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Authors
Khatibzadeh, Nima
Stilgoe, Alexander B
Bui, Ann AM
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Optical trapping of isolated mammalian chromosomes

Nima Khatibzadeh[1], Alexander B. Stilgoe[2], Ann A. M. Bui[2], Yesenia Rocha[1], Gladys Cruz[1], Timo A. Nieminen[2], Halina Rubinsztein-Dunlop[2] and Michael W. Berns [1,3]

[1]Beckman Laser Institute and Medical Clinic, University of California, Irvine, 92612, USA, [2]School of Mathematics and Physics, The University of Queensland, St Lucia, 4072, Australia, [3]Institute of Engineering in Medicine, University of California, San Diego, 92093, USA

ABSTRACT

We have estimated the mitotic forces exerted on individual isolated mammalian chromosomes using optical trapping. The chromosomes were trapped by an optical tweezers system created by a continuous wave ytterbium laser at 1064 nm. Individual chromosomes were trapped at different *in situ* powers in the range of ≈20-50 mW. The corresponding trapping forces were determined by a viscous drag method. In the range of laser powers used, the preliminary data show a linear relationship between the chromosome trapping forces and *in situ* powers. We have calculated the dimensionless trapping efficiency coefficient (*Q*) of the chromosomes at 1064 nm and the corresponding effects of trapping power on *Q*. The value of *Q* in our experiments was determined to be ≈0.01. The results of this study validate optical tweezers as a non-invasive and precise technique to determine intracellular forces in general, and specifically, the spindle forces exerted on the chromosomes during cell division.

Keywords: Optical trapping, isolated chromosomes, trapping efficiency, cell division

1. INTRODUCTION

The processes of mitosis and meiosis (cell division) are conserved over virtually all eukaryotic organisms. The proper motion of chromosomes in mitosis/meiosis is necessary for successful cell division. During cell division, the chromosomes are first precisely aligned at the midplane of the spindle in metaphase (the metaphase plate), followed by poleward movements during anaphase to pull the sister chromatids apart[1, 2]. The motion of the chromosomes is mediated by the forces exerted on their kinetochores via microtubules and associated proteins that ultimately result in the sister chromatids being successfully pulled to the respective opposite cell poles. Therefore, determination of the forces on the chromosomes during cell division is essential to understand the underlying mechanics of chromosome motion, and ultimately to develop molecular models to explain the process.

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The forces exerted on the chromosomes can be estimated by hydrodynamic equations dealing with the motion of cylindrical bodies in a viscous medium. To achieve this, chromosome dimensions, movement velocities, and the viscosity of the surrounding intracellular media must be known[3, 4]. Besides the need for monitoring the cell division process to determine the chromosome movement velocities, these indirect methods of force measurements are hampered by significant variation in intracellular viscosity between species, as well as variations at different locations inside the same cell. Thus, devising a direct measurement method to probe the forces on chromosomes during cell division would be of great value.

In the past, mechanical chromosome force sensors involved hooking onto a motile chromosome from outside of the cell via a micro-needle whose tip deflection was calibrated to yield the force value[5]. This direct force measurement method was invasive, and required stretching the cell membrane and cytoskeleton to reach the chromosome. The method required simultaneous measurements of the deflection of the needle tip as a measure of the force. The development of a non-invasive and non-contact force measurement method with piconewton and sub-piconewton sensitivity would have great value in determining the real forces exerted on chromosomes by the mitotic/meiotic spindle.

Optical trapping is an ideal technique for this purpose given its extensive application to study a multitude of cellular and subcellular biological phenomena, such as determination of the motility forces of motor proteins kinesin and myosin[6, 7], and determination of the forces involved in axonal growth[8]. Using optical tweezers eliminates the potential force measurement artifacts associated with mechanical methods, such as the ones arising from stretching the cell membrane and its underlying cytoskeletal structures, and pushing the chromosome against the surface in order to halt its movement[5]. It also eliminates the need for determination of intracellular viscosity. The forces exerted on the chromosome can be calculated by determination of the optical trapping power needed to stop chromosome movement. This value is converted to force using the dimensionless trapping efficiency of the chromosome.

