Relaxation of interkinetochore tension after severing of a k-fiber depends on the length of the k-fiber stub

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Abstract
During mitosis, tension forces acting on kinetochores are required for passage through the spindle assembly checkpoint and for chromatid segregation. It is generally thought that the interkinetochore tension is generated by molecular events occurring at the ends of k-fibers, bundles of microtubules bound to kinetochores. However, recent work has shown that bridging microtubules, which interact laterally with sister k-fibers and act as a bridge between sister kinetochores, contribute to interkinetochore tension. We set out to test the role of the bridging fibers in the origin of interkinetochore tension during metaphase in human U2OS and rat-kangaroo PtK1 cells. We severed k-fibers at different distances from the kinetochore and measured the change in the interkinetochore distance, which we use as a readout of the change in interkinetochore tension. We found that severing of a k-fiber closer to the kinetochore results in a larger relaxation of the interkinetochore tension than severing far from the kinetochore. These findings imply that a long k-fiber stub, which remains connected to the bridging fiber, withstands the interkinetochore tension and prevents relaxation of the interkinetochore distance, whereas a short stub does not. Thus, our results are consistent with a role of bridging microtubules in the origin of interkinetochore tension.

Introduction
During mitosis, correct segregation of genetic material requires that sister chromatids of each chromosome attach to microtubules extending from the opposite spindle poles. The attachment of microtubules to chromosomes is mediated by kinetochores, protein complexes on the chromosome [1]. Microtubules bound to kinetochores form bundles known as k-fibers, which generate tension on sister kinetochores. Interkinetochore tension is required for passage through the spindle assembly checkpoint [2] [3] [4] and for the subsequent segregation of sister chromatids. Even though forces acting on kinetochores are not easy to measure directly [5] [6], changes in interkinetochore tension can be deduced, for example, from the changes in interkinetochore distance after severing of a k-fiber. K-fibers have been cut in several studies, but the results were controversial. Maiato et al. observed that k-fiber ablation did not reduce the interkinetochore distance in *Drosophila* S2 cells [7], whereas others observed a decrease in interkinetochore distance in PtK2 cells [8], flattened RPE cells [9], and HeLa cells [10]. These differences may be due to differences in interkinetochore tension in diverse cell types, or to different locations of the severing. Indeed, Kajtez et al. found a larger relaxation of the interkinetochore distance when the severing was performed closer to the kinetochore [10]. This result can be explained by taking into account bridging fibers, microtubule bundles that link sister k-fibers and act as a bridge between sister kinetochores [10]. Severing close to the kinetochore (up to 1 µm away from the kinetochore) results in detachment of the bridging fiber from the k-fiber and thus in release of interkinetochore tension. Conversely, severing far from the kinetochore leaves the bridging fiber attached, preventing release of tension [10]. Yet, the role of bridging fibers in the regulation of interkinetochore tension has not been explored in cells other than HeLa.

Objective
We set out to test the role of the bridging fiber in the maintenance of interkinetochore tension during metaphase in two cell lines, human osteosarcoma cells (U2OS) and rat-kangaroo normal kidney epithelial cells (PtK1). We severed k-fibers systematically at different distances from the kinetochore and measured the change in the interkinetochore distance, which we use as a readout of the change in interkinetochore tension.

