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Article

Bub1 Kinase and Sgo1 Modulate Pericentric Chromatin in Response to Altered Microtubule Dynamics

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Summary

Background: Tension sensing of bioriented chromosomes is essential for the fidelity of chromosome segregation. The spindle assembly checkpoint (SAC) conveys lack of tension or attachment to the anaphase promoting complex. Components of the SAC (Bub1) phosphorylate histone H2A (S121) and recruit the protector of cohesin, Shugoshin (Sgo1), to the inner centromere. How the chromatin structural modifications of the inner centromere are integrated into the tension sensing mechanisms and the checkpoint are not known.
Results: We have identified a Bub1/Sgo1-dependent structural change in the geometry and dynamics of kinetochores and the pericentric chromatin upon reduction of microtubule dynamics. The cluster of inner kinetochores contract, whereas the pericentric chromatin and cohesin that encircle spindle microtubules undergo a radial expansion. Despite its increased spatial distribution, the pericentric chromatin is less dynamic. The change in dynamics is due to histone H2A phosphorylation and Sgo1 recruitment to the pericentric chromatin, rather than microtubule dynamics.
Conclusions: Bub1 and Sgo1 act as a rheostat to regulate the chromatin spring and maintain force balance. Through histone H2A S121 phosphorylation and recruitment of Sgo1, Bub1 kinase softens the chromatin spring in response to changes in microtubule dynamics. The geometric alteration of all 16 kinetochores and pericentric chromatin reflect global changes in the pericentromeric region and provide mechanisms for mechanically amplifying damage at a single kinetochore microtubule.

Introduction

The fidelity of chromosome segregation relies upon the ability of the cell to monitor chromosome biorientation on the mitotic spindle. This monitoring system is known as the spindle assembly checkpoint (SAC). The checkpoint relays structural information from the centromere and/or kinetochore to the cell-cycle regulatory machinery. The requirements for accurate segregation include attachment of spindle microtubules to the kinetochore and biorientation of sister kinetochores to opposite poles [1, 2]. The centromere designates the position of the kinetochore within the chromosome. The kinetochore is a multisubunit protein/DNA complex containing more than 65 proteins organized into 8–9 distinct biochemical complexes [3, 4]. In mammals, the kinetochore is a multiple microtubule attachment site (20–25 microtubules per kinetochore), whereas in budding yeast the kinetochore binds a single microtubule [5]. The kinetochore couples chromosome movement to microtubule dynamics and undergoes conformational changes as a function of tension between the sister kinetochores [6].

Cohesin, the protein complex that binds sister DNA strands following DNA replication, is required for generating tension between sister chromatids [7, 8]. Cohesin, together with a related complex, condensin, are enriched in pericentric chromatin in budding yeast [7–9]. Cohesin and condensin are cylindrically arrayed around the mitotic spindle with cohesin radially displaced and condensin proximal to the microtubule spindle axis [10, 11]. The complexes are required for chromatin compaction and spindle length regulation and therefore contribute to mitotic force balance mechanisms [11]. Cohesin at the pericentromere is protected from premature degradation in meiosis I by Shugoshin (Sgo1). Shugoshin is recruited to the centromere via the Bub1 kinase [12–14] where it contributes to mechanisms responsible for orienting sister centromeres to opposite poles [15].

In metaphase, bioriented sister kinetochores separate into two foci that reside at the kinetochore microtubule plus-ends (Figure 1A). Each focus represents the aggregate of 16 individual microtubule attachment sites. The separation between sister kinetochores is about 800 nm, similar to the separation found between sister kinetochores in mammalian cells [16–19]. This finding, together with recent studies on the position of proteins within the kinetochore, reveals remarkable conservation in both their number and spatial positions in yeast and mammals [20–22]. Thus, the study of how the 16 clustered kinetochores behave in budding yeast is likely to inform our understanding of how a kinetochore with multiple attachments behaves. The focus containing 16 kinetochores is larger than a diffraction limited spot and the architecture (radius, shape) of the cluster is dictated by the position of individual sites within the multiple attachment site. Likewise, the dimensions of the cylindrical array of cohesin provide positional information for the organization of pericentric chromatin. Whereas individual kinetochore proteins and cohesin subunits are stable throughout mitosis [23–25], the aggregate kinetochore cluster as well as the pericentric chromatin and cohesin barrel are dynamic and exhibit geometric changes in response to reduced microtubule dynamics. We demonstrate that the Bub1 kinase regulates these structures through histone H2A phosphorylation and recruitment of Sgo1 to the pericentric chromatin. This study reveals a new mechanism for how histone modification can amplify small changes in tension into large scale geometric changes in living cells.

Results

The Kinetochore Cluster Exhibits an Anisotropic Flare at the Chromosome Surface

The geometric size and shape of the clustered 16 kinetochores in metaphase can be determined using the mitotic spindle as reference. The metaphase spindle is on average 1.3 μm in length and 0.25 μm in diameter. The full width of a two-dimensional Gaussian function fitted to each kinetochore cluster is measured perpendicular (y axis) and parallel (x axis) to the spindle axis (denoted as height and width, respectively)

