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Authors

Berns, MW
Wright, WH
Tromberg, Bj
[et al.](#)

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Use of a laser-induced optical force trap to study chromosome movement on the mitotic spindle

MICHAEL W. BERNIS, WILLIAM H. WRIGHT, BRUCE J. TROMBERG, GLEN A. PROFETA, JEFFREY J. ANDREWS, AND ROBERT J. WALTER

Beckman Laser Institute and Medical Clinic, University of California, Irvine, 1002 Health Sciences Road East, Irvine, CA 92715

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ABSTRACT A laser-induced optical force trap was used to alter the movement of chromosomes in mitotic cells *in vitro*. The trap was produced by using a 1.06- μm neodymium YAG (yttrium/aluminum garnet) laser focused through a phase-contrast microscope. The trap was applied to one side of centrophilic chromosomes off the mitotic spindle and to late-moving chromosomes on the mitotic spindle. In both situations, chromosome movement was initiated in the direction opposite to that of the applied force. When the force was applied, chromosomes moved at velocities 10–20 times normal. These studies verify and extend the feasibility of using this new technique to study factors that influence organelle motility.

The cell organelles and molecules involved in the organization and function of the mitotic spindle have been well characterized. However, the nature of the forces that are responsible for the orderly movement of chromosomes is not well understood. Part of the reason for this gap in our knowledge is the difficulty associated with studying forces in an object as small as a single living cell without perturbing the cell's normal function. The only direct measurements of spindle forces in living cells have been performed by using a microneedle attached to a pressure transducer designed to impale single chromosomes on the spindle of grasshopper spermatocytes (1). Though these early experiments provided the first direct measurement of forces associated with chromosome movement, the technical difficulties associated with impaling single chromosomes and the invasive nature of the technique were major limiting factors. In the present paper, we report the application of a single-beam optical force trap as described by Ashkin and colleagues (2–5) to the division of *Potorous tridactylus* (PTK2) cells *in vitro*. The trap is noninvasive (i.e., it does not induce physical rupture of the cell membrane) and can be applied easily through any conventional microscope. Moreover, it can be applied to a large number of cells quickly and by a technically "unskilled" individual. The trap was used to affect chromosome movement in prometaphase and metaphase cells. Though it was possible to hold chromosomes off the metaphase plate with the optical force, the most frequent observation was high-velocity chromosome movement in a direction away from the applied trapping ("grabbing") force. These surprising results support the concept introduced by Nicklas (6) of a mitotic motor that senses an opposing force. In addition, this study further shows that the optical trap can be used to study problems of organelle motility, as shown by Ashkin *et al.* (5).

MATERIALS AND METHODS

PTK2 cells were grown in standard Rose tissue culture chambers. Each chamber was assembled with high-quality

