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Mitotic Tethers Connect Sister Chromosomes During Anaphase A in PtK2 Cells

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Abstract: Laser microsurgery directed between sister telomeres reduced strain in anaphase PtK2 chromosomes. Optical trapping of severed chromosome ends suggests this strain arises from a physical element which links sister chromatids during early anaphase.

1. Introduction

Various biochemical and mechanical cues guide the controlled segregation of chromosomes during mitosis. The proper harmony of these cues is necessary to preserve cellular function and avoid aneuploidy in the resulting daughter cells. Failure to transport exactly one copy of each chromosome per daughter cell compromises tissue health and increases the risk of tumor development. The exact cellular machinery and conditions involved in this precise segregation of genetic material is still an area of active interest.

Mechanical conditions of subcellular structures have increasingly been shown to play crucial roles in the regulation of mitosis. Recently, tension in the kinetochores of chromosomes has been shown to cue the metaphase to anaphase transition and reposition centromere substructures to improve chromosome movement [1-5]. Here we discuss the chromosomal stress resulting from the kinetochore motility force in the anaphase A stage of mitosis. During the anaphase stage of mitosis sister chromatids are divided and the chromosomes transported to the poles of the cell by a Brownian ratchet mediated kinetochore force and microtubule depolymerization [6-8]. Many useful physical parameters in the cell such as tension, strain, and elastic modulus can be found or shown through biosensors, observation, and *ex vivo* experimentation [1,9-13]. However, the underlying physical forces which move chromosomes in the anaphase cell (motility force) are often determined through theory and *in silico* simulation. Mathematical models derived from *Melanoplus* and *Drosophila* data place the motility force on the order of 1- 10 pN [12,14]. In early micromanipulation experiments with *Melanoplus* spermatocytes R.B Nicklas impaled moving chromosomes with a glass needle and determined a maximum motility force on the order of 700 pN[13]. These data from direct manipulation methods do not reconcile with mathematical models, and require physical interference with the active mitotic spindle raising concerns as to the preservation of the mechanical states under observation. As such current chromosomal anaphase force estimates which are largely derived from parameters determined by *ex-vivo* experiments and passive observations, do not reconcile with the maximum motility force found by Nicklas.

For these studies we understand the chromosome as a body whose observable deformation emerges as a result of real physical forces and strains. Thorough assays of chromosomal micromechanics have shown chromosomes to be linearly extensible bodies whose stiffness largely depends on chromosomal substructure [9,10]. Thus when strained in the lengthwise direction chromosomes can be conceptualized as springs whose elongation reflects an applied force. Elongation of chromosomes during anaphase is often attributed to intracellular drag force acting along the chromosome's length and varying with velocity, thereby forming the basis for minimum motility force estimates. This method assumes chromosome elongation is due entirely to drag and thermal fluctuations. Alternatively, LaFountain et. al found that two of four sister chromatids in crane-fly spermatocytes were physically tethered by an element linking their telomeres such that a backwards force, directed away from the proper pole, elongates segregating chromosomes [15]. Laser microsurgery can help deduce the mechanistic basis for chromosomal elongation by attempting to sever the tether and examining length change, or by generating chromosome fragments—which are presumably attached to the tether—and examining their travel. Furthermore, optically trapping the chromosome fragments offers a limited but non-intrusive method of measuring forces acting the chromosome.

2. Materials and Methods

Mammalian PtK2 epithelial cells were used in this study for their relatively low chromosome number and less rounding during mitosis, helping to improve imaging clarity. The microscope and conjoined optical cutting and trapping system has been described before [16,17]. Briefly, an inverted microscope with a 1.4 NA oil immersion objective channels both a continuous 1064 nm trapping laser, and a pulsed femtosecond 740 nm cutting laser. A

power of 34.2 mW at the focal plane (irradiance of $4.4 \times 10^8 \text{ W/cm}^2$) was sufficient for cutting trials without damaging the cell membrane. To ensure chromosomes and linking elements are cut entirely, the cut was performed in three focal planes each $\sim 0.5 \mu\text{m}$ apart. Optical trapping of moving fragments was accomplished by placing the trap center over the fragment but slightly ahead of its centroid such that the fragment passes over the trap center where the effective force is negligible. The optical trap power was set at 13mW (irradiance of $1 \times 10^4 \text{ W/cm}^2$) at the focal plane, a power for which heating and photochemistry effects are expected to be small [16].

Chromosome lengths were determined by taking the chromosome profile for each time step (kymograph), along the line which the kinetochore and tail end follow to the pole. Chromosomes that deviated from a single linear path were not used. This same method was used to follow the travel paths of chromosome fragments, stub retractions, and to determine velocities. In the relaxation study chromosome length for 30-60s prior to and following the inter-telomere cut was averaged and a chromosome that shortened by more than the standard deviation prior to cutting was determined to have experienced strain reduction.

