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Directed Movement of Chromosome Arms and Fragments in Mitotic Newt Lung Cells Using Optical Scissors and Optical Tweezers

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A pulsed-laser microbeam at 532 nm wavelength (optical scissors) and a laser-induced optical trap at 1064 nm wavelength (optical tweezers) have been successively combined to dissect and manipulate chromosomes in live newt lung epithelial cells. These preliminary experimental results demonstrated that chromosome fragments dissected by laser microbeam surgery, regardless of their size, could be easily pulled or rotated by optical forces when positioned at the periphery of the mitotic spindle. In addition, chromosome arms which were not subjected to laser microsurgery also could be moved with the optical tweezers at the spindle periphery. In our previous study on rat kangaroo kidney cells (PTK_2), this degree of facility in manipulating chromosome movement was not possible, most likely due to the close proximity of the intermediate filament "cage" to the spindle. It is concluded herein that optical scissors and tweezers can be used in combination to study the interaction of chromosomes with the mitotic spindle in cells where the peripheral regions of the spindle are unobstructed by intermediate filaments. This can be performed on newt cells, where the diameter of the cage can be substantially larger than the diameter of the spindle.

INTRODUCTION

Optical traps, or optical tweezers, consist of a single, strongly focused laser beam [1] that uses radiation pressure to manipulate and hold microscopic objects. Optical trapping inside the cell without damage to the cell wall or membrane was first demonstrated by Ashkin *et al.* [2, 3] using spirogyra and protozoa. Subsequent work [4] demonstrated that it was possible to manipulate other intracellular organelles inside plant cells, including chloroplasts and nucleoli. These experimental results demonstrated that optical trapping can be used as a noninvasive and nondestructive tool for the manipulation of biological objects.

A series of papers has been published demonstrating that optical forces can be used to accelerate the movement of late-moving mitotic chromosome and to hold anaphase chromosomes motionless in rat kangaroo (PTK_2) cells [5–7]. More recently, it was demonstrated that a laser scissors could be used to cut chromosomes into fragments at preselected sites, and the optical tweezers could subsequently be used to hold the dissected chromosomal fragments in place [8]. However, it was not possible to use the optical trap to move the dissected chromosomal fragments outside the PTK mitotic spindle. In PTK cells, intermediate filaments (IMF) composed of vimentin and keratin have been shown to surround the mitotic spindle during mitosis [9]. The IMF "cage," being in close proximity to the mitotic spindle, may hinder movement of the chromosome fragments with the optical trap.

Epithelial cells from newt lungs are an attractive system for the study of the mitotic spindle and chromosome behavior because of the large size of the chromosomes and spindle [10] and also because the cell remains flat during mitosis. More importantly, during prometaphase, these cells contain a region between the spindle and the IMF cage that does not contain organelles and is relatively free of microtubules [11]. We hypothesize that this region of "clear cytoplasm" between the mitotic spindle and the IMF cage permits the manipulation of optically trapped chromosome fragments. As a result of the optical manipulation, the trapping force on the chromosome fragment was estimated using viscous drag theory. This suggests that optical tweezers may be suitable for studying the function and behavior of the force-generating mechanism that moves chromosomes during mitosis.

MATERIALS AND METHODS

Newt lung epithelial cells. Primary lung explants from the newt (*Taricha granulosa*) were grown in Rose chambers according to well-

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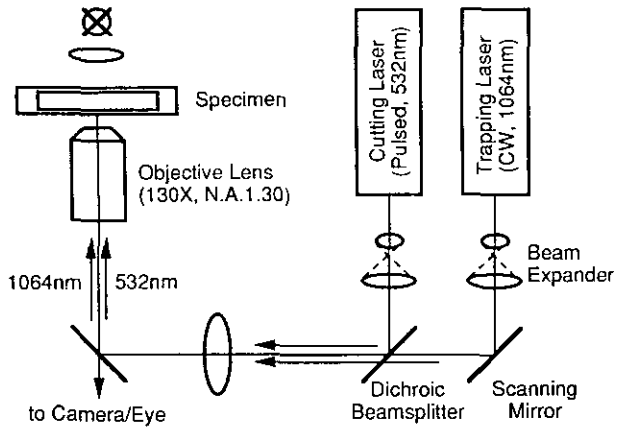

FIG. 1. Simplified instrument configuration for the combined use of laser scissors and laser tweezers.

defined methods [10]. Large, flat, mitotic cells displaying epithelial characteristics were selected for irradiation 1–2 weeks after initiation of the culture.

