How Kinetochores CCAN Resist Force

Andrew D. McAinsh[1,*]
[1]Mechanochemical Cell Biology Building, Division of Biomedical Cell Biology, Warwick Medical School, University of Warwick, Coventry CV4 7AL, UK
*Correspondence: a.d.mcainsh@warwick.ac.uk
http://dx.doi.org/10.1016/j.devcel.2014.09.007

Kinetochores orchestrate chromosome segregation during mitosis and must cope with dynamic forces generated by attached microtubules. In this issue of *Developmental Cell*, Suzuki et al. (2014) demonstrate that the constitutive centromere-associated network (CCAN) displays a complex architecture that plays a crucial role in resisting these forces.

Kinetochores play a critical role in ensuring proper chromosome segregation during mitosis and comprise one of the most complex protein machines within the eukaryotic cell. They contain >150 proteins, most of which are present in multiple copies, leading to an overall mass of several megadaltons. The resulting 1-μm-diameter disk-shaped structure engages with the dynamic plus ends of up to 25 individual microtubules. The coordinated assembly or disassembly of these microtubules, and the kinetochore's capacity to remain attached during both of these phases, generate pulling and pushing forces that maneuver chromosomes, ultimately segregating them to daughter cells in anaphase (Rago and Cheeseman, 2013). A new study from Suzuki and colleagues in this issue of *Developmental Cell* now reveals that the constitutive centromere-associated network (CCAN) of kinetochore proteins is required for the overall structure to resist forces generated by spindle microtubules, thus allowing normal chromosome segregation (Suzuki et al., 2014).

The CCAN protein network consists of 16 protein components, termed CENPs, that form the core of the inner kinetochore (Westhorpe and Straight, 2013) (Figure 1A). An outstanding challenge is to decipher the function of the CCAN network within the kinetochore. Evidence suggests a structural role for CCAN in kinetochore assembly, based largely on the finding that the CCAN interacts with both centromeric chromatin on its interior side and the outer kinetochore KMN network on its exterior side via the CCAN linker proteins CENP-T and CENP-C. The KMN network, a set of three multiprotein complexes (Ndc80, Mis12, and Knl-1), mediates the direct interaction of kinetochores with the lattice of bound microtubules (Figure 1A). CENP-N binds the nucleosome component CENP-A and, together with CENP-T/C, recruits additional CCAN proteins. Unsurprisingly, depletion or mutation of CCAN subunits gives rise to pleiotrophic mitotic phenotypes (Westhorpe and Straight, 2013).

The use of superresolution imaging has provided major insights into how individual kinetochore components are spatially organized within the larger overall structure, pinpointing the relative distance between kinetochore components with subpixel accuracy (Wan et al., 2009). Now, Suzuki, Salmon, and colleagues (Suzuki et al., 2014) use the same methods to generate a nanometer-scale map of the amino and carboxy termini of 13 CCAN subunits, providing new insights into how these proteins are organized within metaphase kinetochores and how this complex responds to spindle forces. Their experiments confirm the expected position of many CCAN proteins but also reveal a number of exciting new features. First, CENP-I, which is believed to be restricted to the inner kinetochore that contacts centromeric chromatin, in fact extends outward toward the microtubule network by 18 nm, placing the extended CCAN in close proximity to the plus end of microtubules (Figure 1A). Second, the CENP-Q/U dimer spans 15 nm from the position of the microtubule plus end toward the centromeric chromatin. This is in agreement with biochemical studies showing that CENP-Q binds microtubules in vitro (Amaro et al., 2010) while the budding yeast orthologs can bind DNA (Hornung et al., 2014).

One surprising finding to emerge from these studies was that all of the relationships between kinetochore components were found to be noncompliant, i.e., delta (the relative distance between positions of kinetochore components) remains constant even as microtubule forces pull the kinetochores away from each other. This includes the linkage between Ndc80 and CENP-A, which, based on earlier measurements, was shown to be compliant (Wan et al., 2009). Additionally, the authors found that CENP-T molecules are only extended by ~19 nm in response to microtubule forces, despite possessing a potential extension of 90 nm in vitro (Suzuki et al., 2011). These results suggest the presence of mechanisms to prevent hyperstretching of the inner kinetochore.

In support of this, the authors provide evidence for a "stretch-restriction" mechanism that is mediated by CENP-T, CENP-C, and a complex containing CENP-H (HIKM). Loss of any one of these linkers causes the inner kinetochore (defined as the distance between CENP-A and the Ndc80 complex; Figure 1A) to extend to the length of the remaining linkers, while the outer kinetochore (defined by the length of the Ndc80 complex; Figure 1A) retains a fixed length (Figure 1B, middle). The maximum stretch observed was seen following small interfering RNA (siRNA)-mediated knockdown of CENP-H and CENP-C, where extension of CENP-T from 16 to 40 nm (Figure 1B, bottom) consequently caused the inner kinetochore to become compliant and, surprisingly, resulted in decompaction of centromeric chromatin (Figure 1B). This may reflect previous studies that implicated CCAN in the deposition of CENP-A nucleosomes (Hori et al., 2013). Overall, these experiments led the authors to conclude that the kinetochore is "stiff," but some conformational changes are still possible: this is exemplified by the finding that the CENP-A/CENP-T/Ndc80 linkage

becomes 10 nm longer between late prometaphase and metaphase (Figure 1B, top). The question remains as to whether this conformational change is dependent upon microtubule tension.

