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Defining the kinetochore's rules of engagement

Quantitative analyses and computer modeling reveal how a "molecular lawn" fine-tunes the interactions between kinetochores and microtubules.

Focal Point

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(Left to right) Anatoly Zaytsev, Lynsie Sundin, Keith DeLuca, Ekaterina Grishchuk, and Jennifer DeLuca provide a new model of the kinetochore–MT interface by revising the rules of engagement for MT-binding kinetochore proteins such as Ndc80. By analyzing and modeling the effects of phosphorylating the Ndc80 subunit Hec1, the researchers reveal that vertebrate kinetochores can't be organized as a series of repetitive sites (second from right), each containing multiple Ndc80 molecules (red) dedicated to binding a single MT (green). Instead, the kinetochore functions as a molecular lawn (far right), in which Ndc80 molecules can dynamically switch between multiple MTs in their vicinity. This arrangement allows cells to fine-tune kinetochore–MT attachments during mitosis.

During mitosis, kinetochores connect chromosomes to microtubules (MTs), helping sister chromatids to align on the metaphase spindle and segregate to opposite poles in anaphase. Yeast kinetochores contain a number of MT-associated proteins (MAPs) that together attach to a single MT. Vertebrate kinetochores carry a similar complement of MAPs, but each kinetochore attaches to 20–30 MTs, perhaps by forming a repetitive series of yeast-like attachment sites, with each site operating independently to bind a single MT. Zaytsev et al. reveal that the vertebrate kinetochore–MT interface is better described as a "molecular lawn," in which individual MAPs can dynamically switch between multiple MTs (1).

The Ndc80 complex is an essential MAP at both yeast and vertebrate kinetochores. The Aurora B kinase regulates Ndc80's association with MTs by phosphorylating the complex's Hec1 subunit at up to nine different sites. Jennifer DeLuca's lab at Colorado State University in Fort Collins has previously shown that phosphorylating all nine sites on Hec1 strongly inhibits kinetochore–MT interactions, whereas blocking Hec1 phosphorylation induces hyperstable attachments to the mitotic spindle (2, 3). The overall level of Hec1 phosphorylation declines as mitosis proceeds, although which of the nine sites are phosphorylated at any one time is unclear. "We wanted to know how differential Hec1 phosphorylation affects kinetochore function," DeLuca says.

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DeLuca and her colleagues Lynsie Sundin and Keith DeLuca therefore analyzed cells whose endogenous Hec1 had been replaced with mutant versions of the protein in which each of the nine phosphorylation sites had been mutated to either nonphosphorylatable alanine or phosphomimetic aspartate residues (1). None of the mutants supported proper chromosome segregation—indicating that Hec1 phosphorylation must be dynamically regulated—but metaphase cells expressing Hec1 mutants with a single phosphomimetic substitution recapitulated several important properties of wild-type cells. Their kinetochores showed normal movements on the metaphase spindle, and they attached to similar numbers of MTs. "It didn't matter where the substitution was. Any single phosphomimetic substitution could recapitulate metaphase chromosome movements," DeLuca explains. As the number of phosphomimetic substitutions increased, however, the number of kinetochore–MT attachments gradually decreased and chromosome movements became progressively more erratic. Accordingly, in vitro measurements showed that increasing Hec1 phosphorylation gradually decreased the Ndc80 complex's affinity for MTs.

To further investigate how Hec1 phosphorylation influences kinetochore function, DeLuca and colleagues turned to Ekaterina Grishchuk and Anatoly Zaytsev at the University of Pennsylvania in Philadelphia, who recently developed a mathematical model of the kinetochore–MT interface that treats the structure as a repetitive series of independent binding sites containing multiple Ndc80 complexes (4). "Changing the phosphorylation status of Hec1 in cells elicits a gradual response in kinetochore–MT attachment, but our model predicted a much stronger, switch-like response," Grishchuk says. In the repetitive sites model, she explains, each binding site can only interact with a single MT and is oblivious to events at neighboring attachment sites. Changes in Hec1 phosphorylation and Ndc80's binding affinity therefore have a dramatic effect on the number of kinetochore-attached MTs.

Zaytsev et al. therefore changed the rules of engagement for kinetochore MAPs, modeling the kinetochore–MT interface as a "molecular lawn" in which Ndc80 molecules aren't constrained to a single MT-binding site and can instead interact with any MT in their vicinity. "This significantly improved the model's fit with the experimental data," says Grishchuk. Moreover, the new model correctly predicted that Hec1 mutants with three phosphomimetic substitutions would best recapitulate the dynamics of kinetochore–MT interactions during prometaphase.

"The lawn model allows for the dynamic reorganization of attachments," DeLuca explains. "If a group of phosphorylated Ndc80 complexes detaches from a MT, they can immediately interact with neighboring MTs, providing a buffering effect that prevents an all-or-nothing response to phosphorylation." This could help cells fine-tune kinetochore–MT interactions to optimize the fidelity of chromosome segregation. With this in mind, the researchers now want to investigate how small changes in Hec1 phosphorylation and MT attachment are sensed by checkpoint signaling pathways.

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4. Zaytsev, A.V. et al. 2013. *Cell Mol Bioeng.* 6: 393-405.