Laser microsurgery. Laser microsurgery (scissors) was performed using the second harmonic 532-nm wavelength from a pulsed (10 ns pulse duration) Nd:YAG laser (Quantel, Model YG481A, Santa Clara, CA). Laser energy was controlled by an adjustable optical attenuator (Karl Lambrecht Corp., Model K1174, Chicago, IL) that was placed in the laser beam path. The laser beam was transmitted through an inverted microscope (Zeiss Axiomat, Thornwood, NY) and focused by a Zeiss Neofluar X100 phase-contrast objective, with a numerical aperture of 1.3. The chromosome was moved under the focused laser using a 0.5-μm step size scanning stage (Zeiss). A dichroic reflector inside the microscope was used to reflect the green microsurgical beam into the microscope while transmitting an image to the video camera (Hamamatsu, Model C-2400 with Newvicon tube, Bridgewater, NJ) attached to one of the observation ports of the microscope. The video image was recorded by a half-inch time lapse VCR (Panasonic, Model AG-6030P, Secaucus, NJ) and displayed on a monochrome monitor. The target chromosomes were exposed to the 532-nm pulsed laser microbeam, having a repetition rate of 10 Hz, while being simultaneously observed by video microscopy.

Laser-induced optical trapping microbeam. A second Nd:YAG laser (Quantronix, Model 116, Smithtown, NY) operating continuous wave (CW) at a wavelength of 1064 nm in the TEM_{00} mode was used to generate the optical trap. The laser was directed by a series of mirrors into a Zeiss Universal microscope and was focused onto the specimen plane by a Zeiss Neofluar X100 phase-contrast objective, with a numerical aperture of 1.3. One of the beam-steering mirrors was tilted by a DC actuator controlled by a joystick, which resulted in the movement of the tweezers in the optical field of the microscope. A dichroic mirror was used to reflect the IR beam into the microscope while simultaneously transmitting light from the specimen to the video camera. The optical tweezers were applied, using a trapping power of 440 mW at the microscope objective, on the dissected chromosome fragment by positioning it under a cross hair that corresponded to the position of the trap in the optical field. The behavior of the trapped chromosome fragment was recorded on videotape for all experiments. The micrographs presented in this paper were reproduced from the videotape images by capturing a single video frame with a Polaroid (Cambridge, MA) FreezeFrame using Kodak PlusX Pan photographic film (Fig. 1).

Force calculations. The velocity of the optically trapped chromosome as it was manipulated was measured from the recorded videotape images. The position of the center of the trapped chromosome was plotted on a video monitor every 0.33 s (10 video frames). The drag force was determined using the expression $F = \mu s \upsilon$, where μ is the viscosity, s is a shape factor that describes the object (units of length), and υ is the velocity. The shape factor for the chromosome was calculated for two orientations [12], that is, for the case where chromosome motion was perpendicular to its long axis