3. Results

Following the lengths of chromosomes in unaltered cells through anaphase revealed a maximum, natively occurring, mean extension of .065 of the chromosomes initial length between 0-1 minutes (Fig.1). Applying laser cuts between sister chromosome ends in a subsequent group of cells resulted in a statistically significant mean retraction of .075 of the starting length ($n=5$) in roughly half of tested chromosomes and no significant retraction in the remaining ($n=4$) chromosomes. When chromosomes were severed during anaphase transport, the remaining

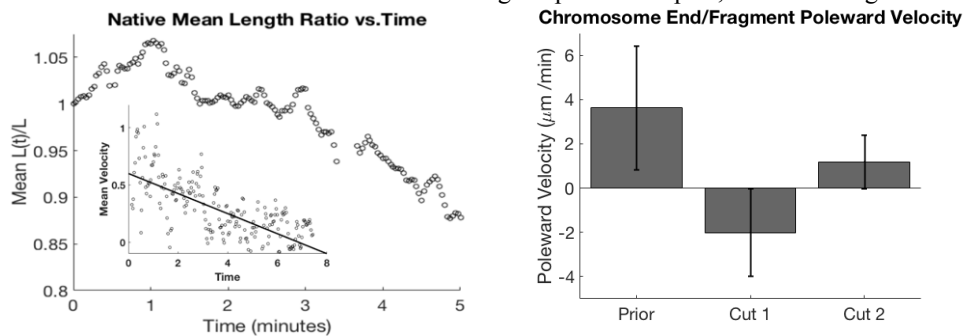


Fig. 1. *Main.* Averaged Length/Initial Length for ($n=13$) chromosomes: Chromosome extension is seen between 0-1 minutes. Fig. 1. *Inlay.* Kinetochore Velocity ($\mu\text{m/minute}$) for the same data set vs. Time (minutes) indicating most kinetochores are still in transport (anaphase A) between $\sim 0-6$ minutes. Fig. 2. Mean Chromosome End/Fragment Poleward Velocities in $\mu\text{m/min}$ ($n=12$) with standard deviations (error bars) for the chromosome: prior to severing (Prior) chromosome ends segregate to the poles, immediately following fragmentation (Cut 1) fragments stop or travel to the opposing pole, and after the inter-telomere cut (Cut 2) fragments stop or switch direction back towards the proper pole.

arm attached to the kinetochore always segregated successfully, while chromosome ends (chromosome fragment) either moved towards the cell equator ($n=4$), crossed the equator ($n=4$), or stagnated completely ($n=2$), and a small fraction of fragments segregated to the proper pole ($n=2$). When a cut was directed in front of fragments moving away from the proper pole all fragments ($n=12$) either stopped or reversed direction to then travel towards the appropriate pole (Fig. 2).

Lastly, when trapping moving chromosomal fragments a trap power of 13mW at the focal plane, corresponding to a $\sim 1.5 \text{ pN}$ force was sufficient for ceasing cross-polar travel as all fragment centroids ($n=3$) were able to move $1.5-2.5 \mu\text{m}$ past the trap center towards the opposite pole. Fragment centroids then gradually returned to the center of the trap nearing the end of anaphase A.

4. Discussion

Accrued chromosome strain in anaphase appears to originate from both drag and tethering forces. Native length ratio extension of 6.5% is seen between 0-1 minutes and does not persist, as the chromosomes assumed a more stable length between 1.5-3 minutes while kinetochores continued chromosome transport. Considering the chromosome as an extending spring, this early extension peak reflects a temporary native accrual of strain. Laser cuts directed in the cellular space between sister chromosome ends revealed a source of chromosome strain independent of drag force. In roughly half of tested chromosomes, cuts between separating chromosome ends resulted in a mean relaxation of 7.5% suggesting 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 of only half of PtK2 chromosomes is consistent with LaFountain et. al's findings that only half of chromatids are tethered in crane-fly spermatocytes. While the 7.5% retraction is strikingly close to the extension seen in native chromosomes, further work is needed to fully distinguish extension due to drag versus tethering. When severing chromosomes during transport, kinetochore attached arms successfully segregated while chromosome fragments either stagnated and then successfully segregated, briefly travelled towards the opposing pole and stopped, or fully travelled to and remained at the sister side. 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, differences in the duration and distance of this cross-polar travel suggest the tethering element may lose structural integrity as anaphase progresses such that it can no longer transmit sufficient force to move a fragment through the cytoplasm. Fragments that continued segregating properly may not have been fully severed from the stub arm or perhaps are otherwise physically linked to segregating spindle structure. Optical trapping of cross-polar travelling fragments appears to provide an upper bound of ~1.5 pN for the tethering force as the fragments were able to travel past the center of the trap where the effective force is negligible but were not able to escape the trap. The tethering force seemingly lessened towards the end of anaphase as fragments returned to the trap center.

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