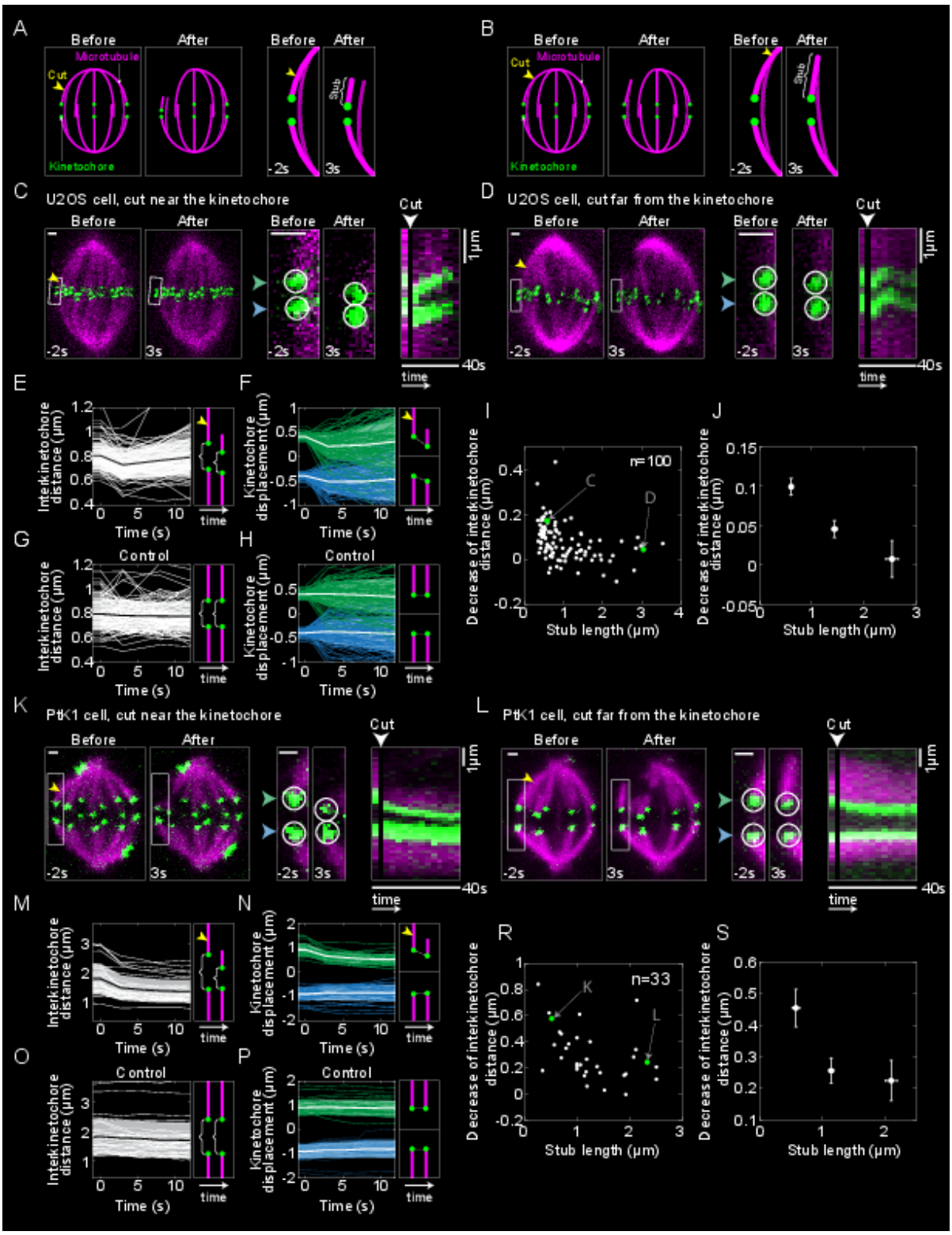

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Figure Legend

Figure 1.

(A) Predicted outcome of the k-fiber severing experiment when the severing spot is close to the kinetochore. The bridging fiber will detach from the k-fiber stub and the interkinetochore distance will decrease.

(B) Predicted outcome of the k-fiber severing experiment when the severing is per-

formed far away from the kinetochore. The bridging fiber and the k-fiber stub will remain linked, and the interkinetochore distance will not change significantly.

(C-J) Analysis of spindles in U2OS cells expressing CENP-A-GFP (green) and mCherry-tubulin (magenta).

(C) Images of a spindle 2 s before (left) and 3 s after the severing (right). The outermost k-fiber was cut with a laser (yellow arrow) ~0.5 µm away from the kinetochore. Enlargements of the region inside the white rectangle show that the kinetochore proximal to the severing spot moved towards its sister, decreasing interkinetochore distance. Kymograph shows a decrease of the interkinetochore distance in the first frame after the cut, followed by an increase of the interkinetochore distance caused by the transport of the stub towards the pole to which it was originally connected.

(D) Images of a spindle before and after the severing of the k-fiber, in a cell where the cut was performed ~3 µm away from the kinetochore. Enlargements of the region inside the white rectangle and kymograph are also shown. The distance between sister kinetochores did not change significantly following the severing.

(E) Interkinetochore distance as a function of time for the kinetochores whose k-fiber was severed. Severing was performed at time 0. Data from individual cells, the mean value, and the standard deviation are shown by thin lines, thick black line, and shaded region, respectively.