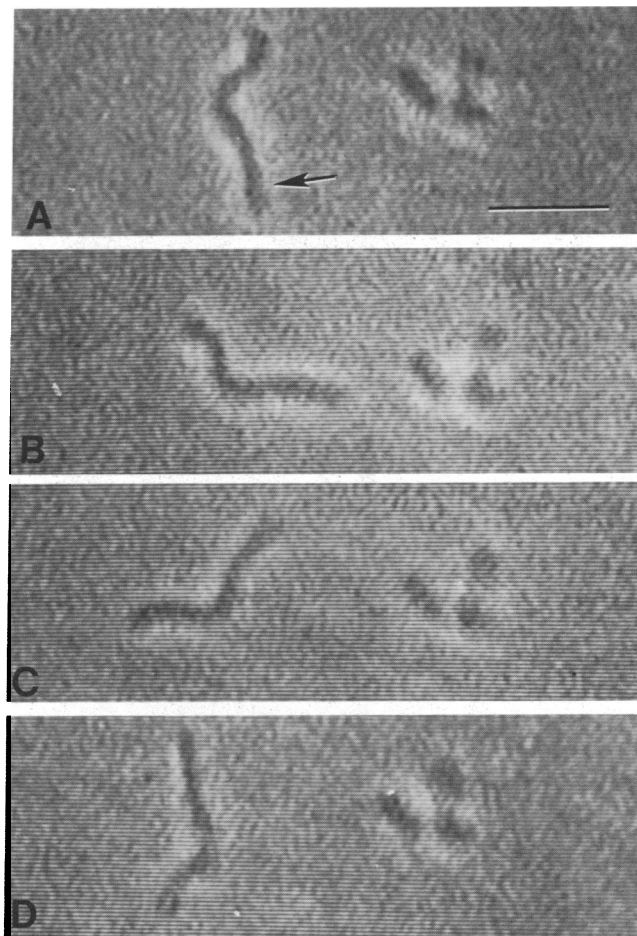


FIG. 1. A chromosome from a lysed cell being rotated 180° by the optical trap. Note small marker chromosomes in the field to the right of the chromosome being rotated. (Bar = 10 μm .)

optical windows to permit optimal visualization with standard phase-contrast optics and time-lapse video analysis. Because of the flat morphology of these cells, the chromosomes and other mitotic structures are clearly visible (7). An air-curtain incubator was directed at the microscope stage to maintain the culture chamber at 35–37°C.