In this study, we use 1064 nm optical tweezers to trap single isolated chromosomes from mammalian Chinese hamster ovary (CHO) cells. The chromosomes were trapped using a range of trapping powers. The corresponding trapping forces were calculated based on application of the viscous drag method in order to determine the chromosome trapping force-power relationship.

2. MATERIALS AND METHODS

2.1 Cell culture

The chromosomes were harvested from Chinese hamster ovary (CHO-K1) cells (ATCC, CCL-61) cultured in advanced minimum essential medium (Gibco), and supplemented with 4% fetal bovine serum (FBS). Cells were incubated at 37 °C in 5% CO_2.

2.2 Chromosome isolation

2.2.1 Colcemid solution

We used a 10 mL colcemid solution (Gibco, 15210-040) prepared in HBSS (Hank's Balanced Salt Solution) to prevent spindle formation during mitosis, thus arresting the cells in metaphase. A Stock solution was prepared with 5mM $CaCl_2$ and 1 mM PIPES (Sigma Aldrich, 6757). A 100 ml working solution was prepared by adding 80 ml of ddH_2O (pH: 6.5) to 10 ml of the stock solution. 11.8 g (≈11 ml) of hexylene glycol (Acros Organics, 150340025) was added and stirred. The final working solution was stored at 4 °C.

2.2.2 Isolation procedure

A standard protocol was followed to isolate the chromosomes[9]. Briefly, the CHO-K1 cells were grown to 80% confluence, treated with the colcemid solution at 0.06 mcg per mL of growth media, incubated for 2 to 4 hours at 37 °C. After incubation, the colcemid containing medium with floating cells was transferred to a 50 ml centrifuge tube and centrifuged at 1000 rpm for 2 minutes at 4 °C. The cell pellet was re-suspended in cold (4 °C) working solution and transferred to a 15 mL centrifuge tube and centrifuged at 2000 rpm for 3 minutes at 4 °C. The cell pellet was re-suspended in ice cold working solution and incubated in a 37 °C water bath for approximately 10 minutes allowing the cells to equilibrate with the hypotonic working solution. When the cells swelled so the chromosomes became clearly visible, they were lysed. Next, the cell suspension was filtered through a 10 micron nylon filter to remove nuclei, cytoplasmic debris, and unbroken cells. The chromosome-rich suspensions were placed in 2mL vials, and stored at 4 °C. For the trapping experiments, a volume of ≈0.5 mL of the chromosome-rich suspension was diluted with ≈1.5 mL of the working solution at room temperature, and the diluted solution was pipetted into a Petri dish.

2.3 Optical tweezers setup

The optical tweezers system is similar to one previously described[10]. Briefly, a continuous wave 1064 nm wavelength ytterbium laser (PYL-20M, IPG Photonics) was collimated and steered through a series of mirrors and lenses, and coupled into the epifluorescence port of an inverted microscope (Zeiss Axiovert-135). A dual band laser dichroic mirror (Chroma Technology, z532/1064rpc) was used to reflect the IR laser beam toward the back aperture of the microscope objective while simultaneously transmitting the light collected from the biological specimen to a CCD camera. The laser beam was focused through a high numerical aperture (NA=1.4), oil immersion, Phase III, 100X objective (Zeiss Plan-Apochromat). The objective transmission efficiency at 1064 nm wavelength was determined ≈25% based on a three objective measurement method[11]. Laser power in the focal spot was varied from ≈20 to 50 mW in each individual experiment.

