Human Ska complex and Ndc80 complex interact to form a load-bearing assembly that strengthens kinetochore–microtubule attachments

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Accurate segregation of chromosomes relies on the force-bearing capabilities of the kinetochore to robustly attach chromosomes to dynamic microtubule tips. The human Ska complex and Ndc80 complex are outer-kinetochore components that bind microtubules and are required to fully stabilize kinetochore–microtubule attachments in vivo. While purified Ska complex tracks with dis-assembling microtubule tips, it remains unclear whether the Ska complex–microtubule interaction is sufficiently strong to make a significant contribution to kinetochore–microtubule coupling. Alternatively, Ska complex might affect kinetochore coupling indirectly, through recruitment of phosphoregulatory factors. Using optical tweezers, we show that the Ska complex itself bears load on microtubule tips, strengthens Ndc80 complex-based tip attachments, and increases the switching dynamics of the attached microtubule tips. Cross-linking mass spectrometry suggests the Ska complex directly binds Ndc80 complex through interactions between the Ska3 unstructured C-terminal region and the coiled-coil regions of each Ndc80 complex subunit. Deletion of the Ska complex microtubule-binding domain or the Ska3 C terminus prevents Ska complex from strengthening Ndc80 complex-based attachments. Together, our results indicate that the Ska complex can directly strengthen the kinetochore–microtubule interface and regulate microtubule tip dynamics by forming an additional connection between the Ndc80 complex and the microtubule.

Significance

Microtubules are dynamic, tube-like structures that drive the segregation of duplicated chromosomes during cell division. The Ska complex is part of a molecular machine that forms force-bearing connections between chromosomes and microtubule ends. Depletion of the Ska complex destabilizes these connections and disrupts cell division. The Ska complex binds microtubules, but it is unknown whether it directly holds force at microtubule tips or indirectly stabilizes the connections. Here, we show that the Ska complex makes a direct force-bearing linkage with microtubule ends and assembles with another microtubule binding component, the Ndc80 complex, to strengthen its ability to withstand force. Our results suggest that the Ska and Ndc80 complexes work together to maintain the connections between chromosomes and microtubule ends.


The authors declare no conflict of interest.

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and the microtubule. Our results suggest the Ska complex and Ndc80 complex directly interact with each other and with microtubules to form a multipartite load-bearing assembly that strengthens kinetochore-microtubule attachments.

Results

Ska Complex Bears Load on Microtubule Ends. The Ska complex is reported to dimerize in solution and to cooperatively bind the microtubule lattice as a dimer or as higher-order oligomers (4, 15–17). Before measuring the strength of its attachments to microtubules, we used total internal reflection fluorescence (TIRF) microscopy to examine the oligomeric state of the Ska complex at the low nanomolar concentrations used in our microtubule binding and rupture force assays. Individual particles of GFP-tagged Ska complex (Ska complex$^{\text{Ska3-GFP}}$) bound and diffused along Taxol-stabilized microtubules, as reported previously, and similarly to the lattice diffusion of other kinetochore components (Fig. 1E) (15, 17–19). The mean residence time of Ska complex$^{\text{Ska3-GFP}}$ particles on microtubules was 5.2 ± 0.1 s, similar to previously measured residence times (Fig. S1A) (15, 17). Particle intensities fell within a unimodal, approximately Gaussian distribution that did not change across a fivefold increase in concentration, and they photobleached or dissociated in single steps (Fig. 1C and D). Moreover, individual Ska complex$^{\text{Ska3-GFP}}$ particles, when bound sparsely onto coverslip surfaces, exhibited single-step photobleaching, and their mean intensity before bleaching matched that of single GFP-tagged yeast Ndc80 complexes (Fig. S1B). Using size exclusion chromatography–multiangle light scattering (SEC-MALS), we confirmed that Ska complex$^{\text{Ska3-GFP}}$ in solution can form a dimer and exists in a monomer–dimer equilibrium at micromolar concentrations (Fig. S1C) (16). However, our TIRF data suggest that at low nanomolar concentrations, the Ska complex binds the microtubule lattice as a single complex.

