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Authors

Ono, Matthew

Preece, Daryl

Duquette, Michelle

et al.

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Revealing the micromechanics driving cellular division: optical manipulation of force-bearing substructure in mitotic cells

Matthew Ono[a], Daryl Preece[a,b], Michelle Duquette[a], Arthur Forer[c], and Michael Berns[a,d]

[a]Department of Bioengineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, USA 92093-0412
[b]Department of NanoEngineering, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA, USA 92093-0412
[c]Department of Biology, York University, 4700 Keele Street, Toronto, ON, Canada M3J IP3
[d]Beckman Laser Institute and Department of Biomedical Engineering, University of California Irvine, CA 92617, USA

ABSTRACT

During the anaphase stage of mitosis, a motility force transports genetic material in the form of chromosomes to the poles of the cell. Chromosome deformations during anaphase transport have largely been attributed to viscous drag force, however LaFountain et. al. found that a physical tether connects separating chromosome ends in crane-fly spermatocytes such that a backwards tethering force elongates the separating chromosomes. In the presented study laser microsurgery was used to deduce the mechanistic basis of chromosome elongation in rat-kangaroo cells. In half of tested chromosome pairs, laser microsurgery between separating chromosome ends reduced elongation by 7±3% suggesting a source of chromosome strain independent of viscous drag. When microsurgery was used to sever chromosomes during transport, kinetochore attached fragments continued poleward travel while half of end fragments traveled towards the opposite pole and the remaining fragments either did not move or segregated to the proper pole. Microsurgery directed between chromosome ends always ceased cross-polar fragment travel suggesting the laser severed a physical tether transferring force to the fragment. Optical trapping of fragments moving towards the opposite pole estimates an upper boundary on the tethering force of 1.5 pN.

Keywords: chromosomes, microsurgery, optical trapping, micromanipulation, mechanics, tethers, mitosis, anaphase

1. INTRODUCTION

Proper cellular division underlies the natural development and repair processes in living tissues. In the anaphase stage of mitotic cell division molecular motors and microtubule depolymerization produce a motility force at the chromosome's kinetochore to transport one copy of each chromosome to the cell poles.[1–3] Failure to transport exactly one copy of each chromosome results in an abnormal chromosome number in the daughter cells, a condition known as aneuploidy. Although aneuploid cells are often not viable, some may continue proliferation and can result in congenital birth defects, miscarriage, and tumorigenesis.[4,5] Numerous biochemical and mechanical cues guide the controlled segregation of genetic material through mitosis. Proper balance and feedback of these cues is necessary to protect cellular functionality and avoid aneuploidy in the new tissue. The cellular structures and machinery behind this vital separation of genetic material remains an active area of interest.

The mechanical involvement of cellular substructure has increasingly been shown to play vital roles in regulating the progression of mitosis. Tension in the chromosome kinetochore has been shown to signal proper microtubule attachment, initiate the metaphase-to-anaphase transition, and reposition centromere substructure to improve chromosome transport.[6–10] While many useful parameters describing mechanical states in the cell such as strain, tension, and elastic modulus can been studied through biosensors and ex vivo experimentation,[6,11–15]

the underlying physical forces responsible for moving chromosomes (motility force) are not well known. Mathematical models and theoretical estimates based on the viscous drag force required to move chromosomes in *Drosophila* and *Melanoplus* cells place motility force between 0.1 and 1 pN.[15–17] In a more direct approach, optical trapping experiments demonstrated that chromosomes could be manipulated in vitro and later in vivo, estimating a 30 pN force to be capable of moving newt chromosomes in cytoplasm.[18–20] Later optical trapping experiments on ex vivo chromosomes extracted from Chinese hamster ovary cells (CHO) found a .1-12 pN needed to move chromosomes,[21] while in vivo anaphase-like chromosome movement was halted in *Mesastoma* cells with 2-3 pN.[22] Optical trapping values are close to the theoretical range of motility forces. However in pioneering micromanipulation work R.B. Nicklas measured anaphase motility force by impaling moving rat-kangaroo chromosomes with a glass needle. By examining the needle flexure Nicklas found a stalling force of 700 pN required to stop chromosome movement.[14] Current theoretical and trapping estimates do not agree with the maximum force found by Nicklas, and further, the method of force production behind the motility force is not well understood. As such, a more complete understanding of the involved cellular substructure is needed to reconcile measurements with theory.