$$s_{\perp} = \frac{32\pi(a^2 - b^2)}{(2a^2 - 3b^2)z + 2a} \tag{1}$$

and also for the case where the chromosome motion was parallel to its long axis

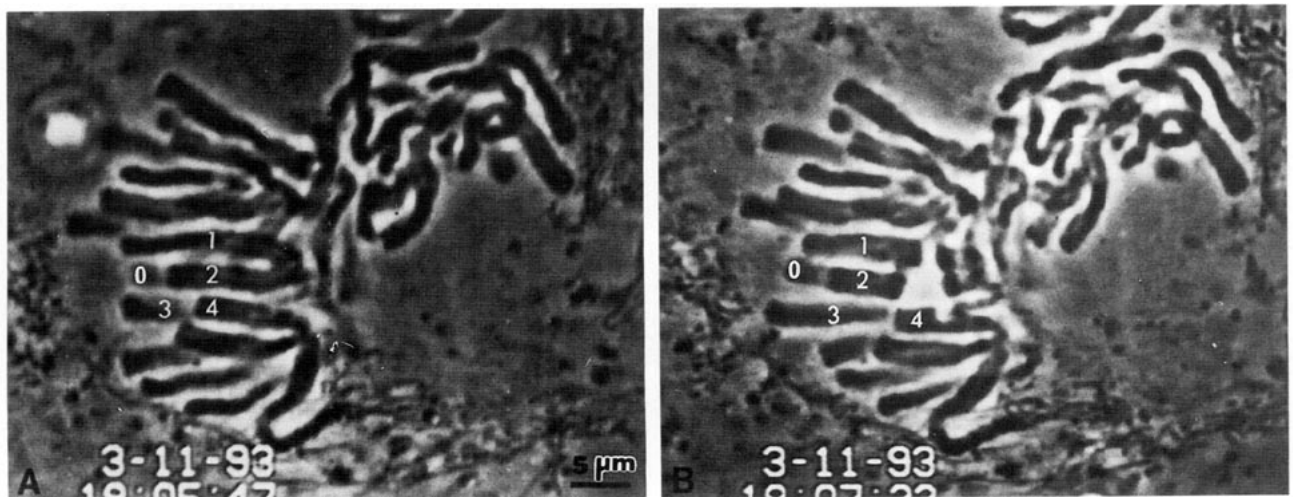

FIG. 2. An example of chromosomal laser microsurgery in newt lung epithelial cell. (A) Before laser microbeam surgery. (B) After laser microbeam surgery. Chromosomes labeled 1, 2, and 3 were dissected completely. Chromosome 4 was partially dissected. Chromosome 0 was partially covered by chromosome 2.

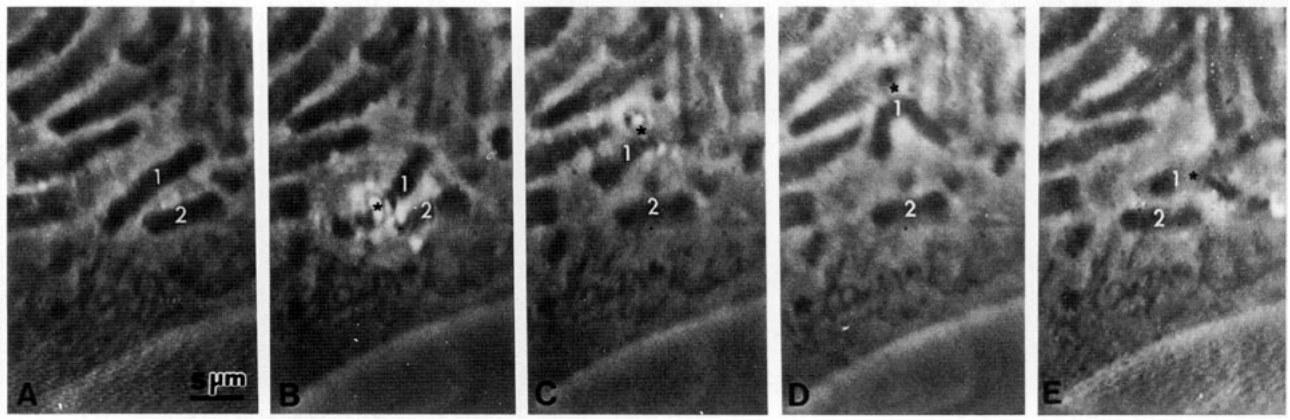

FIG. 3. An example of optical trapping of chromosomal fragments dissected by laser microsurgery, showing sequence of trapped chromosomal fragment movement. Asterisk indicates the position where the trapping force was exerted.

$$s_{\parallel} = \frac{16\pi(a^2 - b^2)}{(2a^2 - b^2)z - 2a} \tag{2}$$

In Eqs. (1) and (2), $a = l/2$, $b = d/1.64$, and

$$z = \frac{2}{\sqrt{a^2 - b^2}} \ln \frac{a + \sqrt{a^2 - b^2}}{b},$$

where l and d are the length and diameter of the chromosome, respectively. An average viscosity of ~2.8 dyn-s/cm^2 (280 cP), measured in the clear area surrounding the spindle [13], was used for the newt lung cell cytoplasm viscosity.

