



# 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 disassembling 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.**

kinetochore | microtubules | optical tweezers | Ska complex | Ndc80 complex

Depolymerizing spindle microtubules generate forces required to separate duplicated chromosomes during mitosis. The kinetochore couples dynamic microtubule ends to chromosomes and harnesses the energy released by depolymerizing microtubules to pull duplicated chromosomes to opposite poles. Kinetochore–microtubule attachments must sustain piconewton-scale loads, especially during metaphase when bioriented kinetochores are subject to tension from opposing spindle microtubules. Attachments that are too strong or too weak can generate erroneous chromosome–microtubule attachments and promote chromosome missegregation during cell division (1). The incorrect segregation of chromosomes leads to aneuploidy and has been linked to chromosomal instability (2, 3). The attachment strength established and maintained between kinetochores and dynamic microtubule ends is fundamental to faithful chromosome segregation and cell division.

In vivo experiments show that the heterotrimeric Ska complex (Ska1, Ska2, and Ska3; Fig. 1A) is important for the stability of kinetochore–microtubule coupling and suggest at least three models for how it might contribute to coupling strength. Purified Ska complex binds directly to microtubules in vitro (4), and loss of Ska complex in vivo delays mitotic progression and has been associated with chromosome congression failure and mitotic cell death (4–7). Based on these observations, one view is that the Ska complex contributes directly to kinetochore–microtubule coupling (4, 7, 8). However, some studies suggest instead that the Ska complex plays a more indirect, regulatory role in kinetochore–microtubule coupling

by recruiting protein phosphatase 1 to the kinetochore, rather than by bearing microtubule-generated forces (9). Ska complex localizes to kinetochores in vivo through interactions with the Ndc80 complex (Hec1, Nuf2, Spc24, and Spc25; Fig. 1A), an essential component of the kinetochore–microtubule interface (10–13). This observation raises a third possibility, that the Ska complex might enhance Ndc80 complex-based coupling independently of its own microtubule binding affinity (14). Purified Ska complex alone tracks with depolymerizing microtubule tips (4) and has also been found to enhance the microtubule lattice binding and tip tracking of the Ndc80 complex (15). While these findings are consistent with a direct role for Ska complex in kinetochore tip coupling, they do not address the load-bearing capacity of Ska complex-based attachments. Thus, it remains uncertain whether the Ska complex can bear significant load on microtubule ends, either alone or in combination with the Ndc80 complex.

Here, we tested the microtubule end, load-bearing strength of the human Ska and Ndc80 complexes, both together and independently. We found that Ska complex bears load at microtubule ends on its own and strengthens Ndc80 complex-based end attachments. Using cross-linking mass spectrometry, we found that the Ska3 unstructured C-terminal region of Ska complex interacts with the coiled-coil regions of the Ndc80 complex. Furthermore, we show that strengthening Ndc80 complex-based attachments requires the Ska complex to simultaneously bind the Ndc80 complex

## 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 microtubules 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.**

