JCB Journal of Cell Biology

SPOTLIGHT

Kinetochores get a grip!

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A new study by Larson and colleagues (2025. *J. Cell Biol.* https://doi.org/10.1083/jcb.202405176) reveals that kinetochores are biased to bind to microtubule plus-ends due to an interplay between subcomplex organization and the intrinsic polarity of microtubules.

Kinetochores are remarkable microtubule-binding complexes that control virtually all aspects of the chromosome segregation process in eukaryotic cells. They link chromosomes to the ends of dynamic microtubule filaments, withstanding the pulling forces of the mitotic spindle in metaphase, prior to the segregation of sister chromatids in anaphase. Kinetochores also ensure that attachment errors are corrected such that sister chromatids are bound to microtubules from opposite spindle poles, a configuration termed biorientation. While individual kinetochore subcomplexes have been studied in some detail, an important and exciting step forward is to investigate fully assembled kinetochores and explore their properties as molecular machines for chromosome segregation. The way in which individual subcomplexes are arranged in kinetochores—in multiple copies, with different conformations, in changing subunit compositions—suggests that the features of kinetochores must arise from more than just the sum of the properties of individual proteins or complexes. Classic studies have explored these properties, purifying kinetochores from cells and asking, for example, how they bind, nucleate, or translocate microtubules (1, 2, 3).

Following in the footsteps of these studies, Larson and colleagues established their own approaches to investigate the properties of kinetochores (4). They used a combination of total internal reflection fluorescence microscopy (TIRF) with single-molecule localization and laser trap assays to analyze the properties of budding yeast kinetochores. The reconstituted kinetochores are assembled in cell extracts on a short piece of centromeric DNA tethered to a glass surface (TIRF) (5) or purified and immobilized on polystyrene beads that can be manipulated in an optical trap. Exploring the properties of budding yeast kinetochores in this way is especially meaningful since the single microtubule capture in cells can be recreated convincingly in vitro and avoids the complexity of fibrous bundle attachments, which are prevalent in higher eukaryotes.

Larson and colleagues find that the immobilized yeast kinetochores are predisposed to bind microtubules at their plus-ends (4). This is remarkable as taxol-stabilized microtubules lack the biochemical marks of dynamic microtubule plus-ends, such as the GTP cap. This finding implies that the inherent polarity of the microtubule surface, composed of alternating copies of two different proteins, alpha- and beta-tubulin, and the cognate microtubule binding elements of the kinetochore must play a key role in selective plus-end binding. Consistent with this idea, the authors find in laser trap experiments that the attachment of kinetochores to microtubule plus-ends is much stronger (threefold increased rupture force) than that to microtubule minus ends. Interestingly, also the lateral attachments of kinetochores to microtubules have a pronounced directional bias. Dragging kinetochore-decorated beads toward the microtubule plus-end requires significantly more force than pulling them toward the microtubule minus-end. Thus, not only the end-on attached configuration but also lateral attachments are highly direction-sensitive. Direct comparisons between the TIRF and laser trap observations are hindered by the use of distinct approaches to reconstitute kinetochores in each setup, likely leading to differences in kinetochore compositions or subunit stoichiometry. A particular limitation of the centromeric DNA-based assembly in extracts is that the outer kinetochore under these conditions seems to have lesser microtubule binding elements than native kinetochores (6). A direction for future experiments is therefore to try and match the physiological outer kinetochore composition even more closely and allow better cross-assay comparison.

What may be the kinetochore "reader" of the structural polarity of the microtubule surface? The prime candidate for this activity is the Ndc80 complex, the conserved microtubule receptor of kinetochores. Ndc80 binds the microtubule surface at a "toeprint" region between the alpha- and beta-tubulin subunits (7). Electron microscopy has revealed that the Ndc80 decoration has a distinct orientation, with the Ndc80–Nuf2 coiled-coil stalks projecting uniformly at a defined angle toward the microtubule plus-end, suggesting a highly stereospecific binding (8, 9). One may think of this as a microtubule version of the classical pointed and barbed end decoration of actin filaments by myosin. Indeed, the authors find that much of the kinetochore binding asymmetry is recapitulated when using just purified recombinant Ndc80 complex in the laser trap assay (Fig. 1 A). Nevertheless, it will be