RESULTS AND DISCUSSION

Preselected prometaphase chromosomes were first cut with the laser scissors. The target chromosome was aligned under the cross hair on the video monitor and brought into focus by the microscopic objective. The laser was fired repeatedly while a chromosome arm was moved through the focused laser beam until the chromosome was completely severed (Fig. 2). As shown in Fig. 2A, the target chromosomes labeled 1, 2, and 3 were intact before laser microbeam surgery. However, after being exposed to the laser, each of the three target chromosomes was cut into two separate chromosome fragments (Fig. 2B). In Fig. 2B, a chromosome just above the lowest target chromosome (labeled 4) was partially cut due to inadvertent laser exposure. Laser microsurgery at 532 nm was successful in cutting chromosomes as described in our previous studies [8, 14]. The power level of each laser pulse was approximately 800 nJ at the specimen plane. Approximately 600 laser pulses (at 10 Hz) were required to cut an entire chromosome. Severed chromosome arms were ejected to the periphery of the spindle as previously described [14]. After completion of the laser microsurgery, the Rose chamber was transferred to the optical trapping microscope and the mitotic cell with severed chromosomes was relocated.

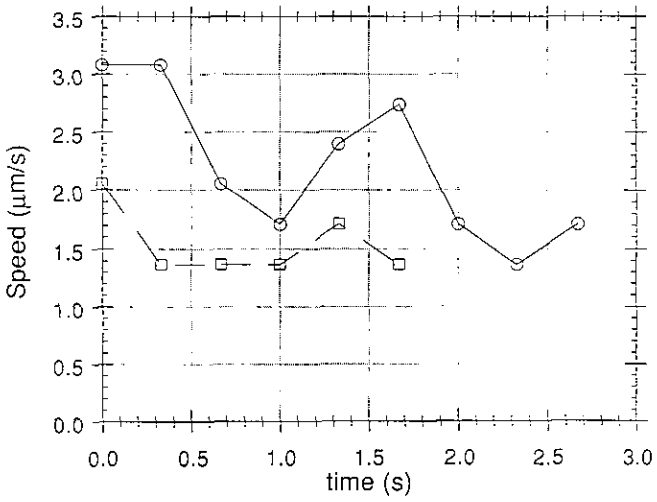

FIG. 4. Trapped chromosome velocity as a function of time for chromosome 1 shown in Figs. 2B–2D. Two separate sequences of movement are shown, as indicated by the separate lines.

Our results indicate that in newt lung cells, optical forces can be used to move and rotate the dissected chromosome fragments at the periphery of the mitotic spindle. The spindle is surrounded by a "cage" consisting of intermediate filaments [11, 13]. The clear area between the spindle and the IMF cage (clear cytoplasm) does not contain organelles typically found in the cytoplasm. The distance that a chromosome fragment could be moved from its original position in the spindle periphery varied in different experiments regardless of the size of the chromosome. For example, a tiny chromosomal fragment was rotated 180° within a radius of ~5 μm while it was being pulled by the optical tweezers. Another chromosomal fragment 6 μm in length was pulled away from its original position up to a distance of ~5 μm. However, it was also noted that a large chromo-

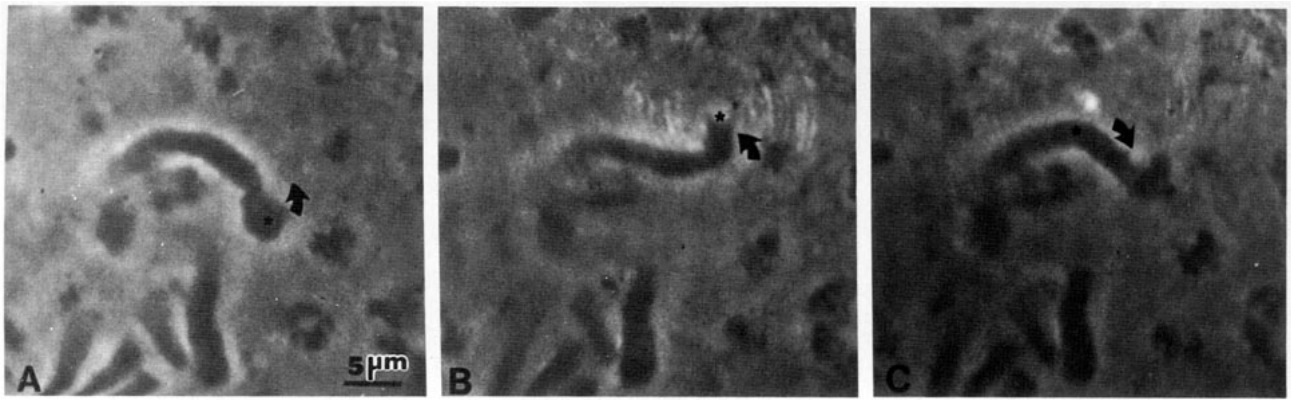

FIG. 5. An example of optical trapping of intact chromosome showing trapped chromosome bending and rotating. Asterisk indicates the position where the trapping force was exerted. Arrow indicates the pulling direction.