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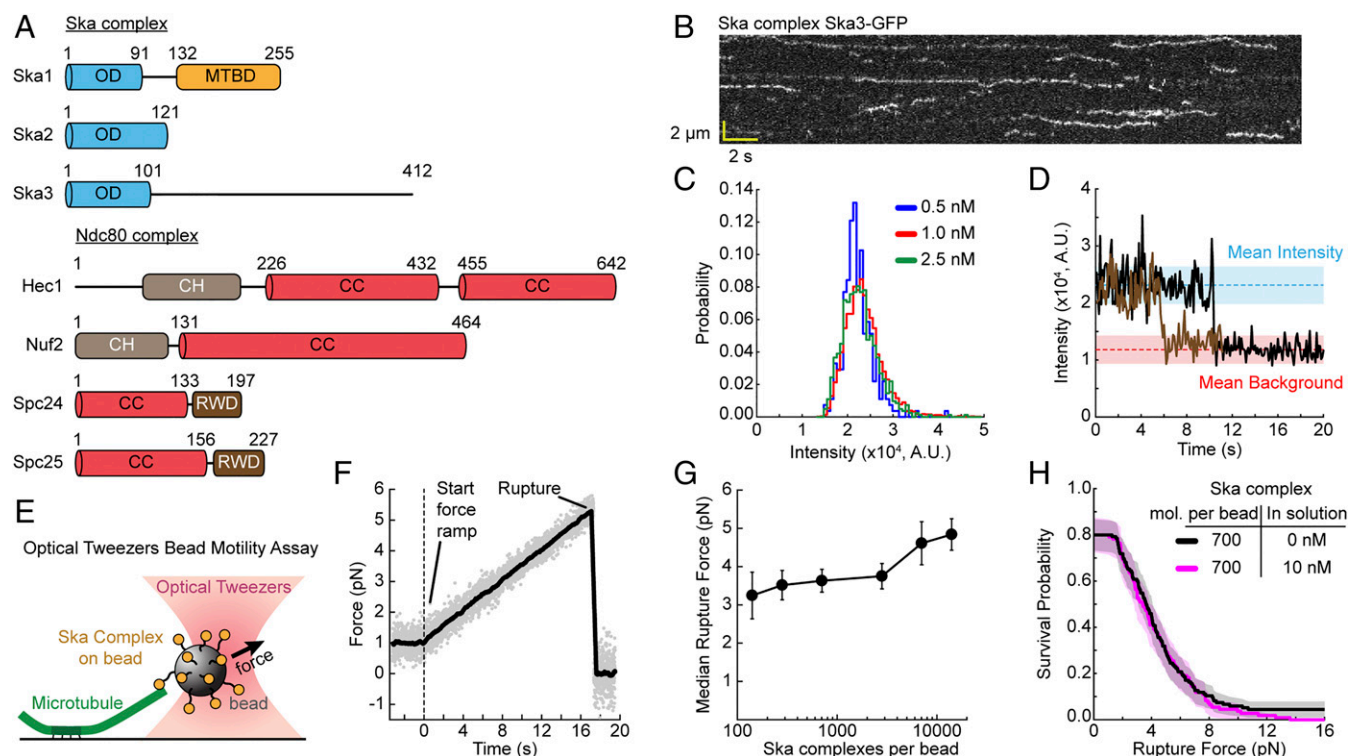
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**Fig. 1.** Ska complex bears load on microtubule ends. (A) Domain architecture of the Ska complex and Ndc80 complex. CC, coiled-coil; CH, Calponin homology domain; MTBD, microtubule binding domain; OD, oligomerization domain; RWD, RING finger, WD repeat, DEAD-like helicases domain. (B) Example kymograph of Ska complex<sup>Ska3-GFP</sup> molecules binding a microtubule. (C) Histogram of tracked Ska complex<sup>Ska3-GFP</sup> particle intensities for three different concentrations. (D) Two example intensity versus time traces of tracked Ska complex<sup>Ska3-GFP</sup> particles. After loss of particle tracking, due to dissociation or bleaching, the background was sampled for several frames to calculate the background intensity. Blue dashed line indicates the mean particle intensity for all tracked molecules; red dashed line indicates the mean background intensity. Shaded regions are SD. (E) Cartoon of the optical-tweezers-based bead motility assay with Ska complex attached to the beads. A bead coated in Ska complexes is bound to the end of a dynamic microtubule. Using the optical tweezers, a force is applied that pulls on the Ska complex–microtubule connection. (F) Raw data of a Ska complex rupture force experiment (gray). Black line is data smoothed using a 50-point sliding window. Vertical dashed black line indicates start of force ramp. (G) Median 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.