Here we discuss the chromosomal stress resulting from the kinetochore motility force pulling chromosomes through the cytoplasm during anaphase A of mitosis. Thorough micromechanical assays have shown that chromosomes are linearly elastic and homogeneous bodies whose stiffness depends largely on chromosomal substructure.[11,12] Thus chromosomes can be understood as elastic bodies whose deformations reflect the influence of physical forces and strains. When strained in the lengthwise direction a chromosome can then be conceptualized as a linear spring whose extension reflects an applied force. In the current model of anaphase transport the motility force works only against viscous drag, and thus chromosome deformation reflects drag force and thereby forms the basis for some theoretical estimates. Alternatively, LaFountain et. al. found that physical *tethers* link half of separating chromosome ends in crane-fly spermatocytes[23] and transfer a cross-polar force, working against the motility force, that elongates chromosomes. Severed chromosome fragments were capable of travelling across the cell to the partner chromosome as confirmed in recent studies, which also found that chromosome elongation can be reduced by severing this tether.[24,25] Optical manipulation can test the tethering model in rat-kangaroo epithelial cells (PtK2) to distinguish mechanical sources of chromosome elongation by: (1) directing microsurgery at the space between chromosome ends to sever a tether and decrease chromosome extension, (2) severing chromosomes to produce a chromosome fragment and examining fragment travel, and (3) optically trapping tethered fragments to estimate the tethering force magnitude.

2. MATERIALS AND METHODS

2.1 Cells and Culture

Mammalian PtK2 cells from rat-kangaroo kidney epithelial tissue were used in this study. PtK cells round less during mitosis and have a relatively low chromosome number helping to improve image clarity. Cells were cultured in DMEM and 10% fetal bovine serum with added pennecillin and streptomycin in 10 cm dishes. Cells were seeded on 35mm glass bottom imaging dishes for experiments and were allowed to incubate until reaching approximately 80% confluence to increase the likelihood of finding an anaphase cell. During experimental trials the temperature, humidity, %CO_2, and % O_2 composition of the sample was maintained with a stage heater and gas mixer.

2.2 Optical System

The microscope and conjoined optical cutting and trapping system in these experiments has been described before.[21,22] Briefly, an inverted microscope with a 1.4 NA, 63x oil immersion objective channels a continuous 1064 nm (Nd:YVO4) trapping laser, and a pulsed femtosecond 740 nm (Ti:Sapphire) cutting laser. Images were captured from a Hammatsu CCD camera while stage control, beam steering, and irradiance exposure were controlled through a LabView VI.

2.3 Laser Microsurgery and Optical Trapping

Laser microsurgery was directed at the intracellular space between separating chromosome ends (inter-telomere cut) to asses possible tethering contributions to chromosomal strain . Chromosome length was averaged for the 30-60s prior to and immediately following the cut. Chromosomes that shortened by more than the standard deviation prior to cutting were determined to have experienced strain reduction. Strain was evaluated by dividing the chromosomes post-cut length by the chromosome initial length as shown with data from a single trial in Figure 1. Chromosome lengths were found by taking the lengthwise profile of the chromosome along the path the kinetochore and end (telomere) follow to the pole at each time step. The profiles were then concatenated in ImageJ to yield kymographs with position-time data. Chromosomes that bent or otherwise strayed from a single linear path were not used.

Laser microsurgery was used to sever chromosome arms and produce chromosome fragments, presumably attached to the tether. In one group fragments were allowed to travel freely. In a subsequent group, cross-polar travelling fragments were allowed to move briefly before applying an inter-telomere cut. In the last group an optical trap was placed over or ahead of the centroid of cross-polar travelling fragments such that the fragment passed through the trap center where effective trapping force is negligible. Fragment travel was also followed by creating position-time kymographs of the fragment profile in ImageJ, from which velocity could be interpolated.

For laser microsurgery, a power of 34.2 mW at the focal plane (irradiance of 4.4 x 10^8 W/cm^2) was sufficient for cutting trials without grossly damaging the cell membrane. Cuts were performed in 3 focal planes, each roughly 0.5 μm apart to ensure chromosomes and tethering elements were fully severed. Optical trap power was set at 13mW (irradiance of 1 x 10^4 W/cm^2) at the focal plane, a power at which heating and photochemistry effects are expected to be small.[21]

3. RESULTS

Roughly half of the tested chromosomes (n=5) showed statistically significant strain reduction following an inter-telomere cut, while the remaining (n=4) chromosomes did not show significant length change. Chromosomes which retracted showed a mean strain reduction of 7±3%.