2.4 Chromosome size measurements

The transverse (x and y) image planes were calibrated by measuring known distances in the x and y directions and correlating them with the corresponding number of the pixels. For this purpose, a micrometer microscope ruler with 10 μm spacing was used. The calibration yielded ≈16.4 pixels per μm in both x, and y directions. The length and diameter of the individual chromosomes were measured under phase contrast microscopy using the 100X objective.

2.5 Chromosome trapping

The chromosome suspension (made from chromosomes-rich suspension diluted with the working solution at room temperature) was pipetted into glass bottom petri dishes (FluroDish, FD35-100) and the chromosomes were trapped with the 1064 nm laser tweezers. If the chromosomes were twisted, or folded upon themselves, they were not selected for trapping. Often the chromosomes were floating horizontally in the suspension medium prior to trapping. When exposed to the trap, these chromosomes usually changed to a vertical orientation. Once a chromosome was in the trap, the motor-driven microscope stage (Ludl Electronic Products, BioPrecision2, NY, USA) was driven at a specific velocity. This resulted in the application of a known viscous drag force on the chromosome. The velocity at which the trapped chromosome dropped out of the trap was determined to be the "escape velocity".

3. RESULTS AND DISCUSSION

Individual isolated chromosomes with mean ± s.d. diameter values of ≈0.95±0.16 μm, length of ≈6.71±1.74 μm, and aspect ratio (length/radius) of ≈14.42±3.89 were suspended in media with 1 cp viscosity prior to optical trapping. The chromosomes were trapped in the ranging of ≈20-50 mW in the focal volume. At each trapping power, the stage velocity was increased to the point that the trapped chromosomes fell out of the trap, the escape velocity. Using the hydrodynamic calculations for Stokes' flow around cylindrical objects[12], the trapping forces were calculated based on the measured escape velocities, the viscosity of the media, and the dimensions of the chromosomes (Eq. 1):

$$F_{\parallel} = \frac{2\pi\mu v L}{\ln\frac{L}{a} - 0.807} \tag{1}$$

where $F_{\parallel}$ is the force on the cylinder with the fluid flow parallel to the cylinder longitudinal axis. The parameter μ is the dynamic viscosity of the surrounding media (N.s/m^2), v is the fluid flow velocity (m/s) at which the chromosome escapes the trap, parameters L and a are the length and radius of the cylinder (m), respectively.

Figure 1a shows the trapping force-power relationship of isolated chromosomes versus the trapping power. The results demonstrate a linear relationship between the trapping force-power within the power range examined.