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<sup>Ska3-GFP</sup>) bound and diffused along Taxol-stabilized microtubules, as reported previously, and similarly to the lattice diffusion of other kinetochore components (Fig. 1B) (15, 17–19). The mean residence time of Ska complex<sup>Ska3-GFP</sup> particles on microtubules was  $5.2 \pm 0.1$  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<sup>Ska3-GFP</sup> 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<sup>Ska3-GFP</sup> 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). Median rupture strengths for populations of Ska complex-coated beads were 3–5 pN, depending on the surface density (Fig. 1G, Fig. S2A, and Table S1). These observations show that tip couplers based on purified Ska complex alone can bear significant loads.

Previous work shows that Ndc80 complex microtubule attachments are strengthened through avidity. Increasing the surface

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. 1*G*, and see Fig. 3*D*). 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. 1*H*). 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.

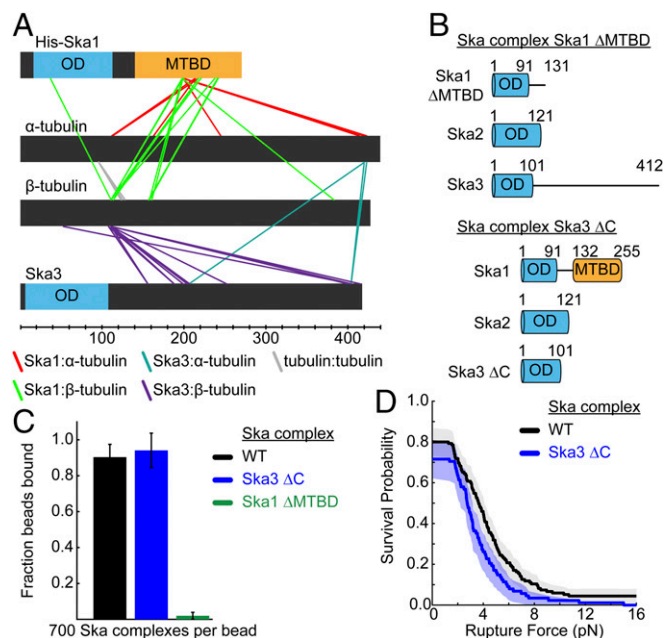
**Ska Complex Ska3 C Terminus Is Not Required for Load Bearing.** To identify interacting regions between the Ska complex and microtubules, we performed cross-linking mass spectrometry of Ska complex incubated with Taxol-stabilized microtubules. In agreement with previous reports, we observed cross-links between microtubules and the Ska1 C-terminal microtubule binding domain (MTBD) as well as between microtubules and the Ska3 unstructured C terminus (residues 102–402) (Fig. 2*A* 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  $\Delta$ MTBD) or the Ska3 C terminus (Ska3  $\Delta$ C) (Fig. 2*B*). Beads coated with mutant Ska complex<sup>Ska1  $\Delta$ MTBD</sup> 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<sup>Ska3  $\Delta$ C</sup> that bound microtubules was similar to wild type (Fig. 2*C*), and their end attachment strength was only slightly reduced (by 1.3-fold; Fig. 2*D* 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. 3*A*). 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. 3*B* and *D*, Fig. S2*B*, 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.