Using an optical-tweezers bead motility assay, we next measured the microtubule end-binding strength of the Ska complex. We coated beads with the Ska complex at various concentrations, to control the surface density of the molecules on the bead (Fig. 1E). Depending on the surface density and molecular structure, one or more molecules can simultaneously interact with the microtubule tip, an arrangement that mimics the multivalency at kinetochore-microtubule interfaces in vivo (20, 21). Individual Ska complex-coated beads were first attached to the growing tips of single microtubules anchored to a coverslip. After an initial low force was applied and the bead was verified to track with tip growth, the force was increased gradually until the attachment ruptured (Fig. 1F). The mean rupture force versus Ska complex molecules per bead. Error bars are SD from bootstrapping analysis of the median. The median values and errors are calculated from the same data shown in Fig. S2A. (H) Rupture force survival probability plot for 700 Ska complex molecules per bead without (black) and with (magenta) 10 nM Ska complex in solution. Shaded areas are 95% confidence intervals from Kaplan–Meier analysis.
density on the beads increases the number of Ndc80 complexes that can simultaneously reach the microtubule end (see below and ref. 18). To test whether the Ska complex behaves similarly, we measured the strength of Ska complex-based attachments as a function of its surface density on beads. We observed only a small, 1.5-fold increase in Ska complex attachment strength over a 100-fold range in surface density; whereas the strength of human Ndc80 complex-based attachments increased more substantially, by 4.2-fold over a 24-fold density range (Fig. 1G, and see Fig. 3D). Furthermore, addition of 10 nM free Ska complex in solution did not increase attachment strength of bead-bound Ska complex, consistent with the lack of Ska complex oligomerization at nanomolar concentrations (Fig. 1H). Taken together, our data show the Ska complex is load-bearing and suggest that its load-bearing capacity is largely established at low molecular surface densities and not strongly enhanced by additional Ska complexes.

**Ska Complex Ska3 C Terminus Is Not Required for Load Bearing.** To identify interacting regions between the Ska complex and microtubules, we performed cross-linking mass spectrometry of Ska complex incubated with Taxol-stabilized microtubules. In agreement with previous reports, we observed cross-links between microtubules and the Ska3 unstructured C terminus (residues 102–402) (Fig. 2A and Fig. S3) (22, 23). To test the importance of these regions for load-bearing interactions between the Ska complex and microtubules, we measured the attachment strength of mutant Ska complexes missing either the Ska1 MTBD (Ska1 ∆MTBD) or the Ska3 C terminus (Ska3 ∆C) (Fig. 2B). Beads coated with mutant Ska complex failed to bind to microtubules, indicating that the MTBD is required for formation of a load-bearing attachment. In contrast, the fraction of beads coated with Ska complex that bound microtubules was similar to wild type (Fig. 2C), and their end attachment strength was only slightly reduced (by 1.3-fold; Fig. 2D and Table S1). These observations confirm that, within the Ska complex, both Ska1 and Ska3 interact with microtubules. The Ska1 MTBD is necessary for load-bearing interactions with microtubules, whereas the Ska3 C terminus makes only a minor contribution.

**Ska Complex Strengthens Ndc80 Complex-Based Microtubule Attachments.** Ska complex increases the affinity of Ndc80 complex for the microtubule lattice and can promote Ndc80 complex tip tracking in the absence of force (15). To determine whether the Ska complex can increase the load-bearing capacity of the Ndc80 complex, we measured the rupture force of Ndc80 complex-based attachments with and without the Ska complex added free in solution (Fig. 3A). Adding the Ska complex strengthened Ndc80 complex-based microtubule end attachments when the Ndc80 complex was at a low surface density on the beads, but not when it was at a high density (Fig. 3B and D, Fig. S2A, and Table S1). The increase in strength afforded by the Ska complex at the low Ndc80 complex surface density was greater than the rupture strength of the Ska complex alone, suggesting a synergistic effect. These results show that the Ska complex strengthens Ndc80 complex-based coupling, particularly when the latter is weak due to low avidity.

