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Cdt1 throws kinetochore-microtubule attachments for a loop

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Abstract

The Ndc80 complex links spindle microtubules to the kinetochore to ensure the proper segregation of chromosomes during mitosis. Analysis of the replication licensing factor Cdt1 during mitosis now reveals a cooperative role with the Ndc80 complex in establishing stable microtubule attachments to the spindle.

The faithful segregation of genetic material during mitosis is required for organisms to safeguard the content of their genome and propagate their species. Interfering with this process induces genomic instability and cell death, and is hypothesized to contribute to cancer development in humans. Chromosome segregation during mitosis requires the action of kinetochores, large protein machines composed of ~100 different proteins that form on centromeres. Kinetochores capture and regulate spindle microtubules and create the spindle assembly checkpoint signal that inhibits mitotic exit until microtubules are properly attached. Following correct kinetochore-microtubule (kMT) attachment, kinetochores act as motors utilizing the energy stored within the microtubule lattice to segregate chromosomes to opposite spindle poles.

Initial capture of spindle microtubules by kinetochores is facilitated in part by ATP-dependent motor proteins including dynein and CENP-E[1]. These initial contacts mature into “end-on” kMT attachments mediated by the KMN (Knl1, Mis12, Ndc80) protein supercomplex. Central to the function of the KMN network is the conserved Ndc80 complex which comprises Hec1 (Highly Expressed in Cancer 1, also known as Ndc80), Nuf2 and Spc24/25[1]. Hec1 consists of an unstructured N-terminal tail followed by a calponin homology (CH) domain and a long coiled-coil, which interact with the CH domain and coiled-coil of Nuf2. Within this shared Hec1-Nuf2 interface, Hec1 extends a conserved unstructured loop region, which allows a bend within the rigid coiled-coil of the Ndc80 complex (Fig. 1a)[1]. At their C-termini Hec1 and Nuf2 bind the Spc24 and Spc25 proteins, which anchor the full complex in the kinetochore (Fig. 1a,b)[1]. Although the Ndc80 complex has been extensively studied and despite our growing understanding of the microtubule binding activities, structure and location of Hec1 within the kinetochore superstructure, how this complex carries out its functions in chromosome movement, spindle checkpoint signaling, and regulation of end-on attached microtubules is not fully elucidated[1,2,3]. On page 593, Varma *et al.* offer further insight into how the human Ndc80 complex generates stable microtubule attachments through the interaction of Hec1 with the DNA replication licensing factor Cdt1[4].

How kinetochores remain attached to depolymerizing microtubules with bonds that can withstand enough force to move chromosomes is a fascinating question. Each Ndc80 complex has at least three microtubule attachment points required for stable kMT attachments in human cells: two on its dual CH domains and one on its N-terminal unstructured tail[3]. Additionally, Ndc80 complexes can oligomerize along microtubule protofilaments (omitted from the figure for clarity)[2]. The current thinking is that strong attachments are built from numerous low-affinity interactions regulated by phosphorylation

by mitotic kinases including Aurora B, and by microtubule polymer conformation[2,3]. In the latter case, the Ndc80 CH domains bind the microtubule on a surface generated from two adjacent tubulin subunits, which is lost during the conformational change that accompanies microtubule depolymerization[2].

The findings of Varma *et. al.* add Cdt1 as another player in the Ndc80-dependent kMT attachment pathway[4]. The authors employed a protocol to assay the activity of Cdt1 during mitosis independently of its origin licensing function during the G1 phase of the cell cycle, and identified a role in chromosome congression. They demonstrated that Cdt1 localizes to kinetochores where it binds the Hec1 loop and is required for the formation of stable microtubule attachments. They also used a super-resolution imaging technique to show that in the absence of Cdt1 the coiled-coil of the Ndc80 complex was highly bent whereas in the presence of Cdt1 it assumed an elongated conformation (Fig. 1c). They propose that this dramatic conformational change induced by Cdt1 mediates kMT attachment stability. Why a DNA replication licensing protein would be used in mitosis is unknown, but one intriguing possibility is that it represents a previously unrecognized mechanism to activate the spindle checkpoint. Replication licensing in many systems happens soon after cells exit mitosis, and cells may have evolved mechanisms to ensure that critical licensing factors are present prior to mitotic exit. According to this hypothesis, cells with low Cdt1 levels would be unable to align chromosomes and trigger the spindle checkpoint until Cdt1 concentrations are high enough to ensure proper licensing in the next G1.