The optical trap used was similar to that described by Ashkin and colleagues (2–5). A continuous wave neodymium YAG (yttrium/aluminum garnet) laser (Quantronix model 116) was deflected into a Zeiss photomicroscope and focused by a Neofluar $\times 100$ (numerical aperture 1.3) objective into the optical field. The laser power in the 1- to 2- μm focused spot was controlled and varied from 10 to 200 mW. The standard equation for force generation when all the light is retroreflected (2) is $F = 2P/c$, where P = laser power and c = speed of light. For the power used, this would be 6.7–133

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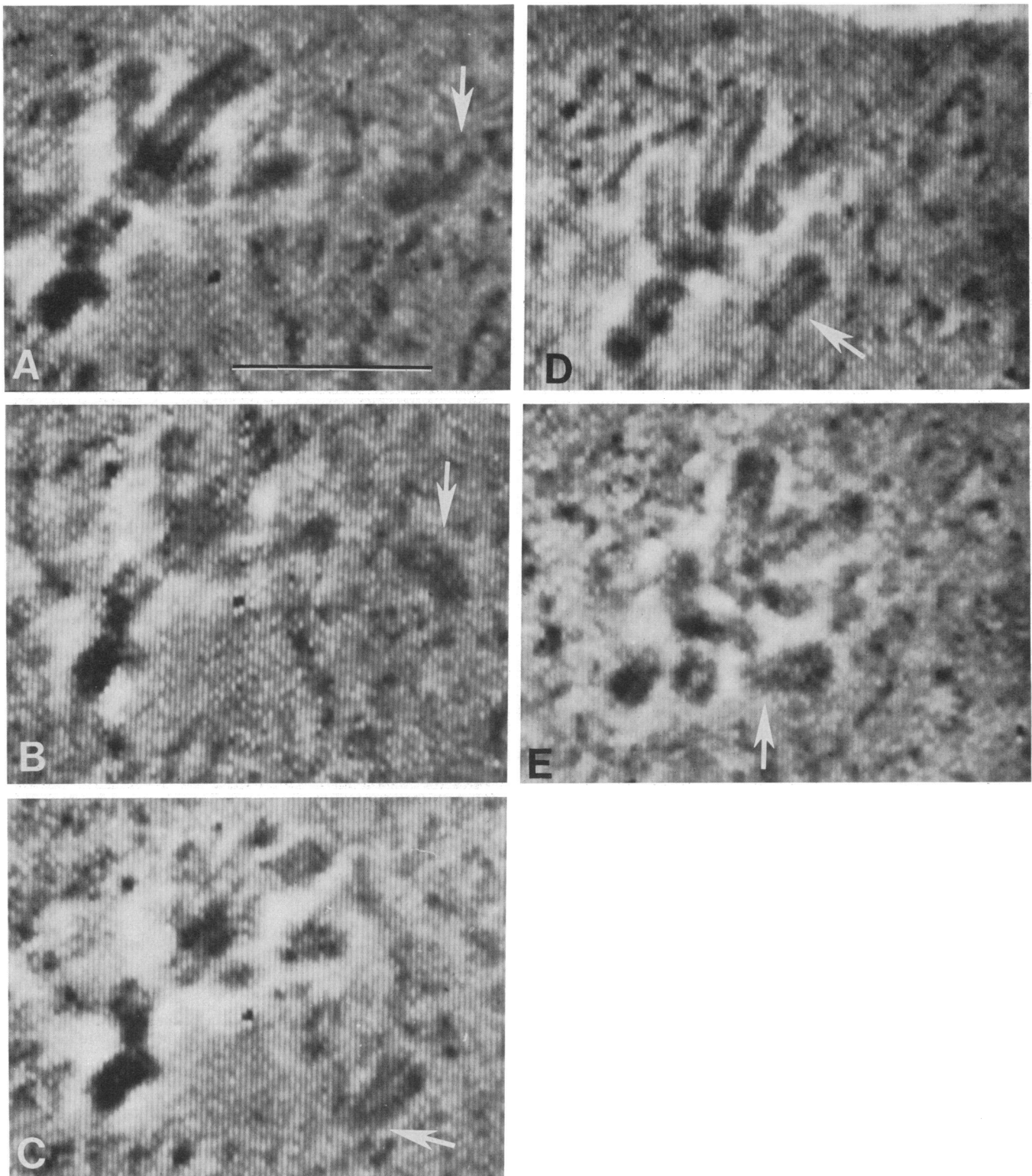