Next, we tested whether the Ska complex could strengthen Ndc80 complex-based attachments that were weakened due to a decreased affinity between the Ndc80 complex and the microtubule. We introduced Aurora B phosphomimetic mutations (serine/threonine to aspartate) in all nine phosphorylation sites in the Hec1 N-terminal tail to generate the mutant, 9D Ndc80 complex. These mutations dramatically decrease the affinity of the Ndc80 complex for microtubules (19, 24, 25). As expected, we found the mutant 9D Ndc80 complex formed attachments that were significantly weaker than those formed by wild-type Ndc80 complex (Fig. 3C). Adding free Ska complex increased the attachment strength of the mutant 9D Ndc80 complex by more than fivefold (Figs. 3C and D). We raised the surface density of the mutant 9D Ndc80 complex on the beads by threefold and found that the Ska complex could also moderately strengthen the attachments formed at this higher density (Fig. 3D and Fig. S2C). Furthermore, we tested a mutant Ndc80 complex lacking the entire unstructured N-terminal 80-aa tail of Hec1 (ΔN Ndc80 complex). As expected, this mutant ΔN Ndc80 complex formed weak attachments on its own that, just like the 9D mutant, could be strengthened by the addition of free Ska complex (Fig. 3E). Together, these results show that the Ska complex strengthening is independent of the Hec1 N-terminal tail.

**Purified yeast Ndc80 complex and native yeast kinetochore particles detach more frequently from disassembling tips than from assembling tips (18, 26). We verified that this difference also occurs for human Ndc80 complex by applying a force clamp. Beads coated with human Ndc80 complex were attached to growing tips and then subjected to a constant tension of ~2 pN. Under this condition, the Ndc80 complex-based couplers tracked continuously with end growth and shortening, remaining persistently attached as the tips switched spontaneously between assembling and disassembling states (Fig. 4A and B). The mean detachment rate for Ndc80 complex-based couplers from disassembling tips was 14-fold higher than from assembling tips, confirming that the coupling was less stable during tip disassembly (Fig. 4C and D and Table S2–S6). Interestingly, adding Ska complex in solution specifically stabilized the coupling during tip disassembly, reducing the detachment rate twofold, with no apparent effect during assembly. Altogether, these results
Upon alignment at the metaphase plate, and \( B \) Ska complex strengthens Ndc80 complex microtubule attachments. and \( \text{– Fig. S2} \) \( B \) | \( C \) and \( \text{Fig. S4} \) Ska complex affects the dynamics of Ndc80 complex and bound microtubules. Multiple studies suggest that the Ska complex and Ndc80 complex govern microtubule behavior and suggests that together they may increase the switching frequency of kinetochore-bound microtubules.

**Ska Complex Binds the Ndc80 Complex Coiled-Coil Through the Ska3 C Terminus.** Multiple studies suggest that the Ska complex and Ndc80 complex interact directly, but the interaction interface between the complexes has not been defined (12, 13). To identify the specific regions involved in their interaction, we performed cross-linking mass spectrometry with Ska complex, Ndc80 complex, and Taxol-stabilized microtubules. The Ska3 unstructured C terminus (residues 102–412) cross-linked robustly with the Ndc80 complex and microtubules (Fig. 5A and Fig. S4). A total of 328 unique cross-links was found between the Ndc80 and Ska complexes. Of these, 97% (318 of 328) were between Ska3 and the Ndc80 complex, distributed across the Ska3 C terminus and among all four Ndc80 complex subunits. Ska3 primarily cross-linked to regions of the Ndc80 complex that are predicted to form coiled-coils. Few Ska3 cross-links were observed with the CH domains of Hec1 and Nuf2 or the RWD domains of Spc24 and Spc25. These results suggest that the Ndc80 complex and Ska complex directly interact through the Ska3 unstructured C terminus that preferentially binds to coiled-coil regions throughout the Ndc80 complex.