Cdt1 is not the only protein to bind the loop domain within the Ndc80 complex coiled-coil. Recent papers demonstrated that the Dis1/Tog microtubule polymerases, which are conserved from yeast to humans[5], bind the loop in fission yeast[6], whereas the unrelated Dam1 complex, which is not conserved in humans[5], binds the loop in budding yeast[7]. Both of these interactions were required for kMT binding activity *in vivo*. Cdt1-loop binding in human cells is surprising, as Cdt1 (unlike Dis1/Tog and Dam1) is not believed to bind microtubules. It is thus important to determine whether Cdt1 binds the Ndc80 complex alone or as part of a larger complex containing a microtubule-binding component. Although Varma *et al.* demonstrate a strong association between Cdt1 binding and conformational changes in the Ndc80 complex in cells *in vivo*[4], how Cdt1 binding mediates this is unclear and thus it would be important to identify additional mitotic binding partners of Cdt1 or the Hec1 loop region in humans. One intriguing possibility is the cooperation of Cdt1 with the Ska complex, which stabilizes Ndc80-microtubule interactions[8]. The Hec1 internal loop was recently reported to recruit Ska in live cells[9], and a crystal structure of the human Ska complex reveals a symmetric structure with potential analogy to the Dam1 complex[8].

Understanding how Cdt1, Ndc80, and other Hec1 loop interacting proteins might work together to allow kinetochores to harvest the energy of depolymerizing microtubules will be an important area for future studies. *In vivo* and *in vitro* data argue that the dual CH domains of the Ndc80 complex can couple microtubule depolymerization to chromosome movement[2,3]. Moreover, recent estimates suggest that there are 20-30 Ndc80 molecules per microtubule and each Ndc80 has at least three weak interactions[3,10]. These could build a sleeve that uses numerous low-affinity interactions to remain bound to a depolymerizing microtubule (Fig. 1d). In this model the Ndc80 complex would reside at the depolymerizing microtubule tip, however, high-resolution imaging techniques have placed the Hec1 loop and Dam1 deeper within the kinetochore[11,12]. This suggests that stabilized microtubules could penetrate beyond the Hec1 CH domain, allowing the Ska or Dam1 proteins to act as sliding clamps that are pushed poleward by the curving protofilaments of a depolymerizing microtubule (Fig. 1d). Sleeves and sliding clamps could coexist to build a robust coupler, which could explain why Dam1 is not essential in fission yeast and chromosomes can align without Ska[8,13]. Alternatively, Dam1 and Ska could bind the loop to act as tension

dependent rescue factors. Modeling studies in budding yeast suggest that such an activity controls chromosome congression to the metaphase plate[14]. The application of pulling forces to a depolymerizing microtubule tip within a ring structure would straighten curling protofilaments, reinstating lateral interactions between adjacent protofilaments and rescuing the depolymerizing microtubule. Depolymerizing microtubules could also be rescued by recruiting a microtubule polymerase, suggesting a possible similarity between the Dam1 complex and Dis1/Tog. Additional studies will be needed to test these possibilities.

Another interesting question is how binding of Cdt1 to the Ndc80 complex is regulated. After origin licensing is completed in G1, Geminin binds and stabilizes Cdt1 and also inhibits its licensing activity through S and G2 phase to prevent genome re-replication[15]. Although Geminin ensures that Cdt1 accumulates in G2 to promote proper chromosome segregation, it is degraded during the metaphase to anaphase transition and it is not found with Cdt1 at kinetochores, suggesting that Cdt1 is released from Geminin early in mitosis, presumably to bind Hec1. Alternatively, Cdt1 may be activated by its association with the kinetochore, which raises the possibility that the Ndc80 complex might be required for DNA replication.

The study by Varma *et al.* highlights the complexity of the core microtubule binding function of kinetochores, which requires the orchestrated activity of functionally diverse factors, as well as three distinct domains of the Ndc80 complex. Future studies will determine how the loop binding factors fit into the existing framework of Ndc80 complex regulation as we begin to piece together the order of events that lead to faithful chromosome segregation.