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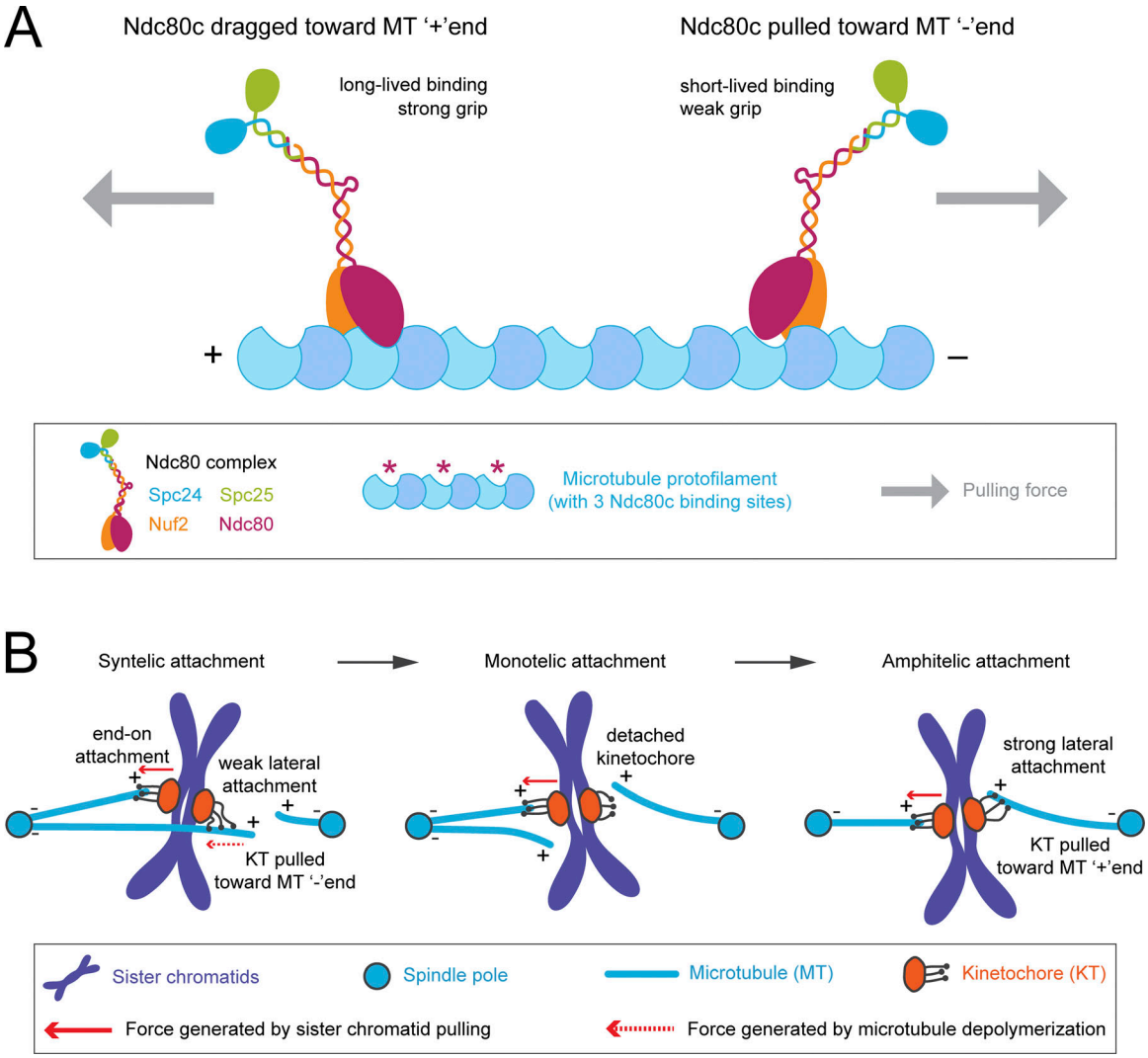

Figure 1. **Kinetochores grip more tightly to microtubule plus-ends than to minus-ends. (A)** The Ndc80 complex binds at the surface of microtubules with asymmetric strength: strongly when pulled toward the microtubule plus-end or weakly when pulled toward the microtubule minus-end. **(B)** Kinetochore–microtubule gripping strength may contribute to the correction of kinetochore attachment errors early in mitosis because it could selectively stabilize those lateral attachments that are about to be converted into correct end-on attachments.

important to define in future experiments, if additional elements of the kinetochore or a special arrangement of Ndc80 complexes may contribute to the observed phenomenon.

While laser trap assays allow the application of defined forces on kinetochore complexes, this is typically not the case in the TIRF experiments. To circumvent this limitation, the authors devised an elegant approach to apply forces to microtubule-bound kinetochores. They reversed the buffer flow in the assay chamber and forced the kinetochore to reorient with respect to the microtubule. Consistent with structural rearrangements that occur under these moderate forces, fluorescent marks on the inner and outer kinetochore separate in distance from one another. Separation of inner and outer kinetochore marks is also detectable on laterally attached kinetochores and is more pronounced when kinetochores laterally move toward the microtubule plus-end than toward the minus end.

Overall, the experiments in the study illuminate how sensitive the attachment properties of kinetochores are with respect to the polarity of the microtubule. The important biological implication is that not only mature end-on attachments are stabilized, but also lateral attachments that are dragged toward the plus end are more stable. As these attachments are "on their way" to form a correct end-on attachment, there is a biological advantage to prefer them over lateral attachments that are dragged toward the minus end (Fig. 1 B). The longer lifetime of this attachment makes it more likely to reach a microtubule plus-end or to hold on to the microtubule surface until a catastrophe event of the same filament would convert it into a correct end-on attachment. Because lateral attachments in cells are typically mediated by molecular motor proteins, the contribution of this mechanism to the physiological process of biorientation is not straightforward to evaluate. The findings in this study might help to explain recent intriguing observations that microbeads coated with only Ndc80/Nuf2 can achieve a biorientation-like state in mouse oocyte spindles (10).

The intrinsic attachment bias revealed in the elegant experiments of Larson and colleagues could therefore be one of the many puzzle pieces that together ensure the exceptional accuracy of chromosome segregation in eukaryotic cells.

Acknowledgments

The authors acknowledge support from the German Research Foundation (SFB1430 Molecular Mechanisms of Cell State Transitions, project ID 424228829).

Author contributions: C. Cozma: Visualization, Writing - review & editing, S. Westermann: Writing - original draft, Writing - review & editing.

Disclosures: The authors declare no competing interests exist.

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