(F) Displacement of the sister kinetochores in the direction parallel to the line joining them, with respect to the position of the midpoint between them before severing (0 µm). Kinetochores proximal and distal to the severing spot are shown in green and blue, respectively. Data from individual cells, the mean value, and the standard deviation are shown by thin lines, thick white line, and shaded region, respectively.

(G) Interkinetochore distance of the outermost kinetochores on the uncut side of the spindle as a function of time. Legend as in E.

(H) Displacement of the outermost kinetochores on the uncut side of the spindle as a function of time. Kinetochores attached to the pole proximal and distal to the cut are shown in green and blue, respectively. Legend as in F.

(I) Relaxation of the interkinetochore distance 3 s after the cut as a function of the length of the k-fiber stub. Data points corresponding to the cells shown in panels C and D are indicated by arrows.

(J) Data from panel I binned by stub length. Binning boundaries are 0–1, 1–2, and 2–3 µm. Error bars represent SEM.

(K-S) Analysis of spindles in PtK1 cells expressing Hec1-GFP (green), injected with X-rhodamine-tubulin (magenta); legend as in (C-J), respectively. In K, the k-fiber stub was ~0.5 µm long, whereas in (L) it was ~2.5 µm long. In (S), binning boundaries are 0–1 µm, 1–1.5 µm and 1.5–3 µm. Scale bars in all images are 1 µm; *n* in graphs denotes the number of cells.

A printer-friendly version of this figure on a white background can be found in Supplementary information.

Cell culture and sample preparation for U2OS cells

Human osteosarcoma U2OS cell line stably expressing CENP-A-GFP, mCherry-α-tubulin, and photoactivatable-GFP-α-tubulin was a kind gift from Marin Barišić and Helder Maiato (Institute for Molecular Cell Biology, University of Porto, Portugal). Cells were grown in Dulbecco's Modified Eagle's medium (DMEM) (1 g/l D-glucose, L-glutamine, pyruvate) with 50 µg/ml geneticin (Life Technologies, Waltham, MA) and appropriate supplements. The cells were kept at 37℃ and 5% CO_2 in Galaxy 170 S incubator (Eppendorf, Hamburg, Germany). To prepare samples for microscopy, cells were cultured in 1.5 ml DMEM with supplements at 37℃ and 5% CO_2 in 35 mm glass coverslip dishes (MatTek Corporation, Ashland, MA, USA) for 24–48 h. Before imaging, the medium was replaced with Leibovitz's L-15 CO_2-independent medium supplemented with fetal bovine serum (Life Technologies).

Imaging and laser severing of k-fibers in U2OS cells

U2OS cells were imaged using Bruker Opterra Multipoint Scanning Confocal Microscope (Bruker Nano Surfaces, Middleton, WI). The system was mounted on a Nikon Ti-E inverted microscope equipped with a Nikon CFI Plan Apo VC 100x/1.4 numerical aperture oil objective (Nikon, Tokyo, Japan). The system was controlled with the Prairie

View Imaging Software (Bruker). During imaging, cells were maintained at 37°C in Okolab Cage Incubator (Okolab, Pozzuoli, NA, Italy). For excitation of GFP and mCherry fluorescence, a 488 and a 561 nm diode laser line were used, respectively. The excitation light was separated from the emitted fluorescence by using Opterra Dichroic and Barrier Filter Set 405/488/561/640. Images were captured with an Evolve 512 Delta EM-CCD Camera (Photometrics, Tucson, AZ) using a 500 ms exposure time. To bring the xy-pixel size in the image down to 83 nm, a 2x relay lens was placed in front of the camera. Z-stacks were acquired comprising 5 focal planes at a 0.5 µm z-spacing. Image acquisition was performed for 10–20 time frames at 5 s intervals. Severing of k-fibers was performed using a Mikan femtosecond laser oscillator (Amplitude Systemes, Pessac, France), which was coupled to the photoactivation module of the microscope, at a wavelength of 1030 nm and exposure time of 2 s. Thus, severing was performed 2 s after acquiring the last image stack of the intact spindle, and the next image stack was acquired 3 s after the severing. This time resolution allowed us to compare our data to those from [10].
Cell culture, sample preparation, imaging, and laser severing of k-fibers in PtK1 cells was performed as described in [10].