FIG. 2. (A) A metaphase cell with a single centrophilic chromosome in the cytoplasm off the spindle. The arrow indicates the site immediately adjacent to the chromosome where the optical trap was applied. (B) A few seconds after exposure to the trapping force, the chromosome has reoriented and initiated movement towards the lower spindle pole. (C) The chromosome has reached the spindle pole, moving a distance of 11 μm in 45 sec; the optical trap has been moved to the spindle pole and applied at the point indicated by the arrow. (D) The chromosome is moving from the spindle pole towards the metaphase plate. (E) The chromosome has reached the metaphase plate. (Bar = 10 μm .)

$\times 10^{-6}$ dynes. The range of forces produced for an idealized situation where all of the light is refracted by the object would be 1/3rd to 1/10th of this. Since the objects to be moved (the chromosomes) have an index of refraction that is higher than the surrounding medium (the cell cytoplasm), the optical

force will be due to refraction (not reflection), and the direction of the net force will be back towards the laser focal point (i.e., the chromosome will be "pulled" by the laser beam). In all of the experiments, the target chromosomes were exposed to the optical trap while continually being

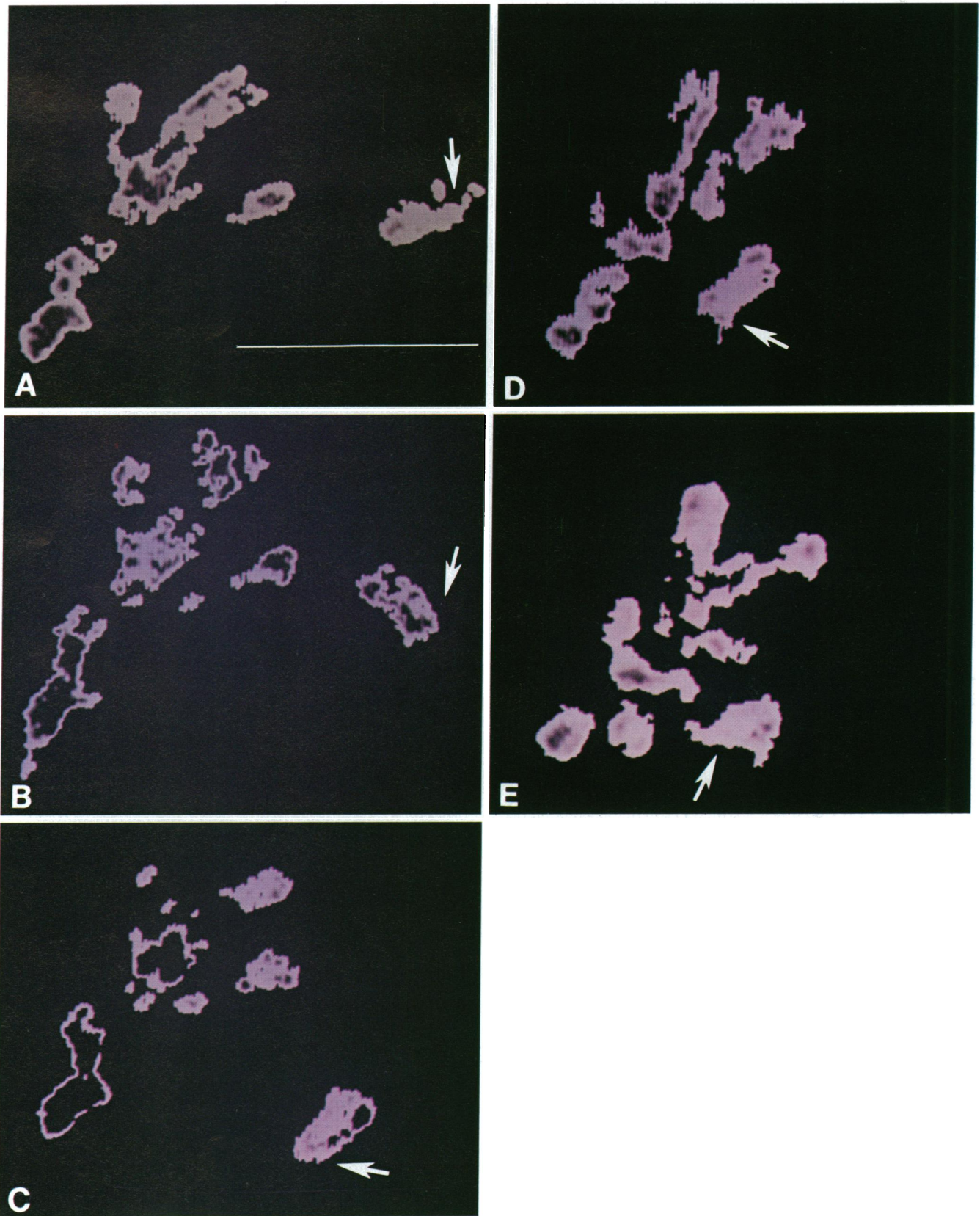


FIG. 3. The same cell sequence as in Fig. 2. Images have been digitally enhanced to accentuate the chromosomes and exclude all other structures (see refs. 8 and 9).

monitored by time-lapse video microscopy. Because of the low resolution of video tape images, digital processing was used to improve image quality and gather quantitative data (8-10).

RESULTS

To demonstrate that the optical trap could be used to manipulate chromosomes, a 50- to 100-mW trap was focused on

the edge of chromosomes in suspension (from lysed cells), and chromosomes were pulled easily (Fig. 1). The largest chromosome in the karyotype was rotated 180°. The arrow in Fig. 1A indicates the location and side of the chromosome exposed to the trapping (pulling) force. In mitotic cells an optical force trap of 130 mW similarly was focused on the edges of individual chromosomes in two situations: (i) centrophilic chromosomes located off the mitotic spindle between one pole and the outer cell margin and (ii) late-moving chromosomes located between the metaphase plate and one mitotic pole. The optical force was also applied in control situations to regions within the mitotic spindle zone and to cytoplasmic regions outside the mitotic spindle. In all cases, mitosis proceeded normally where no chromosomes were exposed to the optical force.

