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Investigating Kinetochore Structural Dynamics during Mitosis by Employing Combinations of Several Microscopic Techniques

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The kinetochore attaches a chromosome to the mitotic spindle and harnesses forces that move the chromosome [1]. Recent studies indicate that kinetochores possess compliant linkages that contribute to mitotic checkpoint signaling [2]. Deformation of these elements are proposed to account for the differences in the distance between populations of labeled inner and outer plate proteins (intrakinetochore stretch) observed by light microscopy (LM). However, the underlying structural basis for intrakinetochore stretch remains unknown. To investigate this, we employ combinations of several microscopic techniques to investigate the change of the kinetochore from an expanded form to a contracted one during microtubule (MT) interaction, and how this change is reversed by drugs affecting MT polymerization. Fluorescent markers for outer and inner kinetochore proteins and for the spindle poles determine the orientation of sister kinetochores with respect to poles and to the centromere and allow precise determination of intrakinetochore stretch for individual kinetochores. By superimposing LM fluorescence on the distribution of gold particles in the corresponding EM images, we compare the shapes of kinetochores with high verses low values of intrakinetochore stretching (Figures 1 and 2).

The human cell line RPE1 is used which is chromosomally stable, maintaining a near-diploidy number of 46 chromosomes. The microscopic techniques employed in different combinations are immuno-LM (GFP-tagged and FluoroNanogold-tagged), immuno-EM (FluoroNanogold-tagged), DIC, serial section EM, and EM tomography. An important point of this study is that we can locate individual kinetochores and their sisters, among the 92 kinetochores present in a single cell, in both the LM images and EM images, and superimpose the LM image over the EM one.

We found that kinetochores are expanded early in spindle formation and are contracted during metaphase when the chromatin between sister kinetochores is the most stretched, creating the most tension. Drugs that effect MT polymerization reverse this process by expanding contracted kinetochores that have lost their MT attachments. The changes in size occur at the outer kinetochore region, while the inner region remains unchanged. Rather than a plate, the outer kinetochore region radically expands and contracts, depending on its interactions with MTs.

References:
[1] I. M. Cheeseman, Cold Spring Harb. Perspect. Bol. **6**(7) (2014), a0 15826.
[2] X. Wan *et al*, Cell **137**(4) (2009), 672.