Image analysis

Image processing was performed in ImageJ (National Institutes of Health, Bethesda, MD, USA). Quantification and statistical analyses were performed in MatLab (MathWorks, Natick, USA). Kinetochores were tracked using Low Light Tracking Tool, an ImageJ plugin [12]. Tracking of kinetochores in the xy-plane was performed on individual imaging planes or on maximum-intensity z-projections of up to three planes. The length of the k-fiber stub was measured in the first frame after the cut from the center of the corresponding kinetochore to the end of the ablated stub. Graphs were generated in Matlab. ImageJ was used to produce kymographs, to scale the images, and to adjust brightness and contrast. Figures were assembled in Adobe Illustrator CS5 (Adobe Systems, Mountain View, CA).

Results & Discussion

We hypothesize that the bridging fiber is linked laterally to a k-fiber in the region away from the kinetochore, whereas these two fibers separate from each other near the kinetochore [10]. Therefore, if a k-fiber is severed close to a kinetochore, this kinetochore will move towards its sister kinetochore because the bridging fiber will detach from the k-fiber stub that remains bound to the kinetochore after the severing, and thus will not be able to balance the tension between sister kinetochores (Fig. 1A). Conversely, if the severing is performed far away from the kinetochore, the bridging fiber will remain connected to the severed k-fiber and continue balancing the interkinetochore tension; thus, the kinetochores will not move significantly towards each other (Fig. 1B) [10].
We used laser ablation to sever k-fibers at various distances from the kinetochore, from 0.3 µm to more than 3 µm, in human U2OS cells stably expressing CENP-A (a kinetochore protein) tagged with green fluorescent protein (GFP) and tubulin tagged with mCherry. Such severing leaves a k-fiber stub attached to the kinetochore, while the other fragment of the severed k-fiber depolymerizes [7] [11]. The k-fiber stub moves first away from the spindle axis, due to the release of compressive forces [10]. Subsequently, the stub moves towards the proximal spindle pole and back towards the spindle axis [8] [9] [10]. We identified successful k-fiber severing by observing the movement of sister kinetochores and the attached k-fiber stub away from the spindle axis immediately after the severing, or later their movement towards the pole. We measured the movement of kinetochores between the last image before the severing and the first image taken 3 s after severing (see Methods), in order to study the response of the system to the release of forces due to severing, before new forces start acting on the k-fiber stub pulling it towards the pole. We did not analyze the cells in which reconnection of the stub to the spindle microtubules occurred less than 3 s after severing. In order to measure the length of the stub without interference from neighboring k-fibers, we severed only the outermost k-fibers. Examples of severing close to and far from the kinetochore are shown in figure 1C and 1D, and Movies S1 and S2, respectively.

The distance between sister kinetochores before severing was 0.81±0.01 μm (n = 100 cells; all results are given as mean±SEM unless otherwise indicated). We found that the distance between sister kinetochores decreased, on average, by 0.08±0.01 μm 3 s after the severing (Fig. 1E). Afterwards, the average interkinetochore distance slowly increased, due to the movement of the stub towards the proximal spindle pole. These results are in qualitative agreement with previous studies [9] [10].

To assess the contribution of the movement of each sister kinetochore to the decrease in interkinetochore distance, we measured the movement of each kinetochore with respect to the position of the midpoint between the sister kinetochores before the cut. We found that 3 s after k-fiber severing the kinetochore proximal to the severing spot moved by 0.20±0.02 μm towards the midpoint, while its sister moved by 0.11±0.02 μm away from the midpoint, on average (n = 100 cells; Fig. 1F; note that the difference between these mean values is somewhat larger than the average decrease of the interkinetochore distance given above, due to the fact that the interkinetochore axis rotates slightly after severing). These data show that the relaxation of the interkinetochore distance was caused predominantly by the movement of the kinetochore whose k-fiber was severed.

To test whether the directed movement of kinetochores was a local response to severing of the associated k-fiber, rather than a non-specific response of the whole spindle to laser irradiation, we analyzed the movement of the outermost kinetochores on the side of the spindle opposite from the severing location. We found no significant change in the interkinetochore distance (0.01±0.01 μm; n = 100 cells) and no preferred direction of kinetochore movement on the uncut side of the spindle (Fig. 1G, H). Similarly, we measured no significant change in the interkinetochore distance for kinetochore pairs adjacent to those whose k-fiber was severed (0.01±0.02 μm, n = 16 cells; we analyzed only the cells in which the k-fiber adjacent to the severed k-fiber undoubtedly remained intact and the associated kinetochores could be tracked). These control measurements show that the response to severing was localized to the kinetochores associated with the severed k-fiber, rather than a consequence of non-specific effects of laser ablation.

