Choreography of the Chromosome Dance during Mitosis

June 17, 2013

GMR Deyter

Chromosome segregation requires the physical attachment of chromosomes to microtubules of the mitotic spindle. Kinetochores are large protein complexes that interact with chromosomes and microtubules to facilitate chromosome movement. Kinetochore-microtubule (K-Mt) attachments must achieve a specific geometry for chromosome segregation to proceed flawlessly. Otherwise, chromosomes can become fragmented during segregation, and it is believed that this type of chromosome instability can fuel tumorigenesis. One mechanism by which cells achieve proper K-Mt attachments is by destroying improper attachments via phosphorylation of kinetochore proteins that bind microtubules. Aurora B kinase has a pivotal role in detaching kinetochores from microtubules, and many of its substrates have been identified and studied using reconstituted subcomplexes. However, the effect of Aurora B phosphorylation on whole, intact kinetochores had not been elucidated previously. Recently, the laboratory of Dr. Sue Biggins (Basic Sciences Division, Fred Hutchinson Cancer Research Center), in collaboration with the laboratory of Dr. Chip Asbury (Physiology and Biophysics Department, University of Washington), used purified yeast kinetochores and single molecule studies to ascertain how phosphorylation affects microtubule attachment to kinetochores. Dr. Krishna Sarangapani of the Asbury Lab is the lead author on the study.

The authors isolated a variety of kinetochore mutants to determine the affect of phosphorylation on K-Mt interactions. The kinetochores were incubated with dynamically growing microtubules attached to a coverslip. A tether on the kinetochores allowed Sarangapani *et al.* to apply force in the direction of the growing microtubules (see figure). In this way, the strength of the K-Mt attachment of the kinetochore mutants could be measured, since the amount of force that can be applied to the kinetochore corresponds to the affinity of the K-Mt attachments.

The researchers focused on two kinetochore proteins important for direct binding to microtubules: Ndc80 and Dam1. Aurora B phosphorylation sites had been identified on both proteins, and Sarangapani *et al.* isolated kinetochores that contained the phospho-mimetic Ndc80-7D and Dam1-4D proteins (with 7 and 4 Aurora B sites mutated to aspartic acid to mimic the negative charge of phosphate addition). They found that wild-type, non-phosphorylated kinetochores ruptured (i.e. fell

off of the microtubule due to the force applied) at ~9 pN while the Ndc80-7D and Dam1-4D kinetochores disconnected from microtubules at ~5.7 pN. Furthermore, kinetochores containing both Ndc80-7D and Dam1-4D mutants ruptured at ~3 pN. These data revealed that phosphorylation of Ndc80 and Dam1 inhibits the ability of kinetochores to bind microtubules in an additive manner.

In a second line of investigation, Sarangapani *et al.* analyzed the ability of the kinetochores to maintain their microtubule attachments under a constant amount of force (~2.5 pN). In this scenario, K-Mt detachment would be the result of changes in microtubule growth and shortening caused by the phospho-mimetic kinetochores. As expected, the average lifetimes for tip attachments of the phospho-mimetic kinetochores were significantly shorter than control kinetochores given the known role of phosphorylation in promoting K-Mt detachment *in vivo*. Unexpectedly, however, changes in microtubule dynamics were also evident in experiments with the Dam1-4D kinetochores. These mutant kinetochores exhibited a three-fold increase in rate of microtubule disassembly, indicating that Aurora B phosphorylation can also promote K-MT detachment indirectly by destabilizing the attached microtubule.

In summary, the work by Sarangapani *et al.* is one of the first *in vitro* single molecule studies exploring phosphorylation-dependent changes in kinetochore behavior using purified kinetochores and dynamically growing microtubules, with the inclusion of tension parameters. These components form an *in vitro* experimental system that comes close to reconstituting the way in which kinetochores bind to the mitotic spindle in a living cell. Their experimental model allowed the authors to decipher the phospho-regulation of kinetochores by Aurora B, revealing independent contributions of this kinase to multiple aspects of K-Mt attachments. Future experiments using this powerful experimental system will likely uncover how phosphorylation alters the binding properties of other kinetochore components.

Sarangapani KK, Akiyoshi B, Duggan NM, Biggins S, Asbury CL. 2013. Phosphoregulation promotes release of kinetochores from dynamic microtubules via multiple mechanisms. *PNAS* 110(18):7282-7.

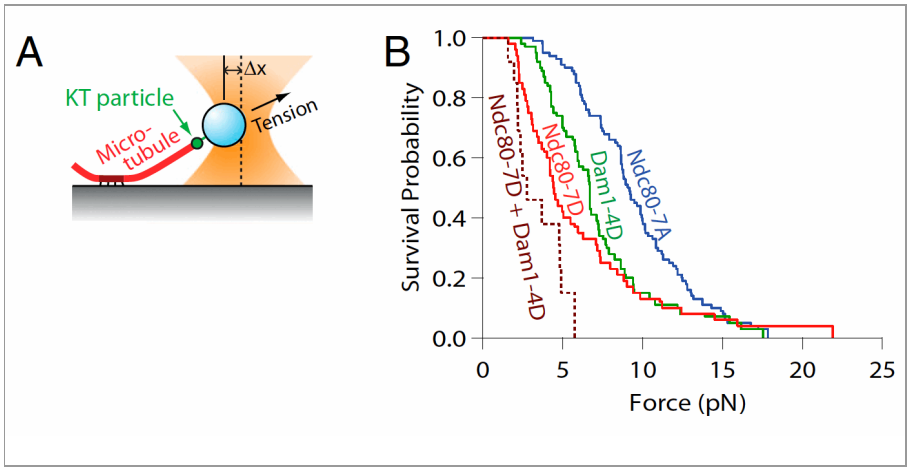

Image modified from the original manuscript

Mimicking phosphorylation on kinetochores reveals deficiencies in obtaining high-affinity microtubule interactions. (A) Schematic of K-Mt manipulations. A kinetochore (green) is bound to a bead (blue) that can be pulled in the direction of microtubule growth (Tension arrow). Kinetochores can only withstand a certain amount of force and will fall off of the microtubule under high tension. (B) Graph depicting the probability that the denoted kinetochores will remain bound to a microtubule tip upon increasing force applied to the kinetochore away from the microtubule. Kinetochores that have phospho-mutant Ndc80-7A, a key kinetochore microtubule-coupling protein, are able to withstand forces up to 15 pN (blue). Phospho-mimetic Dam1-4D (green) and Ndc80-7D (red) kinetochores can maintain their interactions with microtubules at intermediate applied forces. The further diminishment of force that the double phospho-mimetic kinetochores can withstand (brown) indicates that the Ndc80 and Dam1 complexes make synergistic contributions to microtubule attachments that can be individually tuned by phosphorylation.