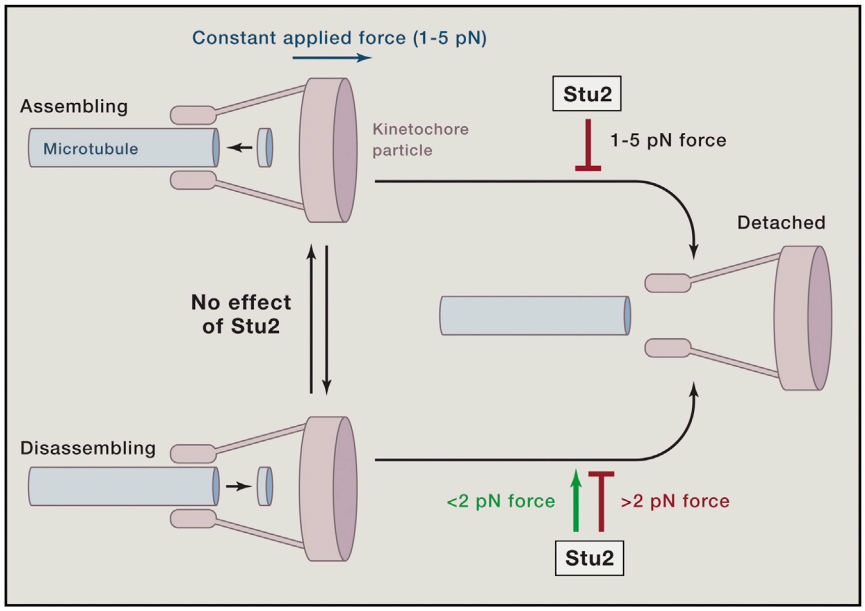

Figure 1. The TOG Domain Microtubule-Binding Protein Stu2 Controls the Persistence of Dynamic Kinetochore-Microtubule Attachments in a Tension-Sensitive Manner

Stu2 is a tension-sensitive modulator of the lifetime of attachments between kinetochore particles and microtubule ends, but does not control the dynamics of the attached microtubule end. Stu2 inhibits detachment at high tension at both assembling and disassembling ends (red inhibitory symbols) but promotes detachment from disassembling ends at low tension (green arrow).

The big surprise in the study came when Miller et al. (2016) analyzed the mechanism by which Stu2 enhanced kinetochore particle-microtubule end attachments. Since XMAP215 family proteins function as microtubule polymerases, the most straightforward hypothesis was that kinetochore-localized Stu2 modulated microtubule-plus-end dynamics in response to tension. To test this hypothesis, the authors turned to an optical trap-based "force-clamp" assay, in which a constant force is applied in the direction of microtubule growth throughout the lifetime of the attachment (Figure 1): this constant force was varied in the 1–5 pN range in different experiments. This approach enables measurement of the parameters describing dynamic instability of kinetochore-attached microtubule ends as well as the lifetime of the kinetochore particle-microtubule end attachment at a fixed applied force. Contrary to the expected result, Stu2 did not affect any of the microtubule dynamics parameters across the range of tested forces. Instead, the presence of Stu2 significantly altered kinetochore particle detachment rate from microtubule ends (Figure 1). At assembling microtubule ends bound to kinetochore particles, Stu2 reduced the detachment rate as expected from the prior results (Figure 1). However, at disassembling microtubule ends bound to kinetochore particles, something remarkable happened: under low tension (below ~2 pN applied force), Stu2 *increased* the detachment rate, indicating that Stu2 *destabilizes* attachments between kinetochore particles and disassembling microtubule ends. This Stu2-dependent destabilization was suppressed at higher tension (Figure 1). Thus, Stu2 acts as a tension-sensitive toggle, differentially controlling the lifetime of dynamic kinetochore particle-microtubule end linkages at low versus high tension (Figure 1). A simple two-state kinetic model developed previously by the authors confirmed that these Stu2-dependent changes in detachment rate were sufficient to explain the differential tension-dependent behavior of kinetochore particle-microtubule attachments.

The surprising results from Miller et al. (2016) place Stu2 as being central to the tension-selective behavior of kinetochore particle-microtubule end attachments in vitro and raise two important questions—first, what is the mechanism by which Stu2 promotes detachments at disassembling ends under low tension while strengthening attachments at both assembling and disassembling ends at high tension, and second, what is the significance of this intrinsic tension-selective behavior to control of kinetochore-microtubule attachments in vivo. With respect to the first question, the significant effort placed on understanding the mechanism by which Stu2 and other XMAP215 microtubule polymerases work (e.g., Ayaz et al., 2014), in conjunction with the ability to complement Stu2-deficient kinetochore particles with purified Stu2, should enable detailed analysis of Stu2 in this context. As the attachments observed with the kinetochore particles in vitro are significantly dependent on the Dam1 complex (Akiyoshi et al., 2010)—a ten-subunit complex also recruited by the Ndc80 complex that oligomerizes on the microtubule lattice and is essential for stabilizing bi-oriented attachments in vivo—Stu2 may act by influencing the Ndc80-Dam1 complex interplay. This possibility should be addressable employing available purified components in vitro. Perhaps more significant is determining the extent to which intrinsic tension-selective behavior contributes to accuracy of chromosome segregation in vivo. A starting point will be to generate and analyze alleles that selectively affect the Ndc80-complex-Stu2 interaction. In vivo, the absence of Ipl1 (budding yeast Aurora B) kinase activity is known to stabilize incorrect attachments lacking tension, resulting in extensive missegregation (Biggins et al., 1999; Tanaka et al., 2002). Thus, it will also be important to integrate the fascinating Stu2-dependent intrinsic tension-selective behavior described by Miller et al., (2016) into the context of phosphoregulation of kinetochore-microtubule attachment stability.

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