Next, we analyzed how the relaxation of the interkinetochore distance depends on the length of the k-fiber stub. We found larger relaxations for shorter stubs, i.e., for the cuts closer to the kinetochores (Fig. 1I, J). The average relaxation was 0.10±0.01 μm for stubs shorter than 1 μm, whereas it was only 0.04±0.01 μm for longer stubs.

To test the contribution of bridging fibers to interkinetochore tension in other mammalian cells, we performed equivalent experiments and analysis as above on rat-kangaroo PtK1 cells. The cells expressed Hec1-GFP as a kinetochore marker and were microinjected with X-rhodamine-tubulin (Fig. 1K, L and Movies S3, S4). The distance between sister kinetochores before severing was 1.83±0.09 (n = 33 cells). We found that 3 s after severing of a k-fiber, the interkinetochore distance decreased by 0.31±0.04 μm (Fig. 1M). After severing, both kinetochores moved towards the midpoint (Fig. 1N). However, the kinetochore closer to the severing spot moved by 0.26±0.04 μm, whereas its sister moved by 0.06±0.02 μm (n = 33 cells). Thus, the decrease of the interkinetochore distance was caused mainly by the movement of the kinetochore whose k-fiber was severed, as in U2OS cells. Yet, it is not clear why the sister kinetochore, whose k-fiber was not severed, moved towards the midpoint in PtK1 cells, whereas it moved away from the midpoint in U2OS cells. As in U2OS cells, control measurements in PtK1 cells showed that the kinetochores on the opposite side of the spindle displayed neither a significant change in the interkinetochore distance, nor directed movement following the severing (n = 33 cells; Fig. 1O, P). Importantly, we found that the reduction of the interkinetochore distance was larger in spindles in which the k-fiber stub was shorter (Fig. 1R, S). The average relaxation was 0.46±0.06 μm for stubs shorter than 1 μm, whereas it was only 0.24±0.04 μm for longer stubs.

We have shown that severing of a k-fiber closer to the kinetochore results in a larger relaxation of the interkinetochore tension than severing far from the kinetochore, in human U2OS cells and in rat-kangaroo PtK1 cells. Our results on these two cell lines are in qualitative agreement with each other and with the previous measurements on HeLa cells [10]. Yet, the three cell lines show quantitative differences in the kinetochore behavior. The average interkinetochore distance in intact metaphase spindles is largest in PtK1 cells (~1.8 μm), intermediate in HeLa cells (~1 μm), and smallest in U2OS cells

(~0.8 μm). After k-fiber severing, in the spindles with the k-fiber stub smaller than 1 μm, the average relaxation of the interkinetochore distance follows the same pattern: it is largest in PtK1 cells (~0.5 μm), intermediate in HeLa cells (~0.2 μm), and smallest in U2OS cells (~0.1 μm). In all the cell lines, there is a transition in the relaxation of the interkinetochore distance at the stub length of roughly 1 μm (Fig. 1J, S and Fig. 5d from [10]). This finding suggests that the bridging fiber is linked laterally to the k-fibers in the region more than ~1 μm away from the kinetochore, whereas these fibers are disconnected up to ~1 μm from the kinetochore.

Conclusions

We have shown in U2OS and PtK1 cells that severing of a k-fiber closer to the kinetochore results in a larger relaxation of the interkinetochore tension than severing far from the kinetochore. Our data imply that a long k-fiber stub is able to withstand the interkinetochore tension and prevent relaxation of the interkinetochore distance, whereas a short stub is not able to do so. This result, which is in agreement with the findings from Kajtez et al. [10], challenges the current view that the tension on kinetochores is generated by molecular events occurring primarily at the ends of the k-fiber. We conclude that the bridging fiber, which links sister k-fibers and acts as a bridge between sister kinetochores, is required to balance the interkinetochore tension.

It will be important to identify the proteins that cross-link the bridging fiber with sister k-fibers, thereby allowing for regulation of interkinetochore tension. To this end, the assay described here can be used in combination with knock-down or overexpression of candidate proteins. Such experiments will provide information about the role of different microtubule cross-linking proteins in the generation of interkinetochore tension during metaphase.

