Cell

# Implications for Kinetochore-Microtubule Attachment from the Structure of an Engineered Ndc80 Complex

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DOI 10.1016/j.cell.2008.03.020

## SUMMARY

Kinetochores are proteinaceous assemblies that mediate the interaction of chromosomes with the mitotic spindle. The 180 kDa Ndc80 complex is a direct point of contact between kinetochores and microtubules. Its four subunits contain coiled coils and form an elongated rod structure with functional globular domains at either end. We crystallized an engineered "bonsai" Ndc80 complex containing a shortened rod domain but retaining the globular domains required for kinetochore localization and microtubule binding. The structure reveals a microtubule-binding interface containing a pair of tightly interacting calponin-homology (CH) domains with a previously unknown arrangement. The interaction with microtubules is cooperative and predominantly electrostatic. It involves positive charges in the CH domains and in the N-terminal tail of the Ndc80 subunit and negative charges in tubulin C-terminal tails and is regulated by the Aurora B kinase. We discuss our results with reference to current models of kinetochore-microtubule attachment and centromere organization.

## INTRODUCTION

The mitotic spindle, a microtubule-based scaffold, captures, aligns, and separates the replicated chromosomes (sister chromatids) during mitosis (O'Connell and Khodjakov, 2007). The spindle assembly checkpoint detects defects in chromosome-spindle attachment and delays anaphase to prevent errors in chromosome segregation. These errors generate imbalances in chromosome numbers that are frequently observed in cancer cells (Musacchio and Salmon, 2007).

Stable chromosome-spindle attachments are formed through kinetochores, scaffolds of ~100 different proteins assembled at the periphery of centromeric DNA nucleosomes containing the histone H3 variant CENP-A (Cleveland et al., 2003; Maiato et al., 2004). *Saccharomyces cerevisiae* has compact kinetochores that attach to a single microtubule (Cleveland et al., 2003; Joglekar et al., 2006; McAinsh et al., 2003; Meraldi et al., 2006; Tanaka et al., 2005). Most eukaryotes assemble larger kinetochores with multiple attachment sites (15–30) for the plus ends of spindle microtubules, which are organized in kinetochore fibers (Cleveland et al., 2003). At low resolution, the kinetochore of vertebrates appears as a trilaminar disk, with an electron-dense inner plate at the periphery of centromeric chromatin, an electron-lucent middle layer, and an electron-dense outer plate, the site of end-on binding of microtubule plus ends (Cleveland et al., 2003). Electron tomography-based reconstructions of the outer plate revealed a fibrous structure that undergoes significant reorganization upon end-on microtubule attachment (Dong et al., 2007).

The Ndc80 complex is a core component of the end-on attachment sites for kinetochore microtubules (Ciferri et al., 2007; Kline-Smith et al., 2005). Its depletion perturbs the architecture of the kinetochore outer plate and reduces the number of attached microtubules (DeLuca et al., 2005; Liu et al., 2006). The four subunits of the Ndc80 complex, named Ndc80 (human Ndc80 is also known as Hec1, for *h*ighly *e*xpressed in *c*ancer *1*), Nuf2, Spc24, and Spc25, assemble into a 170–190 kDa complex in different species (Ciferri et al., 2007; Kline-Smith et al., 2005; Maiato et al., 2004). All four subunits contain long coiled coils (Figure S1 available online). Low-resolution structural analyses of yeast and human Ndc80 complexes (Ciferri et al., 2005; Wei