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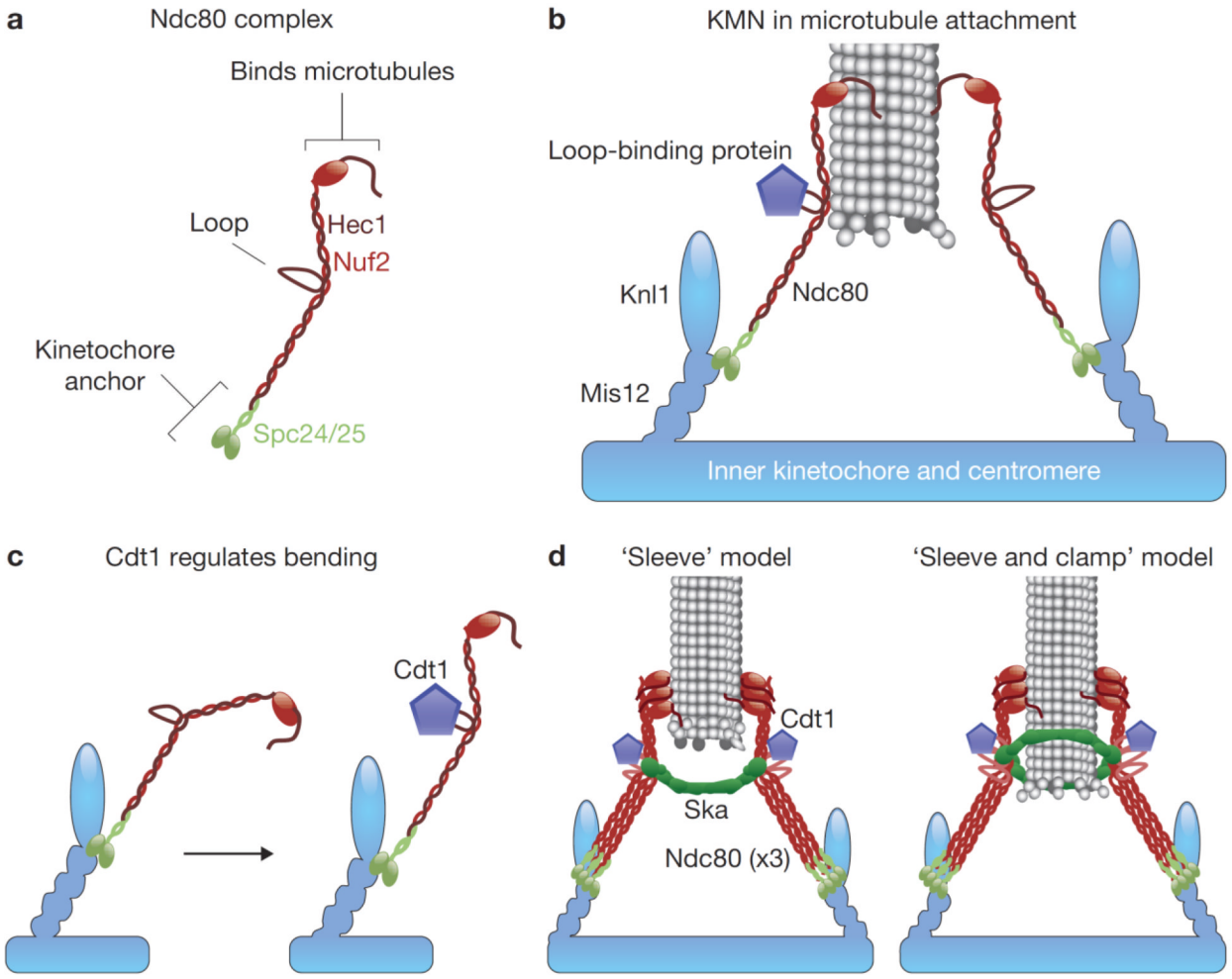

Figure 1.

The role of the Ndc80 complex in kinetochore and spindle microtubule attachments. (**a**) The Ndc80 complex is composed of Hec1 (Ndc80), Nuf2, Spc24, and Spc25. The Hec1 and Nuf2 calponin homology (CH) domains and the Hec1 unstructured tail bind microtubules. The unstructured loop of Hec1 allows the complex to bend and binds protein partners. A long Hec1-Nuf2 coiled-coil interacts with Spc24 and Spc25 which anchor the complex in the kinetochore. (**b**) The KMN network (Knl1, Mis12, Ndc80) anchors the Ndc80 complex to the inner kinetochore, whereas the Ndc80 CH domain and N-terminal tails bind microtubules. (**c**) Cdt1 binds to the Ndc80 unstructured loop and participates in a conformational change. (**d**) Putative models of Ska and Cdt1 cooperation with the Ndc80 complex to promote microtubule attachment. In the "sleeve" model, Cdt1 would cause a conformational change in Ndc80 as in (c) and Ska binding would cluster Ndc80 proteins to properly assemble a sleeve. In the "sleeve and clamp" model Ska and Cdt1 cooperate with the Ndc80 loop region to form a stabilizing ring that would topologically hold the depolymerizing microtubule plus ends and complement the binding activity of the Ndc80 CH domain and unstructured tail.