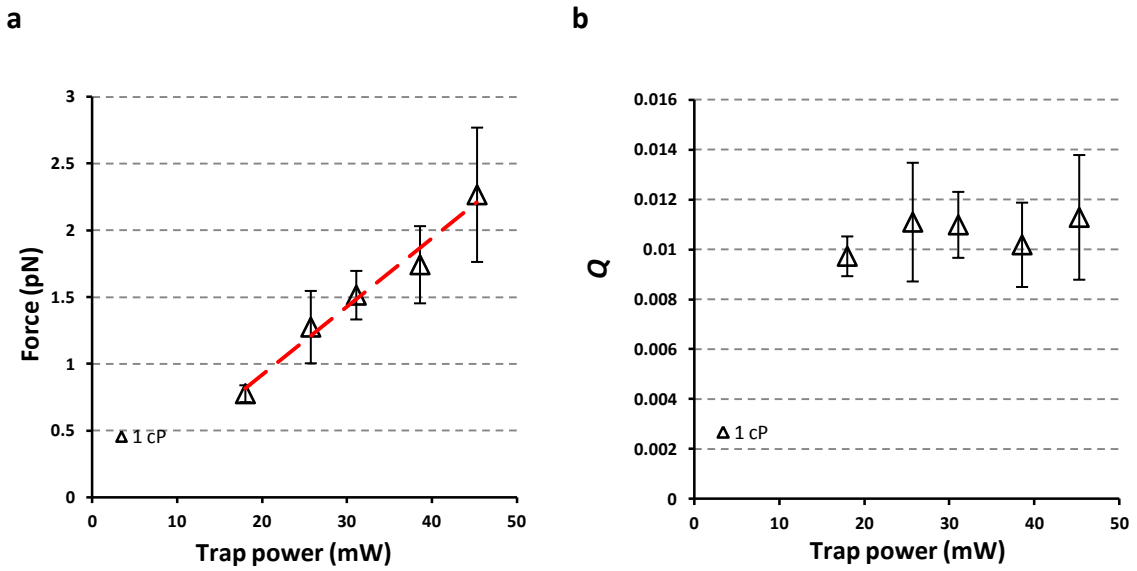

Figure 1- Optical trapping of the isolated CHO chromosomes with laser tweezers at 1064 nm wavelength in 1 cP viscosity medium. a, The chromosomes trapping force-power measurements. **b,** Calculated transverse trapping efficiency (*Q*) of the isolated chromosomes.

The trapping efficiency of the chromosomes (*Q*) at each trapping power was calculated based on the trapping force and power measurements (Eq. 2)[13]:

$$F = \frac{QPn}{c} \tag{2}$$

where *F* is the trapping force (N), *P* is the incident laser power at the specimen (*W*) (*in situ* power), *c* is the speed of light (m/s) in a vacuum, and *n* is the refractive index of the surrounding medium.

The calculated trapping efficiency of the isolated chromosomes is shown as a function of the trapping power in Figure 1b. The results present an almost unchanged value of ≈0.01 for the parameter *Q* of the chromosomes over the range of the trapping powers studied.

CONCLUSIONS

Optical trapping at 1064 nm is an ideal technique to study the motility forces on chromosomes during cell division. It is a sterile, non-contact, and non-invasive method with low energy absorbance, and thermal effects in biological samples. In a dividing cell, the forces exerted on chromosomes by the mitotic spindle through the microtubule-kinetochore

attachments can be estimated by optically trapping a motile chromosome, and increasing the *in situ* trapping power to fully stop the chromosome movement. Next, the forces on the chromosome can be calculated by knowing the trapping efficiency factor (*Q*) which relates trapping power to the force. The parameter *Q* indicates the fraction of the incident light momentum transferred to the trapped object and varies from 0 to 1[13, 14]. The magnitude of *Q* for chromosomes obtained in our experiments (0.01) demonstrates a small trapping efficiency of the chromosomes. This indicates that a small fraction of the incident light momentum is transferred to the trapped chromosomes at 1064 nm while the higher fraction of the light momentum is transmitted through its structure. This is, perhaps, due to the structure of the chromosome. It is composed of elongated nucleoprotein fibrils with interstices between the chromatin fibers. It has been demonstrated by optical refractometry experiments[15] and theoretical modeling of mechanical stress relaxation[16] that chromosome structure permits in and out flow of small molecules. This may also explain the transmission of the high fraction of the incident light (photon) momentum through the interstices of the chromosomes and the subsequent small trapping efficiency.

The trapping forces on the chromosomes examined in this study are in the range of ≈0.7-3 pN (Fig. 1a). The intracellular viscosity of the cells varies from 1-3 cP in the aqueous phase of the cytoplasm in mammalian cells[17-21] to 140 cP in cellular vesicles[22]. For the cell cytoplasm surrounding the mitotic spindle, the reported average viscosity values are ≈300 cP[2, 3, 23], and in a more recent study it was reported as high as ≈1200 cP[24]. During anaphase, the chromosomes move with the average velocity of ≈1 μm/min[25], and can move about five times faster in some cells. Given the maximum average velocity of 5 μm/min, and published viscosity values as high as 1200 cP, the corresponding viscous drag forces exerted on the chromosomes estimated by Eq. 1 would only be up to ≈3 pN. This lies within the range of the forces measured in this study, and is orders of magnitude smaller than reported in earlier studies[5].

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