The first example presented (Fig. 2) is on a stationary centrophilic chromosome that had not moved onto an otherwise normal-appearing metaphase plate. The optical trap "pulling" force was applied to the side of the chromosome between the cell margin and the chromosome, not on the side facing the nearest spindle pole (Fig. 2A; the arrow indicates the point of force application). Within 2–3 sec of force application, the chromosome began to move out of the laser trap focal point towards the nearest spindle pole. The centromere reoriented towards the pole (Fig. 2B), and the chromosome moved to that pole (a distance of 11 μm) in 45 sec (Fig. 2C). [See Fig. 4 for a plot of chromosome velocity as a function of time after force application. The maximum velocity of 48 $\mu\text{m}/\text{min}$ was 24 times faster than normal values reported for these cells (7).] When the chromosome reached the pole (Fig. 2C), it stopped. The optical trap was then moved from its original location and reapplied to the side of the chromosome between the chromosome and the pole (as opposed to the side of the chromosome closest to the metaphase plate). The chromosome started moving towards the metaphase plate within 2–3 sec of force application, and it reached a maximum velocity of 4.4 $\mu\text{m}/\text{min}$. This velocity was twice the normal velocity. The chromosome reached the metaphase plate (Fig. 2E) and stopped moving. The cell subsequently completed mitosis with all chromosomes moving in a normal fashion. Fig. 3 depicts the chromosomes and single-chromosome movement (arrows) in images that have been digitally enhanced to accentuate only the chromosomes in Fig. 2. Digital enhancement was undertaken because of the poor quality of images stored on video tape. Because of the speed with which the observed events occurred, standard microscopic photography was not possible, and images had to be reproduced by photography directly from the video screen (Figs. 2 and 5). In the second series of experiments,

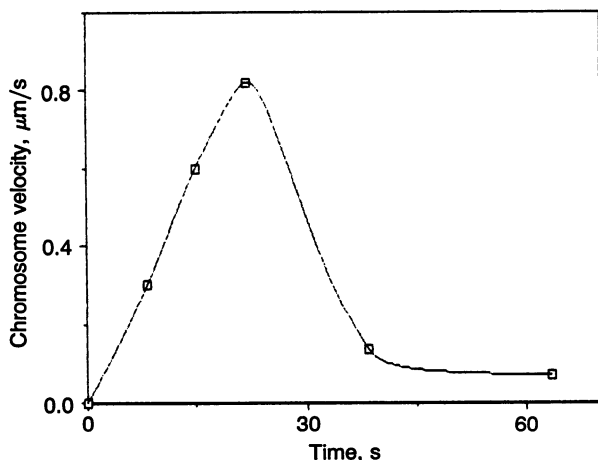


FIG. 4. A graph of chromosome velocity versus time for the cell in Figs. 2 and 3.

a 40- to 50-mW optical trap was applied to late-moving chromosomes on the spindle (Fig. 5). In all cases the target chromosomes were stationary at the time of irradiation, and they were located within the spindle between the metaphase plate and one pole. The force was always applied to the chromosome side closest to the nearest spindle pole. The site of force application is indicated by the arrow in Fig. 5A. The chromosome began moving towards the metaphase plate within 2–3 sec of force application and attained a velocity of 20 $\mu\text{m}/\text{min}$ (it moved 5 μm in 15 sec). In a second cell treated the same way, the chromosome moved 8.8 μm in 27 sec (18 $\mu\text{m}/\text{min}$). Both chromosomes moved at velocities 10 times the normal rate, and both stopped moving as soon as they reached the metaphase plate. The cells subsequently completed mitosis with all chromosomes undergoing anaphase movements.

