

Complications dawn for kinetochore regulation by Aurora

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Organisms must faithfully segregate their chromosomes during cell division; mistakes in this process can be costly and even fatal to the organism (1, 2). During mitosis, replicated chromosomes attach to the spindle, a dynamic system of microtubules organized around two poles. Chromosomes attach to the spindle via kinetochores, structures that form on centromeres and bind the ends of microtubules. For accurate segregation, kinetochores on sister chromosomes must attach to microtubules from opposite poles; incorrect attachments lead to mis-segregation (3). In PNAS, Umbreit et al. (4) expand our understanding of how kinetochore–microtubule interactions can be regulated to correct improper attachments. The authors use *in vitro* studies to demonstrate that a component of the kinetochore, the Ndc80 complex, can directly influence the dynamics of the microtubules it is bound to and how the complex can be regulated to correct errors in chromosome attachment.

Kinetochores are complicated machines. They can stay attached to microtubule ends as they grow and shrink, regulate the dynamics of microtubules, regulate their own activity, and signal to the remainder of the cell. The outer layer of the kinetochore contains the dumbbell-shaped Ndc80 complex (5): One globular domain [the N-terminal domains of Hec1 (Ndc80 in budding yeast) and Nuf2] binds microtubules (6) and is connected by a long coiled coil to the other globular domain (composed of the C-terminal domains of Spc24/Sp25), which connects to other kinetochore components (7) (Fig. 1A). Hec1 contains a conserved calponin homology domain and an unstructured N-terminal tail: Both regions can bind to microtubules independently, but they must act together to produce high-affinity binding (5–8). When sister kinetochores attach to opposite spindle poles (bi-orientation), the linkage between kinetochores and microtubules is placed under tension and this tension stabilizes the kinetochore–microtubule linkage. However, if the two kinetochores attach to the same pole (mono-orientation), there is no tension and kinetochores release their microtubules, allowing the kinetochores another chance to orient on the spindle correctly. A conserved protein kinase, Aurora B, is required for kinetochore release and phosphorylates components of the kinetochore, including the N-terminal

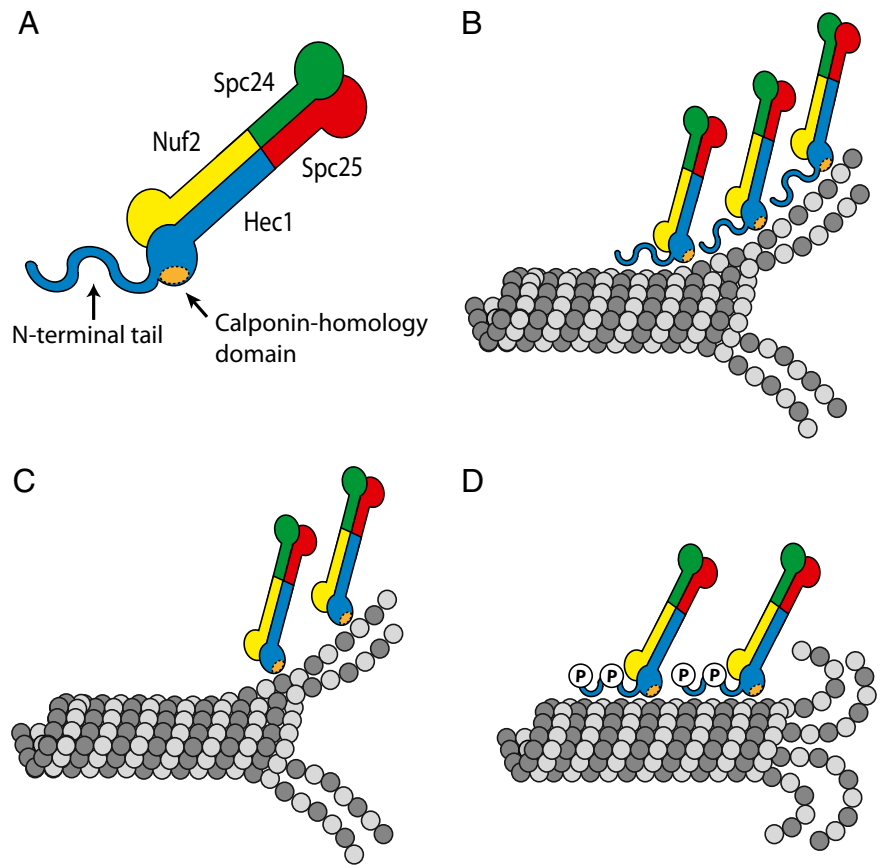


Fig. 1. (A) Human Ndc80 complex: Hec1 (blue) contains a calponin-homology domain (orange) and an unstructured N-terminal tail, Nuf2 (yellow), Spc24 (green), and Spc25 (red). (B) WT Ndc80 complex slows microtubule disassembly, promotes rescue, and stabilizes straighter protofilaments. (C) Mutated Ndc80 complex with a deleted N-terminal Hec1 tail has a lower affinity for microtubules but still slows disassembly, promotes rescue, and stabilizes protofilaments. (D) Alternative mutant form of the Ndc80 complex with phosphomimetic mutations on the Hec1 tail has a lower affinity for microtubules and is still able to slow disassembly but cannot promote rescue or stabilize straighter protofilaments. P represents the residues in the N-terminal tail mutated to mimic phosphorylation by Aurora B kinase.

tail of Hec1 (9, 10). The prevailing model for correcting mono-orientation is that Aurora B phosphorylates Hec1, causing microtubule release (11–13). Umbreit et al. (4) demonstrate that our understanding of the Aurora B mechanism needs to be revisited: Hec1 phosphorylation alters microtubule dynamics at the Ndc80 complex–microtubule interface as well as reducing the affinity of the Ndc80 complex for microtubules.