Thirteen chromosome fragments were produced with laser microsurgery. Fragments exhibited cross-polar travel (n=9), did not move at all (n=2), or segregated to the proper pole (n=2). Magnitude and timescale of this cross polar travel varied greatly as illustrated in Figure 2. The remaining chromosome body attached to the kinetochore always completed segregation to the proper poles.

In the second group only chromosome fragments exhibiting cross polar travel (n=12) were examined. Inter-telomere cuts always ceased cross-polar travel and all fragments either ceased motion or reversed direction to then travel towards the proper pole (Fig. 3).

In the final group (n=3) cross-polar travelling chromosome fragments were all stopped by a 13 mW optical trap, corresponding to a force of 0.4-1.5 pN[21,22] . Fragment lengths varied from 1-3 μm and fragment centroids were able to move past the trap center where effective force is negligible, and towards the cross-polar side of the trap rim (Figure 4).

4. DISCUSSION AND CONCLUSIONS

Inter-telomere cuts revealed a source of chromosome strain independent of viscous drag force. In inter-telomere trials the mean relaxation of 7±3% in half of tested chromosomes suggests that the laser severed a physical linkage, thereby reducing chromosomal strain. Chromosomes that did not shorten may not have been affected as the laser failed to sever a tether, or the chromosomes were not tethered at all. The relaxation in half of PtK2 chromosomes is consistent with previous findings that half of chromosome arms are tethered in crane-fly spermatocytes.[23–25]

Severed chromosome fragments showed different travel patterns: across the equator, towards the equator, stagnant, and towards the proper pole. Moreover, travel to the sister side was stopped or reversed when a laser cut was directed at the space between chromosome ends, severing the tether. Fragment travel showed that the backwards tethering force is capable of dragging fragments across the cell. However the differing patterns of

cross-polar travel suggest the tethering element may lose strength or elasticity during anaphase such that it can no longer move fragments through the cytoplasm. Fragments which continued proper segregation may not have been entirely severed from their chromosome body or perhaps are otherwise physically linked to segregating spindle structure.

For optically trapped fragments the tethering force must be less than 0.4-1.5 pN as fragments travelled through the trap center and remained at the opposing trap edge without escaping. The tethering force seemingly lessened towards the end of anaphase as fragments returned towards the trap center. While tethering force seems to lessen through the course of anaphase and carries implications as to anaphase forces at large, this will not be covered in this study but more widely addressed in an upcoming article by Forer et. al.[25] In summary these findings indicate that tethers transmit force between chromosomes which may exceed some current minimum motility force estimates. Tethering force must therefore be considered when estimating chromosomal motility force during anaphase.

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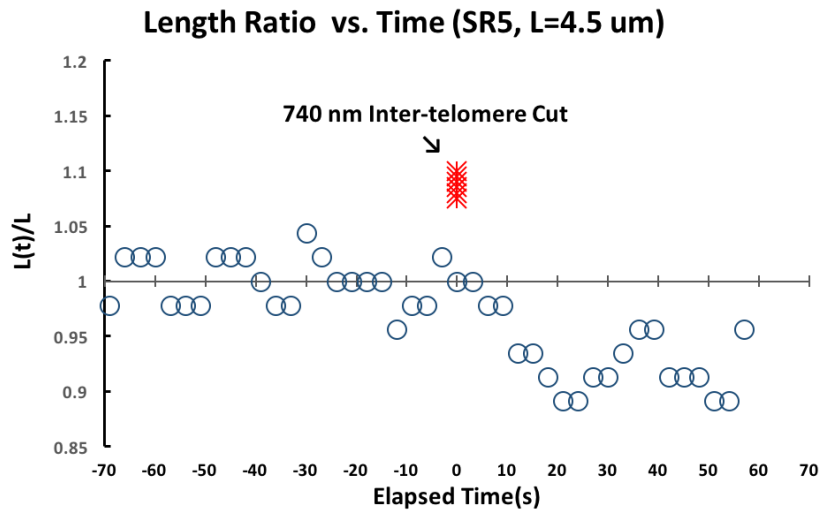
Figure 1. Length Ratio vs Time (seconds) for a single chromosome showing relaxation following an inter-telomere cut (red bar).
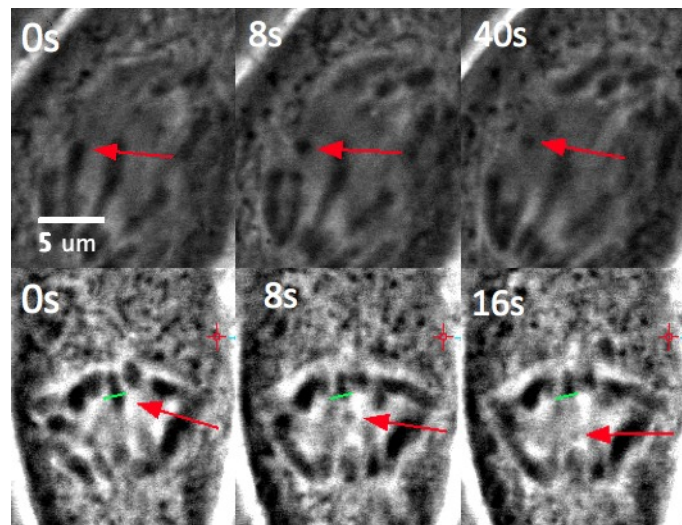