DISCUSSION

The preliminary experiments reported here establish that laser-induced optical forces can be used to study chromosome movement during cell division. Though interpretation of the observations presented here may be open to debate, three facts are unequivocal: (i) stationary chromosomes initiated movement upon application of the force, (ii) the chromosomes moved in a direction opposite to that of the applied "pulling" force, and (iii) the chromosomes moved at peak velocities 10–20 times normal velocity.

In the case of the centrophilic chromosome (Fig. 2 A–D), a possible interpretation is that the mitotic motor "sensed" the force applied to the distal side of the chromosome; in response, microtubule shortening (disassembly) was initiated, causing the chromosome to move towards the pole. This sensing of a tension force is consistent with Nicklas' observations on chromosome behavior under a tension force applied by microneedles (1).

At first glance, our observations may appear to be in disagreement to the predictions of Hill and Kirschner (8), which would suggest that a pulling force should induce polymerization of chromosome-to-pole microtubules. This may have occurred in the few seconds between application of the force and initiation of chromosome movement. However, without structural (electron microscopic) or molecular (antibody staining) information about the chromosome-microtubule relationships, it is simply not possible to be definitive at this time. The high velocity of the chromosome movement in this cell could be explained if there were no microtubule attachment to the kinetochore on the distal side of the chromosome and if the mitotic motor, in response to sensing the opposing force due to the optical trap, were stimulated to a very high rate of activity. This would result in an acceleration of chromosome movement (see Fig. 4) because, as the chromosome was pulled away from the optical trap, the optical holding force on the chromosome decreased.

The fact that, after optical force induced rapid movement, the late-moving chromosomes in the second two cells (see Fig. 5 for one example) stopped at the metaphase plate (rather than moving through the metaphase plate to the opposite pole) is indicative of microtubule attachment to both poles. This observation is consistent with earlier studies in which laser destruction of the kinetochore on one side of a metaphase chromosome always led to premature movement of the double-chromatid chromosome to the opposite pole (7). The observations of the present study are also consistent with the predictions of Hill and Kirschner (8) concerning a pulling force inducing chromosome-to-pole polymerization of microtubules. It is possible that the laser-induced pulling force actually induced the attachment and/or polymerization of microtubules between the chromosome and the pole on the far side of the metaphase plate. Once a normal bipolar microtubule-chromosome linkage was established, then the