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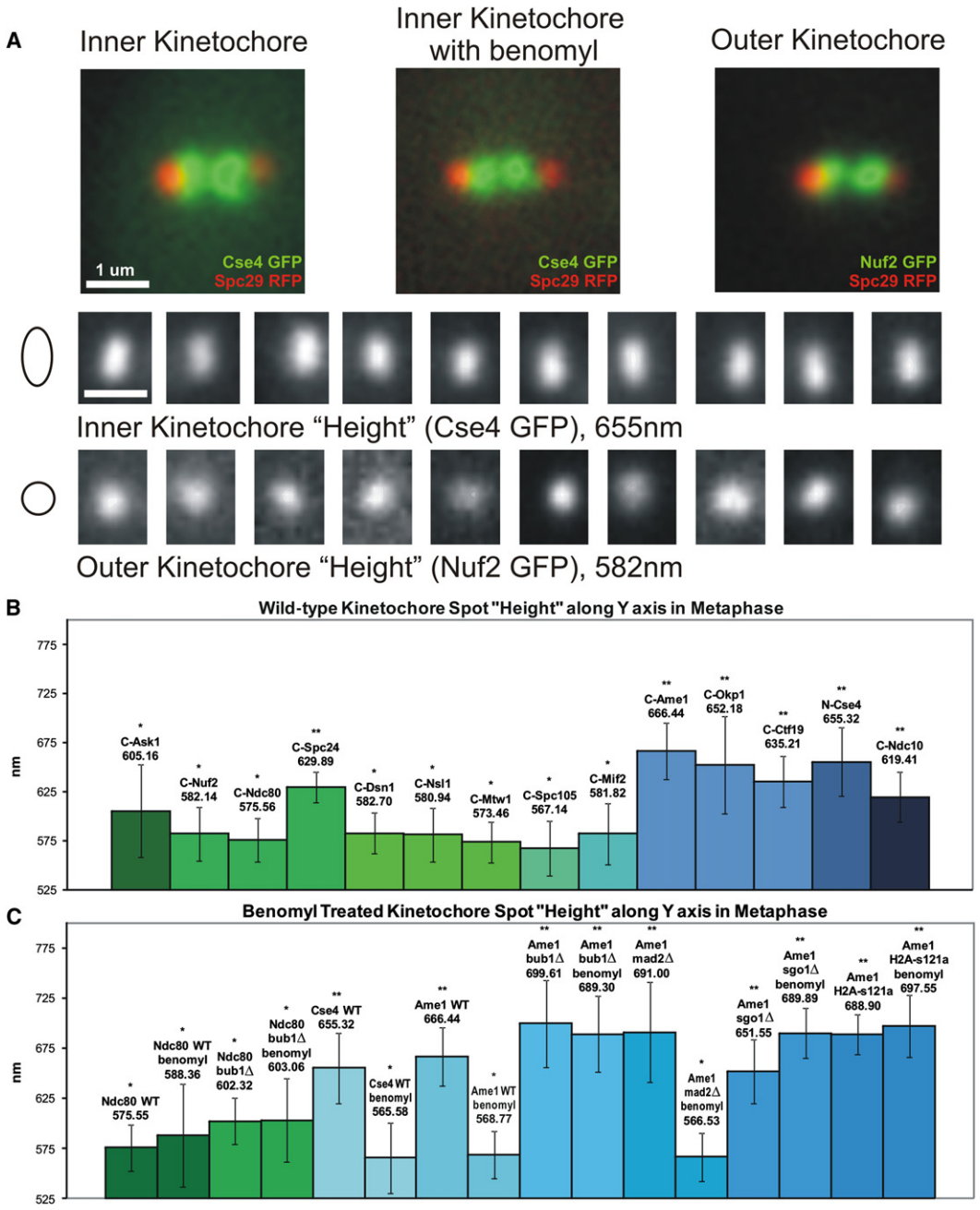

Figure 1. Radial Extension of Inner Kinetochore Proteins

(A) Metaphase spindles in budding yeast. The spindle poles are labeled with Spc29-RFP and kinetochores with Cse4-GFP (left), Cse4-GFP benomyl-treated cells (middle), or Nuf2-GFP (right). Individual panels of fluorescent foci of the centromere histone variant Cse4 and an outer kinetochore protein Nuf2 from metaphase cells are shown. Kinetochore foci were measured by fitting to a Gaussian distribution to the fluorescent spot. The width of the spot was determined from the full-width full-maximum (FWFM) of the Gaussian distribution. Foci of Cse4-GFP extend perpendicular to the spindle axis (655 nm y axis by 520 nm x axis, see Figure S1), as depicted by the oval to the left (upper panel). The microtubule proximal components (outer kinetochore Nuf2-GFP) are 582 nm in height (y axis) and 536 nm in width (x axis, see Figure S1), depicted by the circle to the left (lower panel). The aspect ratio (height/width) for Cse4 is 1.23 versus 1.07 of Nuf2. Scale bars represent 1 μm.

(B) Dimensions of kinetochore clusters. The distribution of fluorescence of the indicated kinetochore proteins fused to GFP perpendicular to the spindle (y axis). The microtubule proximal components (NDC80 complex to Mif2p) are shown in dark green to light green-blue. The COMA complex (Ctf19, Okp1, Ame1) and DNA-binding components Cse4p and Ndc10, are shown in light to dark blue. The asterisks denote statistically significant differences ($p < 0.05$) in the y dimension. The outer kinetochore components (NDC80, MIND, Mif2) are not different from each other (*), but statistically different from the inner kinetochore components (COMA, Cse4, Ndc10) (**), as determined by the Student's t test. Spc24 (of the NDC80 complex) is similar to the inner components (see text). Error bars represent −/+ 1 SD. Each determination represents a minimum of 50 individual measurements.

(C) Dimensions of kinetochore clusters in low concentrations of benomyl. The distribution of fluorescence along the y axis (height) of the inner (Cse4, Ame1) and outer kinetochore components (Ndc80) are shown in WT, *bub1Δ*, *mad2Δ*, *sgo1Δ*, and H2A-S121A with or without exposure to benomyl. The concentration of benomyl (10 μg/ml) results in spindle shortening, not collapse [28]. Outer kinetochore component Ndc80 is unaffected by benomyl treatment (green). Significant changes in the distribution of fluorescence along the y axis are evident in inner kinetochore components (blue) upon benomyl treatment