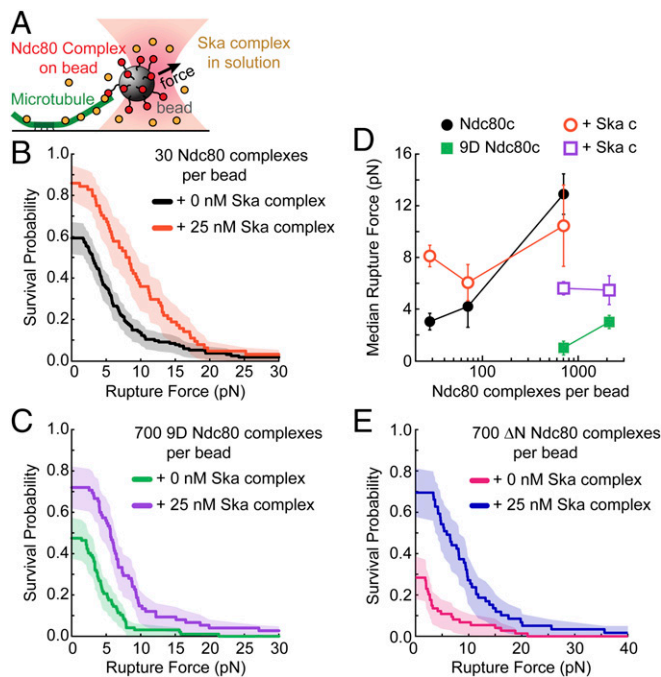
Next, we tested whether the Ska complex could strengthen Ndc80 complex-based attachments that were weakened due to a decreased affinity between the Ndc80 complex and the microtubule. We introduced Aurora B phosphomimetic mutations (serine/threonine to aspartate) in all nine phosphorylation sites in the 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. 3*C*). Adding free Ska complex increased the attachment strength of the mutant 9D Ndc80 complex by more than fivefold (Fig. 3*C* 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. 3*D* and Fig. S2*C*). Furthermore, we tested a mutant Ndc80 complex lacking the entire unstructured N-terminal 80-aa tail of Hec1 ( $\Delta$ N Ndc80 complex). As expected, this mutant  $\Delta$ N 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. 3*E*). 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  $\sim$ 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. 4*A* 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. 4*C* and *D* and Tables 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



**Fig. 2.** Ska3 C terminus is not required for Ska complex load bearing on microtubule ends. (A) Cross-links identified between tubulin and Ska1 or Ska3. Cross-linking reaction with Ska complex and Taxol-stabilized microtubules was performed for 30 min with the amine-to-carboxyl cross-linker EDC. Intra-Ska complex and Ska2 cross-links are not shown for clarity; see Fig. S3 for all cross-links identified and [https://proxl.yeastrc.org/proxlv/viewProject.do?project\\_id=49](https://proxl.yeastrc.org/proxlv/viewProject.do?project_id=49) for all data. (B) Domain architecture of the Ska complex mutants. MTBD, microtubule binding domain; OD, oligomerization domain. (C) Fraction of Ska complex-coated beads that bound to microtubules: Wild-type Ska complex (black), Ska complex<sup>Ska3 $\Delta$ C</sup> (blue), Ska complex<sup>Ska1 $\Delta$ MTBD</sup> (green). Error bars are counting uncertainty. (D) Rupture force survival probability plot for 700 Ska complex molecules per bead (black) and 700 Ska complex<sup>Ska3 $\Delta$ C</sup> mutant molecules per bead (blue). Shaded areas are 95% confidence intervals from Kaplan–Meier analysis.





**Fig. 3.** Ska complex strengthens Ndc80 complex microtubule attachments. (A) Schematic of the optical-tweezers-based bead motility assay with Ndc80 complex attached to the bead and Ska complex free in solution. (B) Rupture force survival probability plot for 30 Ndc80 complex molecules per bead without (black) and with (orange) 25 nM Ska complex in solution. (C) Rupture force survival probability plot for 700 9D Ndc80 complex molecules per bead without (green) and with (purple) 25 nM Ska complex in solution. (D) Median rupture force versus Ndc80 complex molecules per bead. Error bars are SD from bootstrapping analysis of the median. Closed symbols are Ndc80 complex on the bead, and open symbols are Ndc80 complex on the bead with Ska complex in solution. The median values and errors are calculated from the same datasets shown in B, C, and Fig. S2 B and C. (E) Rupture force survival probability plot for 700  $\Delta$ N Ndc80 complex molecules on the bead without (magenta) or with (blue) 25 nM Ska complex in solution. All shaded regions on survival probability plots are 95% confidence intervals from Kaplan–Meier analysis.

