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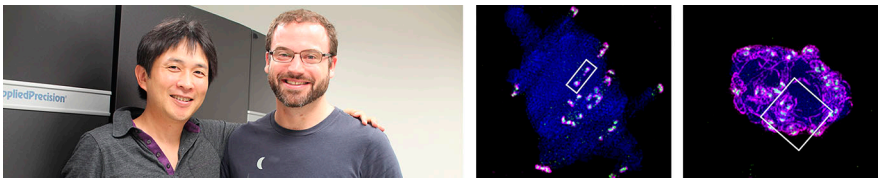
Kinetochores expand their reach

Kinetochore proteins form extended filaments that could promote checkpoint activation and microtubule capture.

Focal Point



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Hironori Funabiki (left) and David Wynne (right) use super-resolution microscopy to examine the structure of kinetochores in *Xenopus* egg extracts and find that, in the absence of microtubule attachments, a subset of kinetochore proteins form expanded structures that may help activate the spindle checkpoint and capture microtubules. In control extracts (left), the checkpoint protein BubR1 (magenta) localizes near to the centromeric marker CENP-A (green) on chromosomes (blue). But when microtubules are depolymerized (right), BubR1 forms extended filaments that also contain other checkpoint proteins as well as the inner kinetochore protein CENP-C and outer kinetochore proteins involved in lateral microtubule attachment.

Kinetochores attach chromosomes to the mitotic spindle. After binding initially to the sides of spindle microtubules, they form stable attachments to microtubule plus ends so that chromosomes can be segregated to the spindle poles during anaphase. In addition, kinetochores recruit the spindle assembly checkpoint proteins that prevent cells from entering anaphase if their chromosomes aren't attached to the spindle correctly. Although the function of individual kinetochore proteins is fairly well understood, how their assembly and disassembly is orchestrated to coordinate the kinetochore's different functions remains unclear. Wynne and Funabiki use super-resolution microscopy to reveal that, in the absence of microtubule attachment, a subset of kinetochore proteins form extended filaments that could help activate the spindle checkpoint and capture microtubules (1).

Kinetochores are thought to assemble in a hierarchical order (2). Inner kinetochore proteins, such as CENP-C and CENP-T, first bind to centromeric chromatin, which is marked by the variant histone CENP-A. These proteins then recruit outer kinetochore proteins involved in microtubule attachment and spindle checkpoint signaling. The inner and outer layers of kinetochores can be distinguished by electron microscopy, but the organization of individual proteins within these layers remains uncertain. David Wynne and Hironori Funabiki, from The Rockefeller University in New York, decided to examine kinetochores using 3D structured-illumination microscopy. "Not many studies have looked at kinetochores using super-resolution microscopy, so we wanted to see what kinds of structures we could visualize," Funabiki explains.

The researchers looked at the assembly of kinetochores on sperm chromosomes added to *Xenopus* egg extracts. "The surprising thing was that several outer kinetochore proteins showed a massive expansion when the extracts were treated with nocodazole to depolymerize microtubules," Funabiki says. These proteins—including the checkpoint proteins Bub1, BubR1, and Mad1, as well as proteins, such as CENP-E and dynein, involved in lateral microtubule attachment—formed long, thin filaments that extended more than a micron away from centromeric chromatin marked by CENP-A. Wynne and Funabiki saw a similar, transient expansion of these proteins in unperturbed egg extracts at the beginning of mitosis.

> "Several outer kinetochore proteins showed a massive expansion when the extracts were treated with nocodazole."

Outer kinetochore proteins have previously been seen to expand their distribution following microtubule depolymerization (3), but not to the extent observed in *Xenopus* egg extracts. Moreover, Wynne and Funabiki saw that the inner kinetochore protein CENP-C also localized to the expanded kinetochore filaments. In contrast, the inner kinetochore protein CENP-T and outer kinetochore proteins involved in end-on microtubule attachment, such as the Ndc80 complex, remained in a tightly focused spot in the absence of microtubules.

Thus, kinetochores appear to be composed of a "core module" involved in stable, end-on microtubule attachments, and an "expandable module" containing proteins involved in checkpoint signaling and initial, lateral microtubule interactions. "We propose that these proteins build a structure that can expand to generate a checkpoint signal and promote lateral microtubule attachment," Funabiki says. In support of this, Wynne and Funabiki found that partially depleting CENP-C reduced both the extent of kinetochore expansion and the ability of the spindle checkpoint to delay mitotic progression.

The expandable kinetochore doesn't seem to assemble through a simple, hierarchical process, however. CENP-C and the outer kinetochore components all depended on each other for their assembly into extended filaments, possibly, Funabiki says, because these structures are formed by the proteins' copolymerization. Wynne and Funabiki found that the assembly process was promoted by the mitotic kinases Aurora B, Plx1, and Mps1, whereas filament disassembly in the presence of microtubules depended on the phosphatase PP1. "We propose that disassembly of the expanded kinetochore promotes the handover of kinetochore function from lateral microtubule attachment and checkpoint activation to end-on attachment and checkpoint silencing," Funabiki says.

Now the researchers want to determine the extent to which kinetochores expand in somatic cells. One possibility is that kinetochore filaments are particularly prominent in egg extracts because they facilitate the rapid cell cycles that occur during early embryogenesis.

1. Wynne, D.J., and H. Funabiki. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201506020
2. Takeuchi, K., and T. Fukagawa. 2012. *Exp. Cell Res.* 318:1367–1374.
3. Hoffman, D.B., et al. 2001. *Mol. Biol. Cell.* 12:1995–2009.