Mapping the kinetochore Delta
Researchers question the role of intrakinetochore tension in mitotic progression.

Kinetochore attach chromosomes to the mitotic spindle and, if a kinetochore fails to attach to spindle microtubules, it can delay mitotic exit via the spindle assembly checkpoint. Some evidence suggests that kinetochores not only monitor whether or not they are bound to microtubules, but also whether these microtubules are pulling on them with an appropriate amount of force. When cells are treated with the microtubule-stabilizing drug taxol, for example, kinetochores are thought to activate the spindle checkpoint because these pulling forces are reduced. Now, however, Magidson et al. cast doubt on a technique used to measure tension levels inside the kinetochore and demonstrate that taxol-treated cells only arrest if some of their kinetochores completely detach from the mitotic spindle (1).

Researchers have used a technique called single-molecule high-resolution colocalization (SHREC) to map the position of kinetochore proteins and how their arrangement is altered upon microtubule attachment (2). As its name suggests, SHREC was originally developed to calculate the distance between individual molecules by measuring the separation of fluorescent spots under a light microscope (3). However, because kinetochores are classically described as thin, trilaminar structures consisting of a central region sandwiched between inner, centromere-associated, and outer, microtubule-binding, plates (4), researchers thought that SHREC could accurately determine the distance, or Delta, between kinetochore proteins in situ. Accordingly, SHREC suggested that spindle microtubules pull outer kinetochore proteins away from the inner kinetochore, increasing Delta values, and that this “intrakinetochore tension” is reduced, leading to lower Delta values, in the presence of taxol (2).

However, when Alexey Khodjakov, Valentin Magidson, and colleagues at the Wadsworth Center in Albany, New York, examined kinetochore architecture in more detail, they realized that kinetochores are not just

simple plates; they are much more complex structures that undergo various changes in response to microtubule attachment or taxol treatment (1). In metaphase cells, for example, microtubule attachment distort the outer kinetochore so that it forms a protrusion visible by electron tomography. Correlative light and electron microscopy, meanwhile, revealed that taxol treatment causes the outer kinetochore to expand and tilt with respect to the inner kinetochore. All of these changes would influence the Delta values obtained by SHREC.

“The model of kinetochores as thin, straight plates doesn’t hold,” Khodjakov explains. “Delta is therefore a multiparametric function that is affected not only by the distance between molecules, but also by changes in the structure’s shape and orientation. That makes interpreting Delta values very difficult.” The decreased Delta values observed upon taxol treatment, for example, might suggest that kinetochores compact in response to this drug, decreasing the distance between inner and outer kinetochore proteins. In reality, however, kinetochores grow thicker in the presence of taxol, and, using correlative light/electron microscopy and structured illumination microscopy, Khodjakov and colleagues were able to visualize this structural reorganization.

It therefore remains unclear how taxol alters intrakinetochore tension levels.

But Magidson et al. found that tension changes aren’t required to explain why taxol induces mitotic arrest. When the researchers added taxol during metaphase, most cells exited mitosis with minimal delay. Some cells arrested, however, and, in each of these cells, Magidson et al. saw that a small number of kinetochores had detached from the mitotic spindle and recruited spindle checkpoint proteins. “Unattached kinetochores are proven to impose mitotic arrest,” says Khodjakov. “So you can’t make any conclusions about signals from other kinetochores that remain attached but are under lower tension.”

Khodjakov says that future studies on the role of intrakinetochore tension should avoid using SHREC as a simple measure of intermolecular distances. As a final note of caution, Magidson et al. also found that Delta values are affected by fixation conditions, which are an increasingly important concern now that superresolution techniques are enabling light microscopists to break the diffraction barrier. “If you want to measure something with nanometer precision, you need to look at the native structure,” Khodjakov says. “Fixation protocols traditionally used in light microscopy aren’t designed to deal with this.”

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2. Wan, X., et al. 2009. Cell. 137:672–684.
3. Churchman, L.S., and J.A. Spudich. 2012. Cold Spring Harb Protoc. 2012:242–245.
4. Brinkley, B.R., and E. Stubblefield. 1966. Chromosoma. 19:28–43.