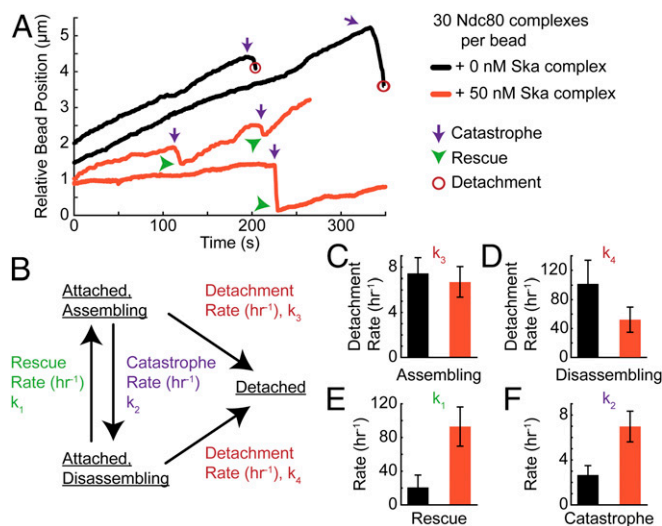
show that Ska complex enhances Ndc80 complex-based attachment in several situations where the coupling would otherwise be relatively poor: when avidity is reduced by lowering the number of participating Ndc80 complexes, when affinity is reduced by adding phosphomimetic mutations in the Hec1 tail or removing the tail, or when attachments are intrinsically destabilized by disassembly of the microtubule tip.

**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. 4 A 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

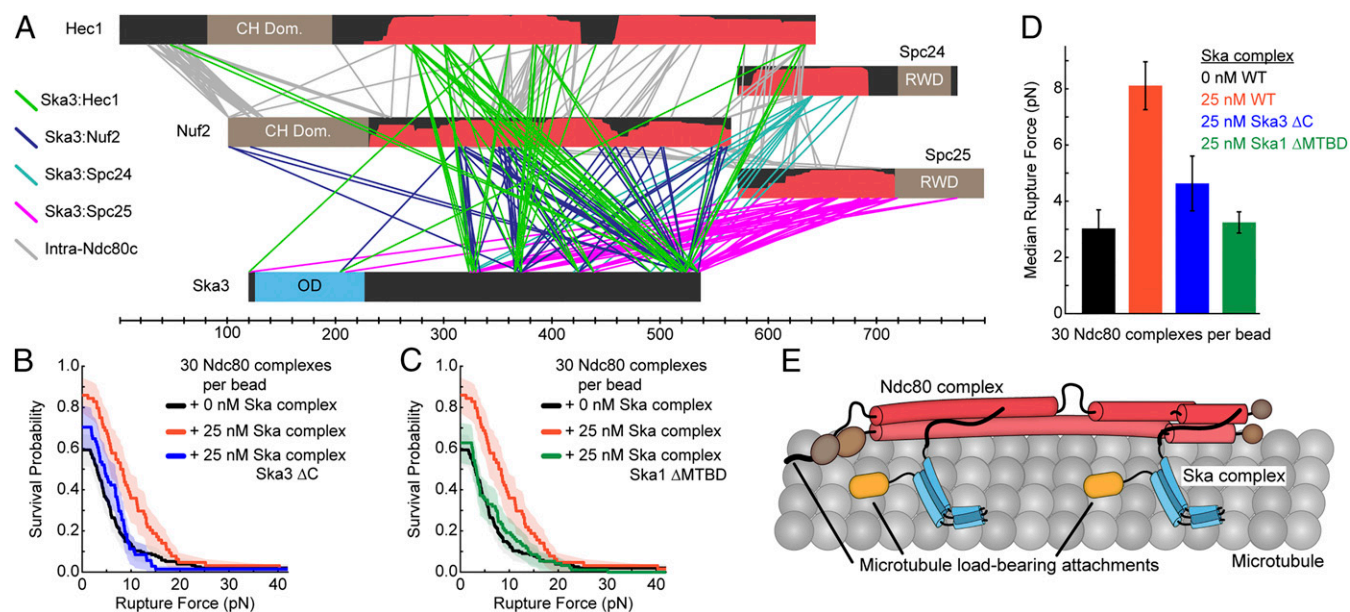
for attached tips by 2.7-fold (Fig. 4F). These results show that the Ska complex changes how the Ndc80 complex governs microtubule behavior and suggests that together they may increase the switching frequency of kinetochore-bound microtubules.