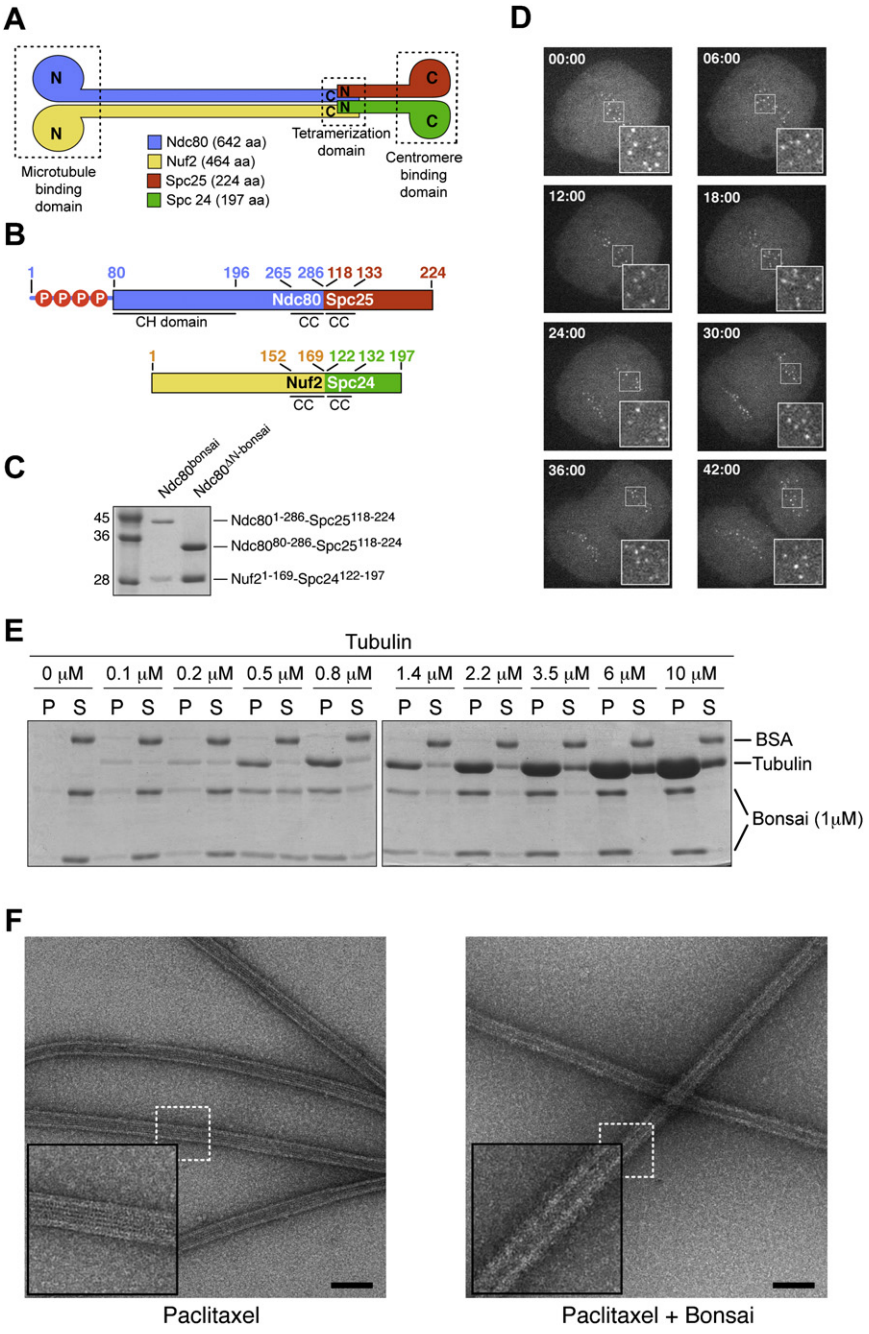


**Figure 1. Properties of Ndc80$^{bonsai}$**

(A) Organization of Ndc80 subunits.

(B) Scheme of Ndc80-Spc25 and Nuf2-Spc24 fusion proteins. Residues 1–286 of Ndc80 (Ndc80$^{1–286}$) were fused to residues 118–224 of Spc25 (Spc25$^{118–224}$). Residues 1–169 of Nuf2 (Nuf2$^{1–169}$) were fused to residues 122–197 of Spc24 (Spc24$^{122–197}$). Red circles with "P" mark phosphorylation sites in the Ndc80 N-terminal tail (Cheeseman et al., 2006; DeLuca et al., 2006; Wei et al., 2007).

(C) Ndc80-Spc25 and Nuf2-Spc24 fusions were coexpressed in *E. coli* and purified to homogeneity.

(D) When injected in HeLa cells, Alexa Fluor 488-labeled Ndc80$^{bonsai}$ stained kinetochores throughout mitosis.

(E) Partition of the Ndc80$^{bonsai}$ complex in pellet (P) and supernatant (S) fractions in cosedimentation assay with increasing concentrations of polymeric tubulin.

(F) Negative stain EM images of Paclitaxel-stabilized microtubules in the absence (left) and presence (right) of bound Ndc80$^{bonsai}$. A thick halo of protein surrounds the microtubules bound by Ndc80$^{bonsai}$, giving them a hairy appearance. Insets are 2.5×. Bar = 100 nm.

et al., 2005) revealed a long rod with globular domains at either end (Figure 1A). The globular regions of Nuf2 and Ndc80 occupy one end of the rod and are located near the N termini of these proteins (Ciferri et al., 2005; Wei et al., 2005). A heterodimeric coiled coil engaging Nuf2 and Ndc80 and emerging from the globular region accounts for most of the central shaft (Figures 1A and S1). The C-terminal end of the Nuf2-Ndc80 coiled coil encounters the N-terminal coiled coil of an Spc24-Spc25 subcomplex. The latter extends to the distal end of the rod, which contains a compact globular domain formed by the interacting heads of Spc24 and Spc25 (Ciferri et al., 2005; Wei et al., 2005, 2006). In vitro, Ndc80-Nuf2 and Spc24-Spc25 form stable subcomplexes that can self-assemble into the ~57 nm full-length Ndc80 complex (Ciferri et al., 2005; Wei et al., 2005).

Both globular regions of the Ndc80 complex are functionally important. The globular region of Spc24-Spc25 is important for kinetochore localization. It maps "internally" (i.e., closer to the centromere) relative to the Ndc80-Nuf2 globular head (Bharadwaj et al., 2004; Ciferri et al., 2005; DeLuca et al., 2006; Gillett et al., 2004). Conversely, the globular head of the Ndc80-Nuf2 moiety binds to microtubules (Cheeseman et al., 2006; Wei et al., 2007). The globular region of the Ndc80 subunit folds as a calponin-homology (CH) domain (Wei et al., 2007). CH domains in different proteins have been implicated in actin or microtubule binding (Gimona et al., 2002; Korenbaum and Rivero, 2002). The isolated CH domain of Ndc80 binds poorly to microtubules, suggesting that additional segments of the Ndc80 complex are required to create a functional microtubule-binding interface (Wei et al., 2007).

By combining protein engineering, X-ray crystallography, mass spectrometry, and biochemical methods, we characterized the architecture of the Ndc80 complex and the organization of the microtubule-binding interface of the Ndc80 complex.