Figure 2. Still images from two fragment travel videos. (Top) Chromosome fragment travels briefly between 0-8 seconds and drastically slows cross-polar travel until stopping around 40s. (Bottom) Chromosome fragment travels entirely to the sister chromosome between 0-16s. Of the cross-polar travelling fragments, n=5 crossed the cell equator and n=4 fragments moved towards but did not cross the cell equator.
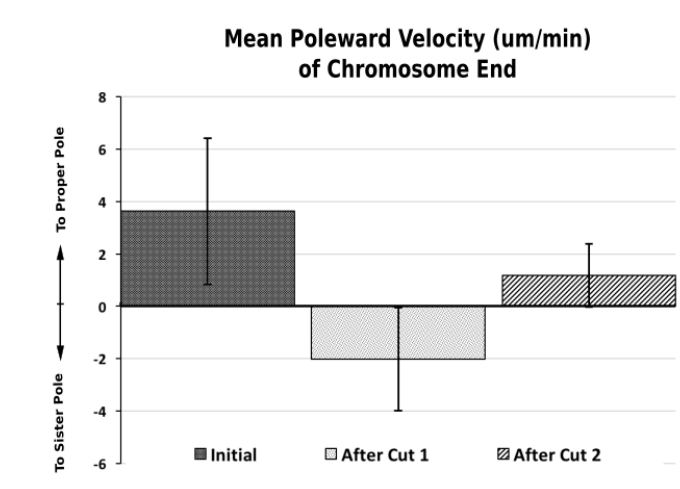
Figure 3. Mean velocity in μm/minute of the chromosome end (n=12) prior to the experiment (initial), after fragmentation (Cut 1), and after the intertelomere cut (Cut 2). Positive velocity indicates travel to the proper pole and negative velocity indicate cross-polar travel towards the sister pole. Error bars are standard deviation for each averaged period.
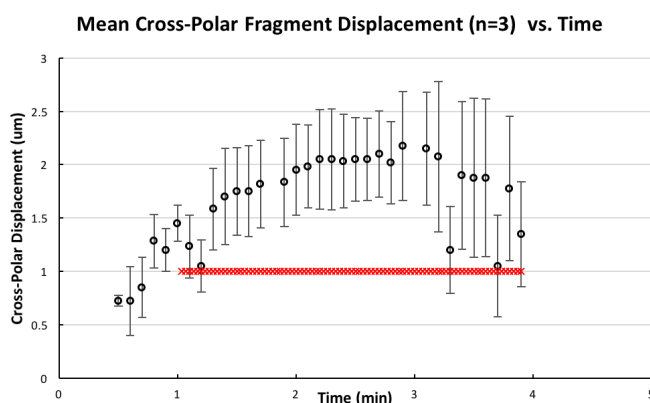

Figure 4. Mean displacement (μm) of fragment centroids towards the sister pole. Positive displacement indicates cross-polar travel. Fragments travelling towards the sister pole were held with a 1064 nm optical trap (red cross-hatches). Fragments moved past the trap center where effective force is negligible, and towards the trap edge on the sister pole side. The trapping force of 1.5 pN was sufficient for holding all tested fragments