somal fragment of 15 μm in length could be pulled by the optical tweezers a linear distance of 12 μm. Displaced fragments could be moved back to their starting position by moving the optical tweezers in the direction opposite to the original motion. All these manipulations were performed within a few seconds.

Figure 3 is an example showing both movement and rotation of a chromosomal fragment by the optical tweezers. The chromosomal fragments labeled 1 and 2 are the same chromosomal fragments 1 and 2 depicted in Fig. 2. When the chromosome fragment is at the position several micrometers away from the optical tweezers, it will be pulled toward the tweezers. Figures 3B and 3C show that the chromosomal fragment moved to the positions, which were set in a horizontal plane perpendicular to the incident laser beam. However, the further movement of the chromosomal fragment was blocked by another chromosome (Fig. 3D). Finally, this chromosomal fragment could be moved back to its original position if the optical tweezers were moved to the opposite side of the fragment (Fig. 3E). The velocity of the trapped chromosome fragment was measured for two separate motion sequences occurring in the interval between Figs. 3B and 3D and is shown in Fig. 4. Using the maximum recorded velocity of 3.1 μm/s, a measured length (l) of 10.2 μm and a measured diameter (d) of 1.2 μm, the drag force on the chromosome fragment while in the optical trap was estimated to be 26–35 pN (2.6–3.5 μdyn), with an average force of ~30 pN for an effective shape factor, s, having equal contribution from parallel ($s_{\|}$ = 29.9 μm) and perpendicular ($s_{\perp}$ = 41.2 μm) orientations of the chromosome. It is likely that the maximum force exerted by the tweezers is somewhat higher than our estimate of 30 pN and could be determined by measuring the speed at which the chromosome fragment "drops out" of the trap. The forces exerted by the optical tweezers compare favorably with a force of 1–74 pN/microtubule produced by nascent kinetochore fibers in the same cells [13]. Hence, calibrated optical tweezers could be used to grasp chromosomes as they attach to the spindle and to measure the maximum force exerted by the nascent kinetochore fibers. In addition, the tweezers may be able to test a model prediction that the total force exerted on the chromosome is not proportional to the number of microtubules once the force-generating sites on the kinetochore have been filled [15].

It was also possible to direct movement of a nonlaser-dissected intact chromosome arm. Figure 5 shows the peripheral end of a long chromosome arm manipulated with the optical tweezers. Figure 5A is at the initiation of optical trapping. When the tweezers were used to pull the proximal arm of this chromosome in the upward direction, the chromosome could be rotated (Fig. 5B). When optical force was then used to pull the chromosome in the downward direction, the chromosome regained its original configuration. As shown in Fig. 5C, when the optical force was switched to the center of the chromosome and pulled upward, the shape of the chromosome changed. These results demonstrate that, under certain conditions, optical tweezers can be used to manipulate intact chromosomes arms, as well as laser-generated chromosome fragments.

In conclusion, we have demonstrated that an optical trap can manipulate cut chromosome fragments and chromosomal arms in the clear cytoplasm of newt pneumocytes. In a previous study [8], while cut chromosome fragments in PTK_2 cells could be held by the optical trap and kept motionless throughout anaphase, it was not possible to actually move (pull) the chromosome fragments. The ability to cut and move severed chromosome fragments as well as to move "intact" chromosomes in newt cells demonstrates that this technology can be applied to studies on spindle function and chromosome movement. Our estimate of the trapping force exerted on a chromosome fragment suggests that optical tweezers may be useful in the future as an intracellular dynamometer. The relative ease of manipulating

chromosomes by optical trapping in newt lung epithelial cells may be attributed to the fact that it is one of the largest of vertebrate somatic cells [10]. More significantly, it appears that the lack of microtubules and organelles in the clear cytoplasm [11] of newt pneumocytes, compared to rat kangaroo cells, is a key factor enabling the use of optical traps for chromosome manipulation during mitosis.

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