Additional Information

Methods

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a wavelength of 1030 nm and exposure time of 2 s. Thus, severing was performed 2 s after acquiring the last image stack of the intact spindle, and the next image stack was acquired 3 s after the severing. This time resolution allowed us to compare our data to those from [10].
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Supplementary Material

Please see https://sciencematters.io/articles/201603000025.

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Ethics Statement

Not applicable.

Citations

[1] Iain M. Cheeseman and Arshad Desai. "Molecular architecture of the kinetochore–microtubule interface". In: *Nature Reviews Molecular Cell Biology* 9.1 (Jan. 2008), pp. 33–46. DOI: 10.1038/nrm2310. URL: http://dx.doi.org/10.1038/nrm2310.

[2] M.Andrew Hoyt, Laura Totis, and B.Tibor Roberts. "S. cerevisiae genes required for cell cycle arrest in response to loss of microtubule function". In: *Cell* 66.3 (Aug. 1991), pp. 507–517. DOI: 10.1016/0092-8674(81)90014-3. URL: http://dx.doi.org/10.1016/0092-8674(81)90014-3.

[3] Rong Li and Andrew W. Murray. "Feedback control of mitosis in budding yeast". In: *Cell* 66.3 (Aug. 1991), pp. 519–531. DOI: 10.1016/0092-8674(81)90015-5. URL: http://dx.doi.org/10.1016/0092-8674(81)90015-5.

[4] Andrea Musacchio and Edward D. Salmon. "The spindle-assembly checkpoint in space and time". In: *Nature Reviews Molecular Cell Biology* 8.5 (Apr. 2007), pp. 379–393. DOI: 10.1038/nrm2163. URL: http://dx.doi.org/10.1038/nrm2163.

[5] J. Ferraro-Gideon et al. "Measurements of forces produced by the mitotic spindle using optical tweezers". In: *Molecular Biology of the Cell* 24.9 (Mar. 2013), pp. 1375–1386. DOI: 10.1091/mbc.e12-12-0901. URL: http://dx.doi.org/10.1091/mbc.E12-12-0901.

[6] R. B. Nicklas. "Measurements of the force produced by the mitotic spindle in anaphase". In: *The Journal of Cell Biology* 97.2 (Aug. 1983), pp. 542–548. DOI: 10.1083/jcb.97.2.542. URL: http://dx.doi.org/10.1083/jcb.97.2.542.

[7] Helder Maiato, Conly L. Rieder, and Alexey Khodjakov. "Kinetochore-driven formation of kinetochore fibers contributes to spindle assembly during animal mitosis". In: *The Journal of Cell Biology* 167.5 (Nov. 2004), pp. 831–840. DOI: 10.1083/jcb.200407090. URL: http://dx.doi.org/10.1083/jcb.200407090.

[8] Mary Williard Elting et al. "Force on spindle microtubule minus ends moves chromosomes". In: *The Journal of Cell Biology* 206.2 (July 2014), pp. 245–256. DOI: 10.1083/jcb.201401091. URL: http://dx.doi.org/10.1083/jcb.201401091.

[9] Vitali Sikirzhytski et al. "Direct kinetochore–spindle pole connections are not required for chromosome segregation". In: *The Journal of Cell Biology* 206.2 (July 2014), pp. 231–243. DOI: 10.1083/jcb.201401090. URL: http://dx.doi.org/10.1083/jcb.201401090.

[10] Janko Kajtez et al. "Overlap microtubules link sister k-fibres and balance the forces on bi-oriented kinetochores". In: *Nature Communications* 7 (Jan. 2016), p. 10298. DOI:

10.1038/ncomms10298. URL: http://dx.doi.org/10.1038/ncomms10298.

[11] T P Spurck et al. "UV microbeam irradiations of the mitotic spindle. II. Spindle fiber dynamics and force production". In: *The Journal of Cell Biology* 111.4 (Oct. 1990), pp. 1505–1518. DOI: 10.1083/jcb.111.4.1505. URL: http://dx.doi.org/10.1083/jcb.111.4.1505.

[12] Alexander Krull et al. "A divide and conquer strategy for the maximum likelihood localization of low intensity objects". In: *Optics Express* 22.1 (Jan. 2014), pp. 210–228. DOI: 10.1364/oe.22.000210. URL: http://dx.doi.org/10.1364/OE.22.000210.