**The Ska Complex and Ndc80 Complex Must Bind Each Other and Microtubules to Strengthen Ndc80 Complex-Based Attachments.** The Ska complex is capable of binding directly to both the Ndc80 complex and to microtubules (4, 12, 13). We have shown that the Ska complex enhances Ndc80 complex-based coupling. Together, these observations suggest that Ska complex might form an extra attachment to microtubules.

**Ska Complex Changes How the Ndc80 Complex Governs Microtubule Switching Behavior.** Upon alignment at the metaphase plate, chromosomes oscillate between poleward and anti-poleward motions, which are partially driven by the switching kinetics of the kinetochore microtubules (27, 28). Altering the microtubule binding affinity of the Ska or Ndc80 complexes independently dampens these metaphase oscillations in vivo (15, 29). To test whether couplers based on the Ndc80 and Ska complexes can affect microtubule tip switching in vitro, we measured the dynamics of tips coupled to Ndc80 complex-decorated beads under a constant force, with or without Ska complex added in solution (Fig. 4A and B and Tables S2–S6). Indeed, the rescue rate for tips attached to Ndc80 complex-based couplers increased 4.5-fold upon addition of free Ska complex (Fig. 4E). This observation is similar to previous findings showing that microtubule rescue rates increase as Ndc80 complex attachments are strengthened (19). Moreover, addition of Ska complex increased the catastrophe rate for attached tips by 2.7-fold (Fig. 4F). These results show that the Ska complex changes how the Ndc80 complex governs microtubule behavior and suggests that together they may increase the switching frequency of kinetochore-bound microtubules.
The Ska complex must bind Ndc80 complex and microtubules to strengthen attachments. (A) Cross-links identified between Ska3 and Ndc80 complex. Cross-linking reaction with Ska complex, Ndc80 complex, and Taxol-stabilized microtubules was performed for 15 min with the amine-to-amine cross-linker BS3. Ska1, Ska2, and tubulin cross-links are not shown for clarity; see Fig. S4 for all cross-links identified and https://proxl.yeastrc.org/proxl/viewProject.do?id=49 for all data. Red shaded regions indicated predicted coiled-coil (Paircoil2) with probability scores from 0.8 to 1.0. (B and C) Rupture force survival probability plot for 30 Ndc80 complex molecules per bead without Ska complex (black, data repeated from Fig. 3B) and with 25 nM Ska complex wild-type (orange, data repeated from Fig. 3B) or Ska complex Ska3 ΔC (C49) mutant (blue) or Ska complex Ska1 ΔMTBD mutant (green). All shaded regions on survival probability plots are 95% confidence intervals from Kaplan–Meier analysis. (D) Median rupture force for 30 Ndc80 complex molecules per bead with the indicated Ska complexes; colors based on B and C. WT, wild-type Ska complex. Error bars are SD from bootstrapping analysis of the median. The median values and errors are calculated from the same datasets shown in B and C. Possible model of how Ska complex and Ndc80 complex directly interact to form multiple microtubule load-bearing attachments.

Discussion

Previous studies have established that depletion of the Ska complex in vivo generally weakens kinetochore–microtubule attachments, thereby (i) diminishing the numbers of attachments that are resistant to cold treatment (4, 7, 8), (ii) causing more frequent kinetochore detachments during congression (30), and (iii) relieving the hyperstabilization of kinetochore–microtubule attachments caused by phospho-blocking mutations in the Ndc80 complex (31). Importantly, many of these weakened microtubule attachment phenotypes were also observed upon specific impairment of the microtubule-binding activity of the Ska complex. These in vivo observations are consistent with the idea that Ska complex makes a direct contribution to load bearing at the kinetochore–microtubule interface. However, the load-bearing capacity of the Ska complex has been unclear, leaving open the possibility that its role is primarily indirect, via recruitment of protein phosphatase 1 (9). We show here that the Ska complex alone can bear load on microtubule ends, that it can enhance Ndc80 complex-based coupling, and that this enhancement requires the Ska complex to bind both microtubules and Ndc80 complex. These observations strongly support the model that the Ska complex strengthens kinetochore–microtubule attachments by forming a load-bearing bridge between the Ndc80 complex and the microtubule (Fig. 5E).