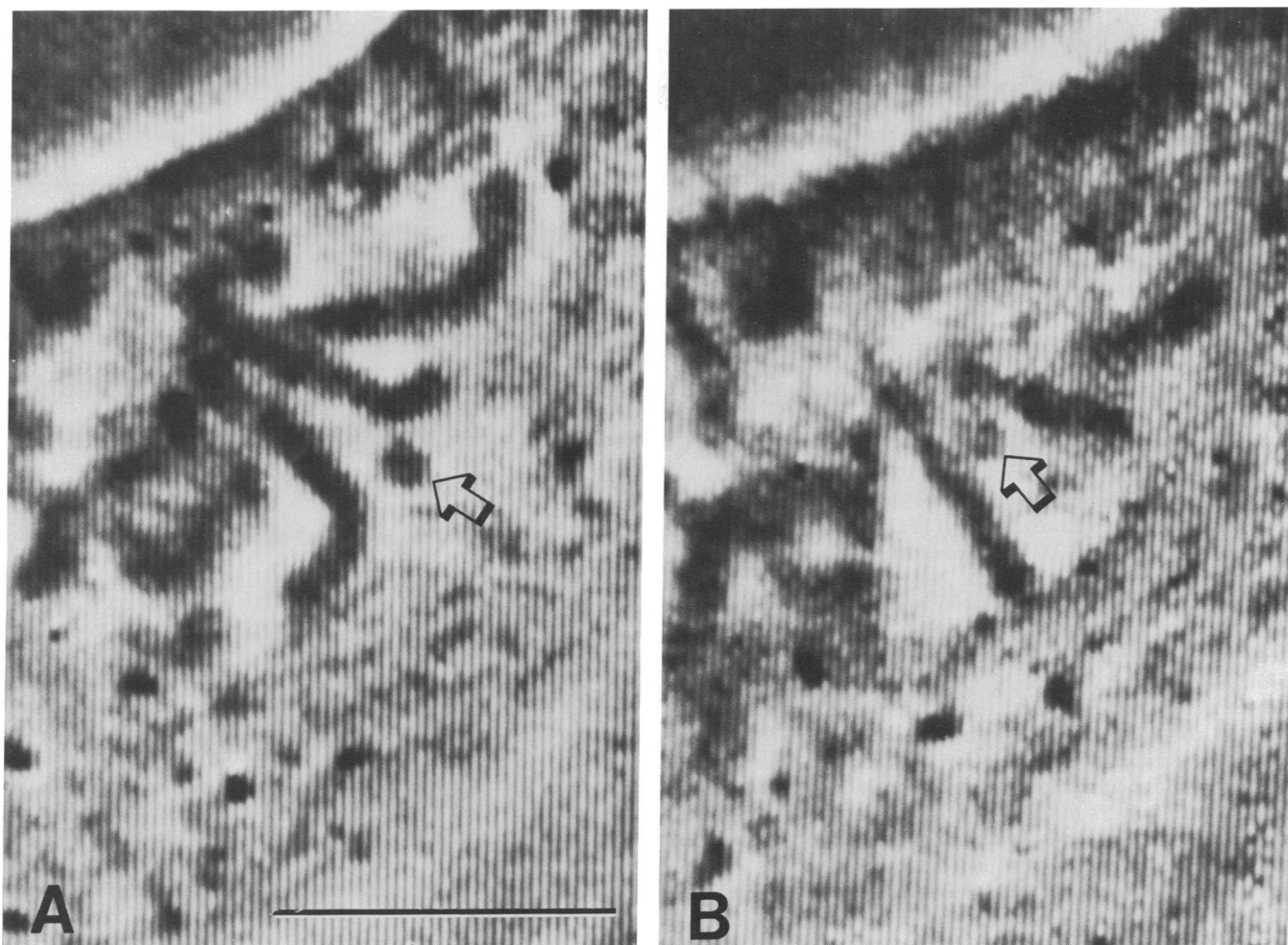


FIG. 5. (A) A metaphase cell with a late-moving chromosome on the spindle. The arrow indicates the point adjacent to the chromosome where the optical trap was applied. (B) The chromosome has moved to the metaphase plate, a distance of $5\ \mu\text{m}$ in 15 sec ($20\ \mu\text{m}/\text{min}$).

continuation of an applied optical force would result in movement of the chromosomes toward the metaphase plate in response to the development of a tension force *vis-à-vis* Nicklas (1). The chromosomes did not continue to move through the metaphase plate to the opposite pole because they had now moved substantially out of the gradient force, and normal bipolar microtubule/spindle dynamics determined subsequent events. The cells, in fact, underwent normal division from this point on. These observations would also argue against the possibility that the optical trap laser beam destroyed the microtubule attachments at the point of the laser focus, since destruction of such attachments causes the chromosome to pass through the equatorial plane and to approach the other spindle pole (7).

In conclusion, we have corroborated the results of Ashkin *et al.* (5) by demonstrating that an optical force trap can be used to manipulate organelle behavior in the mitotic spindle of living cells. Though one might argue with our interpretation of the observed chromosome behavior as it relates to models of spindle dynamics, we do not feel that the observations *per se* are inconsistent with current theories. In fact, the forces that we can apply to the chromosomes to alter their behavior are in the same range as those used by Nicklas (1) in his early studies. A better understanding of these results

will be achieved only with adequate structural (electron microscopic) and molecular studies. Further application of optical forces by laser microbeams should establish optical force traps as a powerful tool in cell biology.

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