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Kinetochores feel the pull

Researchers detail how kinetochores respond to force from spindle.

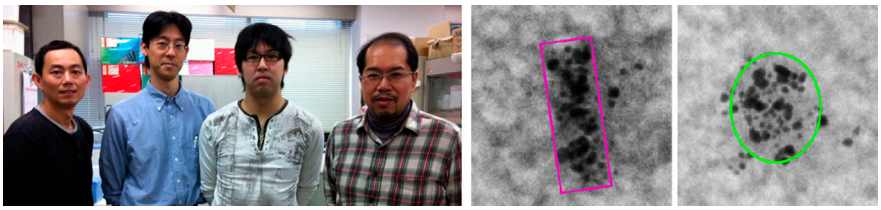
Suzuki et al. (1) take a close look at how kinetochores change shape when spindle microtubules tug on them. They find that only part of the kinetochore stretches and identify a protein that might confer this ability.

Kinetochores, which hook the fibers of the mitotic spindle to chromosomes, are more than passive fasteners. Fashioned from more than 100 proteins, the structures are dynamic and complex (2), and researchers are just starting to discover how they work. The mitotic spindle has to pull hard to separate the chromosomes (3), and light microscopy studies suggest that kinetochores stretch under the strain (4). These distortions allow the cell to advance to anaphase by releasing the spindle assembly checkpoint (5), which otherwise would halt the cell cycle until the spindle is correctly attached to the chromosomes. However, researchers haven't discerned what happens to individual kinetochore molecules.

Using immunoelectron microscopy (EM) and a panel of antibodies to tag eight kinetochore proteins, Suzuki et al. were able to observe the changes in kinetochore structure induced by tension from the spindle. To obtain baseline measurements of kinetochore and protein position, the team scrutinized cells exposed to nocodazole, which breaks down the spindle microtubules and releases the pull on the kinetochores. Suzuki et al. then observed cells dosed with a different compound, MG132, that stalls cells in metaphase, with the kinetochores under tension.

The inner and outer kinetochore plates respond differently to force, the researchers found. The outer plate, to which the mitotic spindle fibers attach, shortens a little but basically maintains its rectangular shape. But the inner plate, which contacts the DNA, becomes shorter and wider, fattening from a rectangle into an oval.

Focal Point



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(Left to right) Tatsuya Nishino, Tetsuya Hori, Aussie Suzuki, Tatsuo Fukagawa, and colleagues (not shown) observed the inner and outer segments of kinetochores by immunoelectron microscopy. When the kinetochores aren't under tension, the inner kinetochore is rectangular (left). On the other hand, when spindle microtubules exert force on the kinetochore, the inner kinetochore widens and rounds off, forming an oval (right).

To confirm that this change resulted from the force on the kinetochores, the researchers turned to cells that manufacture a mutant form of the protein Ndc80, a component of the outer kinetochore plate. This Ndc80 variant weakens the connection to microtubules without modifying the structure of the outer plate. In cells with the faulty version, the inner plate no longer stretched, Suzuki et al. showed.

The next question was what gives the inner plate its flexibility. The researchers used two programs that predict protein structure to search for inner kinetochore proteins that were limber and elongated. CENP-T fit the bill. Again using immuno-EM, the team found that when the spindle is engaged, the N terminus of CENP-T remains in position near the outer kinetochore, but the C terminus shifts in the opposite direction. This result suggests that CENP-T elongates under tension. Another inner kinetochore protein, CENP-N, didn't extend when pulled. The researchers also observed CENP-T's elongation under the light microscope. The two ends of the protein, which the team tagged with green and red fluorescent proteins, were close together in nocodazole-treated cells, but tension caused the ends to move apart.

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Suzuki et al. conclude that a kinetochore morphs under tension because the inner plate widens by about 25 nm while the outer plate retains its shape. Although they don't rule out a contribution from other proteins, the researchers think that CENP-T is the main source of the inner kinetochore's stretchiness. When the spindle isn't engaged, the researchers suggest, CENP-T resembles a yo-yo with a limp string. When the spindle starts tugging, the string extends, broadening the inner kinetochore plate. "The force is very strong, and we did not know what was happening within the kinetochore," says senior author Tatsuo Fukagawa. But now "we see that kinetochore deformation is regulating mitotic progression."

Researchers still need to work out how the shape change by the inner kinetochore shuts down the spindle assembly checkpoint and allows the cell to move into anaphase. Another unknown is how the many kinetochore proteins combine to ensure that the inner and outer plates have different properties.

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