Umbreit et al. (4) express and purify the full-length human Ndc80 complex [previous studies with the human Ndc80 complex used a truncated version (5–7)] and find that it slowed microtubule disassembly. This demonstration proves that a core component of the human kinetochore can directly influence microtubule

dynamics. In agreement with previous *in vitro* studies (5–7), Umbreit et al. (4) find that if the N-terminal tail of Hec1 was deleted or mutated to mimic Aurora B phosphorylation, the complex's affinity for microtubules was greatly reduced. Earlier observations of this reduced affinity led to the model that Aurora B corrects erroneous attachments by releasing microtubules (6, 7). There is *in vivo* support for this mechanism; knocking down the Ndc80 complex results in unattached

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chromosomes (10), and inhibiting Aurora B results in hyperstable attachments (11–13). However, contrary to this model, Lampson et al. (14) found that when they inhibited and then reactivated Aurora B, mono-oriented kinetochores did not release their microtubules; instead, the microtubules depolymerized, reeling the two sister kinetochores to one spindle pole.

Umbreit et al. (4) explain how Aurora B activity can promote both microtubule release and depolymerization at the kinetochore interface. They find that in addition to slowing disassembly, the Ndc80 complex can promote microtubule rescue, the conversion of a shrinking microtubule to a growing microtubule (Fig. 1B). If the N-terminal tail of Hec1 is deleted, affinity for microtubules is reduced but the complex can still rescue shrinking microtubules (Fig. 1C). However, if all sites on the N-terminal tail are mutated to mimic phosphorylation by Aurora B, the ability to rescue microtubules is abolished (Fig. 1D), even though the mutant complex can still slow depolymerization. These results suggest that phosphorylation does not simply abolish the tail's affinity for microtubules but that it actively interferes with the ability of the Ndc80 complex to promote microtubule rescue.

How does the Ndc80 complex promote rescue? Microtubules are tubes composed of 13 linear protofilaments, each of which is a head-to-tail polymer of tubulin dimers. When microtubules depolymerize, the individual protofilaments curl back tightly at the shrinking end (15). Umbreit et al. (4) find that incubating the Ndc80 complex with microtubules produced stabilized microtubule tips whose protofilaments were straighter at their tips and which associated with each other, forming protofilament sheets. Both pro-

erties are likely to favor rescue. The Ndc80 complex with truncated N-terminal Hec1 tails was able to stabilize these straighter protofilaments, but phosphomimetic complexes were not. Alushin et al. (8) have suggested that the calponin-homology domain of Hec1 binds tubulin at an inter-dimer hinge region proposed by Wang and Nogales (16); the current study

Umbreit et al. expand our understanding of how kinetochore–microtubule interactions can be regulated to correct improper attachments.

suggests that this promotes a straighter conformation in isolated protofilaments and that phosphorylation of the N-terminal tail interferes with this function.

Previous studies disagreed about how Aurora B promotes turnover of incorrect attachments, either through immediate release of the microtubules or through depolymerization. Umbreit et al. (4) have advanced our understanding of the Aurora B mechanism by demonstrating that phosphoregulation of the Ndc80 complex can produce both outcomes. Phosphorylation of Hec1 reduces its affinity for microtubules and abolishes its ability to rescue microtubules. Umbreit et al. (4) show that these are separable activities: Microtubule affinity can be reduced without losing the ability to promote rescue. This experimental dissection raises the question of whether these activities are

independently regulated *in vivo*? In the current study, all nine sites in the Hec1 tail were mutated to mimic phosphorylation, but the authors propose that different combinations of site phosphorylation may independently tune these two functions of Aurora B. If there is independent control, do cells use different correction mechanisms for different types of erroneous attachments, such as immediate release of microtubules from single kinetochores that are attached to two poles (merotelic attachment) and depolymerization of microtubules for kinetochore pairs attached to the same pole (syntelic attachment)? Is there a hierarchy of Aurora B functions? For example, do the initial phosphorylations on Hec1 attempt to release microtubules, with additional, later phosphorylations destabilizing any microtubules that have not been released? Another interesting suggestion is that Aurora B may play an important role in normal dynamics and alignment of chromosomes, as well as in error correction. Previous studies have found that mutating phosphorylation sites in the Hec1 N-terminal tail to nonphosphorylatable alanines causes defects in chromosome alignment (10) and suppresses chromosome oscillations about the spindle's equator (9). Inhibiting Aurora B also suppresses oscillations, even though chromosomes are properly attached (17). Perhaps oscillations in the level of Ndc80 phosphorylation at the two sister kinetochores drive these oscillations and they play a role in the proper positioning of the chromosomes on the spindle. More subtle manipulations of Aurora B's activity and Hec1's phosphorylation will be required to answer these questions.

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