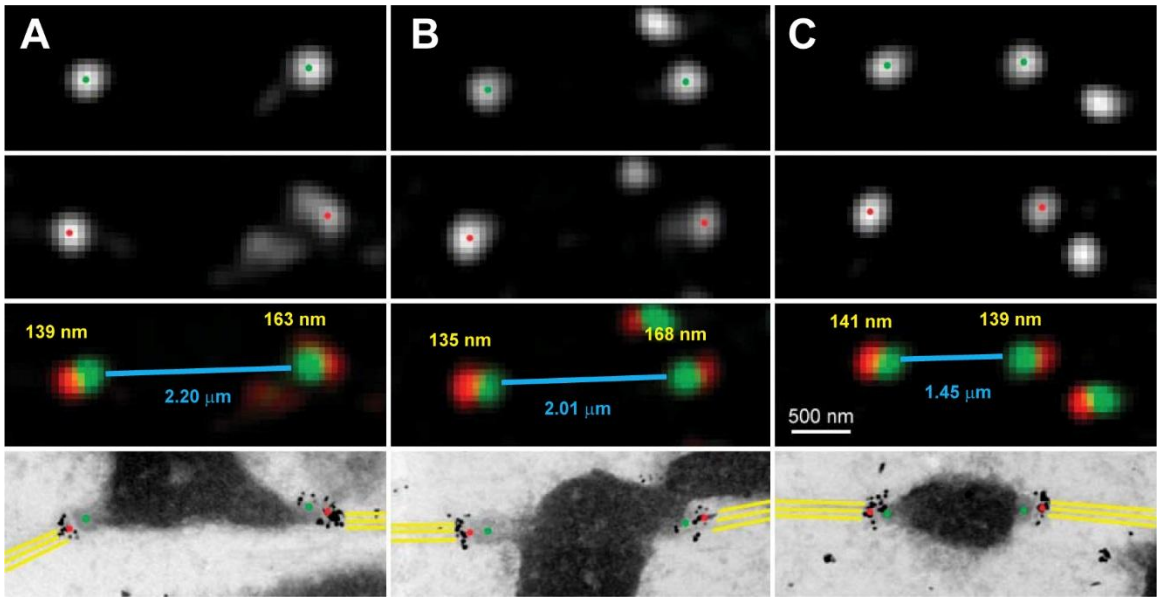

Figure 1. (A-C) Examples of metaphase kinetochores with various level of intra- and inter-kinetochore stretching. Positions of the centroids and exact values of CenpA-Hec1 distance (Delta) as well as interkinetochore distances (Hec1-Hec1) are shown for each kinetochore/centromere. Notice that kinetochores can remain compact even on extremely stretched centromeres (A). Also, deformation of the centromere is often asymmetric (B). Finally, separation of the inner and outer- kinetochore proteins consistently occurs along the axis of the attached microtubule bundle (denoted with yellow lines).

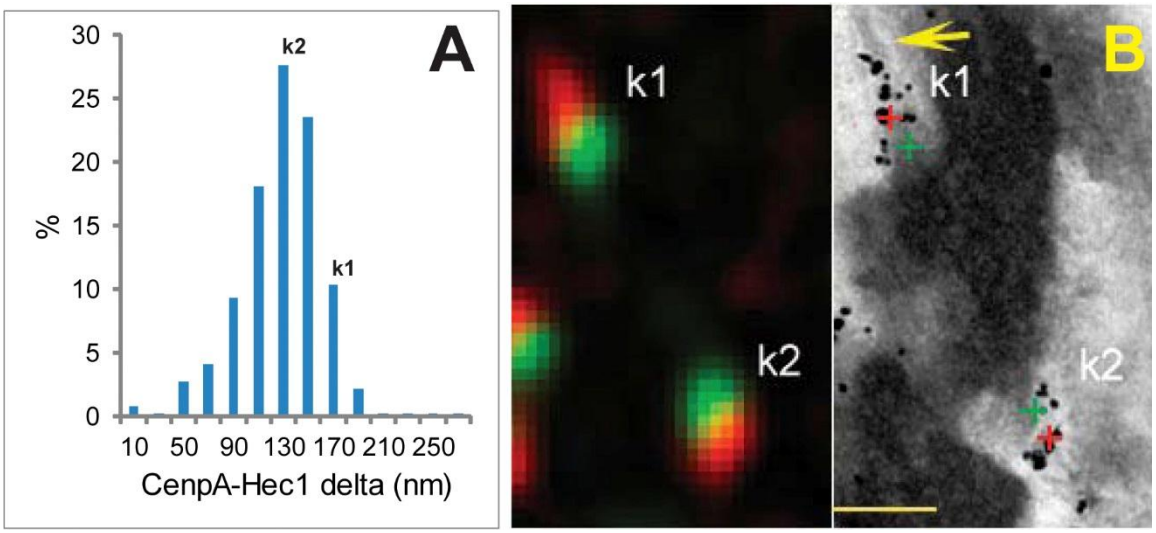

Figure 2. (A) Histogram of CenpA-Hec1 distances (Delta) in metaphase cells. **(B)** Direct LM/EM comparison of Hec1 distribution for metaphase kinetochores with high (k1, CenpA-Hec1=173 nm) vs. average (k2, CenpA-Hec1=137 nm) intrakinetochore stretching. As evident from the distribution of gold particles, the outer plate is more compact (gold particles form a tighter cluster) in the moderately-stretched k2. In highly stretched k1, Hec1 extends along the attached MT bundle. Notice that while GFP is not directly visualized in EM, its position is revealed when LM and EM images are superimposed. Color crosses mark centroids of Hec1 (red) and CenpA (green) distributions. Bar = 500 nm.