Phosphorylation of Ndc80 by Aurora B kinase destabilizes microtubule attachments to the kinetochore. This process allows correction of inappropriate attachments that do not generate sufficient stretching and that might otherwise lead to faulty chromosome attachment (e.g., syntelic or monotelic states) and result in segregation errors during mitosis if not corrected (Lampson and Cheeseman, 2011). Aurora B activity decreases as distance from the centromere increases, and Suzuki and colleagues consistently found that incremental stretching of the inner kinetochore, resulting from siRNA-mediated depletion of CCAN components, correlated with progressive reduction of Ndc80 phosphorylation and stabilization of microtubule attachment. The authors propose that the restriction of stretch seen in prometaphase keeps Ndc80 within range of Aurora B and allows for destabilization and correction of microtubule attachment errors.

Electron microscopy images of the CENP-HIKM complex have recently emerged (Basilico et al., 2014). It will be important to map the positions of CCAN proteins within these structures and integrate this knowledge with the nanometer-scale positioning described by Suzuki et al. However, one caveat is that Suzuki et al. used fixed cells, thus producing mean delta values that may potentially miss dynamic changes in protein architecture. Furthermore, the measurements were calculated as an average of both sister kinetochores, which may also be misleading, as we now know that the forces acting on the two sister kinetochores differ. Indeed, Dumont and colleagues found that the poleward-moving kinetochore is more compressed than its sister (Dumont et al., 2012). Thus, whether hyperstretching and/or conformational changes within CCAN happen on one or both kinetochores will be an important area of future study. Nevertheless, this beautiful work represents a major step forward in understanding how the architecture of the kinetochore allows it to coordinate mechanical and chemical processes that drive the cycles of microtubule attachment, error correction, and force generation necessary for accurate chromosome segregation.

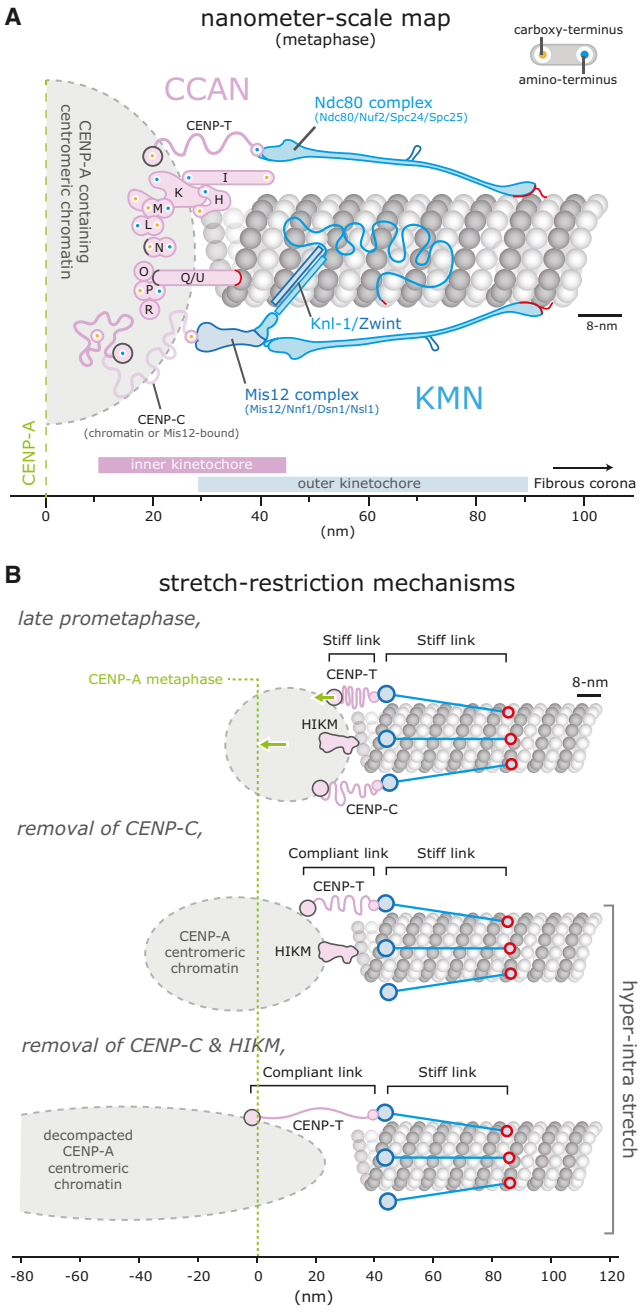

Figure 1. Architecture of the CCAN

(A) Nanometer-scale map of a human mitotic kinetochore during metaphase. Protein components of the KMN and CCAN networks are shown in blue and pink, respectively. Known direct contacts with DNA (black) and microtubules (red) are indicated. Blue and orange spots on proteins denote amino and carboxy termini. Position of microtubule plus end is based on Wan et al. (2009). (B) CCAN components operate as a stretch-restriction mechanism. Top: kinetochore in prometaphase. Blue complexes represent outer kinetochore microtubule-binding complexes. Note the conformational change in CENP-A-to-CENP-T-to-Spc24 linkage (green arrows) that occurs in metaphase. Removal of CENP-C (middle) or CENP-C and CENP-HIKM (bottom) causes a hyperstretching of kinetochore that involves decompaction of the centromeric chromatin.

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