**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



**Fig. 4.** Ska complex affects the dynamics of Ndc80 complex and bound microtubules. (A) Four example bead position versus time traces for 30 Ndc80 complex molecules per bead without (black) and with (orange) 50 nM Ska complex in solution. An average force of 2 pN was exerted on the bead. Purple arrows indicate catastrophe events; green arrowheads indicate rescue events; red open circles indicate detachment events. For clarity, the starting position of each trace is offset by an arbitrary amount. (B) Model of coupler microtubule detachment rates and microtubule switching rates measured from constant-force bead tracking experiments. (C–F) Measured rates for 30 Ndc80 complex molecules per bead without (black) and with (orange) 50 nM Ska complex in solution. Measured rates are (C) detachment rate from an assembling microtubule; (D) detachment rate from a disassembling microtubule; (E) rescue rate; and (F) catastrophe rate. Error bars are counting uncertainty.



**Fig. 5.** 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://proxi.yeastrc.org/proxi/viewProject.do?project\\_id=49](https://proxi.yeastrc.org/proxi/viewProject.do?project_id=49) for all data. Red shaded regions indicated predicted coiled-coil (Paircoils2) 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<sup>Ska3  $\Delta$ C</sup> mutant (blue) or Ska complex<sup>Ska1  $\Delta$ MTBD</sup> 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. (E) Possible model of how Ska complex and Ndc80 complex directly interact to form multiple microtubule load-bearing attachments.

linkage between the Ndc80 complex and the microtubule. However, it is possible that the Ska complex-dependent enhancement of Ndc80 complex-based coupling occurs indirectly, where Ska complex affects microtubule tip structure in a way that enhances tip binding of the Ndc80 complex. To test this possibility, we measured the strength of Ndc80 complex-based tip couplers after addition of a truncated Ska complex, missing the major Ndc80 complex interaction site within the Ska3 C terminus (Ska complex<sup>Ska3  $\Delta$ C</sup>). Deletion of the Ska3 C terminus nearly abolished the ability of the Ska complex to strengthen the Ndc80 complex-based tip attachments (Fig. 5 B and D and Table S1), indicating that direct binding of the Ndc80 and Ska complexes is required for strengthening.

If the Ska complex enhances Ndc80 complex-based coupling by forming an extra linkage between the Ndc80 complex and the microtubule, then removing the major microtubule binding domain of the Ska complex should abolish the enhancement. Indeed, the mutant Ska complex<sup>Ska1  $\Delta$ MTBD</sup> was unable to strengthen Ndc80 complex attachments (Fig. 5 C and D). Cross-linking mass spectrometry with the mutant Ska complex<sup>Ska1  $\Delta$ MTBD</sup> found abundant cross-links between Ska3 and the Ndc80 complex, similar to wild type, suggesting that the mutant Ska complex<sup>Ska1  $\Delta$ MTBD</sup> retains normal interactions with the Ndc80 complex (Fig. S5). Together, these results support a model where Ska complex strengthens Ndc80 complex-based tip attachments by binding the Ndc80 complex directly and providing an additional load-bearing bridge to the microtubule (Fig. 5E).

## 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, tracks with and captures the forces generated by a depolymerizing microtubule tip is critical to understanding how kinetochores 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 (30).

Both the Ska and Ndc80 complexes are important targets of phosphoregulation 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 selective 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-tweezers 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-tweezers microscopy, cross-linking mass spectrometry, and SEC-MALS performed in this study.

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