Cell biological (7, 32), biochemical (15, 33), and evolutionary analyses (34) have suggested that the Ska complex might be a functional analog of the yeast Dam1 complex. However, while the Dam1 complex oligomerizes into microtubule-encircling rings that enhance its tip-coupling performance (35–37), the Ska complex does not appear to form such rings (4). Nevertheless, we find that the Ska complex, like the Dam1 complex, can form load-bearing tip attachments on its own and increase the strength and stability of Ndc80 complex-based couplers. Thus, our results lend further support to the hypothesis that the human Ska and yeast Dam1 complexes are functional analogs. Our cross-linking mass spectrometry shows that the Ska complex interacts with the coiled-coil regions of the Ndc80 complex through the Ska3 C terminus, but the overall architecture of their
assembly at the kinetochore is unknown. Recently, the yeast Ndc80 complex was reported to bind two Dam1 complex rings and perturbations to this two-ring binding created mitotic attachment defects (38). Further structural studies will be needed to determine the assembly stoichiometry and how the Ska complex binds coiled-coil regions along the entire 55-nm-long Ndc80 complex (39). Revealing how this load-bearing unit, composed of the Ndc80 and Ska complexes, works with and captures the forces generated by a depolymerizing microtubule tip is critical to understanding how microtubules translate microtubule depolymerization into chromosome segregation.

Interestingly, the enhancement of Ndc80 complex-based tip attachments upon addition of Ska complex occurred selectively, only when the Ndc80 complex-based attachments were relatively weak. We speculate that this effect might arise because Ska complex preferentially strengthens Ndc80 complex binding to a particular region on the microtubule tip, such as the most terminal tubulin subunits, and that Ndc80 complex-based couplers under weakened conditions rely primarily on bonds in this region. Alternatively, the Ska complex-dependent enhancement might be sterically blocked when Ndc80 complexes bind microtubules with high cooperativity (19, 24). While further studies will be required to understand the molecular basis for this selectivity, the effect could explain how Ska complex specifically prevents kinetochore detachments during episodes of poleward movement in prometaphase (39).

Both the Ska and Ndc80 complexes are important targets of phosphorylation by mitotic kinases. Aurora B phosphorylates Ndc80 complex extensively during early mitosis, thereby reducing its affinity for microtubules and promoting the release of erroneous kinetochore–microtubule connections (29). During this same period, starting in prometaphase, the Ska complex colocalizes with the Ndc80 complex (5, 13). Our finding that the Ska complex can strengthen Ndc80 complex-based microtubule attachments even when all nine Aurora B phosphorylation sites on the Ndc80 complex are mutated to phosphomimetic residues suggests that the Ska complex may partially antagonize the weakening of attachments by Aurora B during early mitosis. The Ska complex itself is also a target of the Aurora B (40), Mps1 (17), and Cdk1 (12) kinases, which are thought to regulate its interactions with microtubules and the Ndc80 complex as well as to promote its oligomerization. The ability of the Ska complex to directly strengthen kinetochore–microtubule coupling implies that the release of erroneous attachments, and stabilization of proper bioriented attachments, may require coordinated phosphorylation of both the Ska and Ndc80 complexes.

Materials and Methods

The human Ska and Ndc80 complexes were expressed from Escherichia coli cells and purified using affinity chromatography and SEC. TIRF and optical-tweezer microscopy as well as cross-linking mass spectrometry were performed as previously described (18, 38). Please see SI Materials and Methods for detailed descriptions of the protein purifications, TIRF microscopy, optical-tweezer microscopy, cross-linking mass spectrometry, and SEC-MALS performed in this study.

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