MICROMANIPULATION STUDIES OF CHROMOSOME MOVEMENT

II. Birefringent Chromosomal Fibers and the Mechanical Attachment of Chromosomes to the Spindle

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ABSTRACT

The degree of mechanical coupling of chromosomes to the spindles of *Nephrotoma* and *Trimeratropis* primary spermatocytes varies with the stage of meiosis and the birefringent retardation of the chromosomal fibers. In early prometaphase, before birefringent chromosomal fibers have formed, a bivalent can be displaced toward a spindle pole by a single, continuous pull with a microneedle. Resistance to poleward displacement increases with increased development of the chromosomal fibers, reaching a maximum at metaphase. At this stage kinetochores cannot be displaced >1 μm toward either spindle pole, even by a force which is sufficient to displace the entire spindle within the cell. The abolition of birefringence with either colcemid or vinblastine results in the loss of chromosome-spindle attachment. In the absence of birefringent fibers a chromosome can be displaced anywhere within the cell. The photochemical inactivation of colcemid by irradiation with 366-nm light results in the reformation of birefringent chromosomal fibers and the concomitant re-establishment of chromosome attachment to the spindle. These results support the hypothesis that the birefringent chromosomal fibers anchor the chromosomes to the spindle and transmit the force for anaphase chromosome movement.

KEY WORDS birefringence · colcemid · micromanipulation · microtubules · mitosis · spindle fibers · vinblastine

The results of the micromanipulation studies presented in the preceding paper (3) demonstrate that from mid-prometaphase through anaphase, flexible but relatively inextensible fibers attach the kinetochores of each bivalent to the spindle poles. These mechanically demonstrable attachment fibers are found to have the same spatial location as the optically detectable birefringent spindle fibers. Although these results suggest that the birefringent chromosomal fibers are the structures which attach the chromosomes to the spindle, they do not rule out the possible participation of a nonbirefringent component of the spindle fiber.

Studies with various physical and chemical agents support the hypothesis that the birefringent chromosomal fibers anchor the chromosomes to

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the spindle. In *Chaetopterus* oocytes, where one spindle pole is attached to the cell membrane, the gradual application of cold (18, 20), high hydrostatic pressure (32, 33), or colchicine (17), results in the shortening of the spindle and the concomitant translation of the chromosomes to the cell surface. However, when high rates of depolymerization are induced with these agents, spindle fiber birefringence fades without spindle shortening. Under these conditions no chromosome motion is observed. Instead, the positions of the chromosomes become random, as if they had been released from their attachment sites (33).

Centrifugation experiments on dividing cells also implicate birefringent spindle fibers in chromosome-spindle attachment. The application of a centrifugal force of 7,000 *g* for 15 min to dividing root-tip cells of *Vicia faba* and *Allium cepa* fails to displace the chromosomes within the spindle (37). However, if the cells are first treated with either colchicine or chloral hydrate and then centrifuged, the chromosomes are completely displaced to the centrifugal end of the cell (37). Since both colchicine and chloral hydrate are known to disrupt spindle fibers, these observations suggest that these fibers anchor the chromosomes to the spindle.

If the birefringent chromosomal fibers are responsible for the observed chromosome-spindle attachment, then they are likely candidates for the traction elements which move the chromosomes in anaphase. However, Forer (12) has concluded from UV-microbeam irradiation studies of crane fly spermatocytes that the mechanical integrity of the birefringent chromosomal fibers is not necessary for chromosome movement and, therefore, that they cannot be the traction fibers. He postulates instead that a second, nonbirefringent component of the chromosomal fiber both produces and transmits the force for chromosome movement.

We have investigated the relationship between birefringent chromosomal fibers and mechanical attachment fibers. We report here studies on the strength of chromosome attachment to the spindle in relation to the stage of division and the degree of development of the birefringent fibers.

MATERIALS AND METHODS

Investigations were carried out on primary spermatocytes of the crane fly, *Nephrotoma ferruginea* Fabricius and the grasshopper, *Trimeratropis maritima* Harris. Crane flies were reared in the laboratory according to the method described by Begg (2). Grasshoppers were periodically collected from a wild population in Brigantine, N. J. and were maintained in the laboratory as previously described (3). Preparation of spermatocyte smears, micromanipulation, microscopy, photography, and data analysis were performed as previously described (3).

Drug Treatment

CRANE FLY: Spermatocytes were treated with either colcemid (CIBA Pharmaceutical Co., Summit, N. J.) or vinblastine sulfate (Eli Lilly & Co., Indianapolis, Ind.) to abolish spindle fiber birefringence. For this purpose a crane fly Ringer's solution was developed which is based upon the ionic composition of the larval haemolymph (10). The Ringer's solution contains 70 mM NaCl, 45 mM sucrose, 25 mM tricine (*N*-Tris [hydroxymethyl]-methyl glycine) pH 7.2, 15 mM $NaHCO_3$, 8 mM KCl, 8 mM $MgCl_2$, and 6 mM $CaCl_2$. Preparations of spermatocytes made with this solution were indistinguishable from those made in the standard way (2). Intact testes were immersed for 10 min in 5 ml of Ringer's solution containing either 1×10^{-6} M colcemid or 5×10^{-6} M vinblastine.

GRASSHOPPER: Spermatocytes were treated with either 5×10^{-6} M colcemid or 1×10^{-5} M vinblastine by immersing 6-10 testicular lobes for 15 min in grasshopper Ringer's solution (28) containing the appropriate drug.

Colcemid Reversal

Aronson and Inoué (1) have shown that the effects of colcemid on dividing sea urchin eggs can be reversed by brief irradiation with 366-nm light. We have used this technique to compare chromosome-spindle attachment in the presence and absence of birefringence in the same *Nephrotoma* spermatocyte. Testes were treated for 5 min with 1×10^{-6} M colcemid and then transferred to 1×10^{-8} M colcemid for an additional 5 min. Spermatocyte preparations made from these testes showed no detectable spindle birefringence. Individual spermatocytes were irradiated with 366-nm light by the method of Aronson and Inoué (1). Irradiation for 2 min resulted in the reformation of birefringent spindles which completed anaphase.

Experimental Protocol

The strength of chromosome attachment to the spindle was tested at various division stages ranging from nuclear membrane breakdown to late metaphase by attempting to displace a chromosome along the interpolar spindle axis (Fig. 1). This manipulation was carried out as follows: The cell was oriented so that movement of the micromanipulator joystick in a north-south direction moved the microneedle along the interpolar spindle axis. A chromosome was engaged with the microneedle with the joystick positioned at the center of its range of movement. The joystick was then moved in a single, continuous motion to the end of its range of movement,

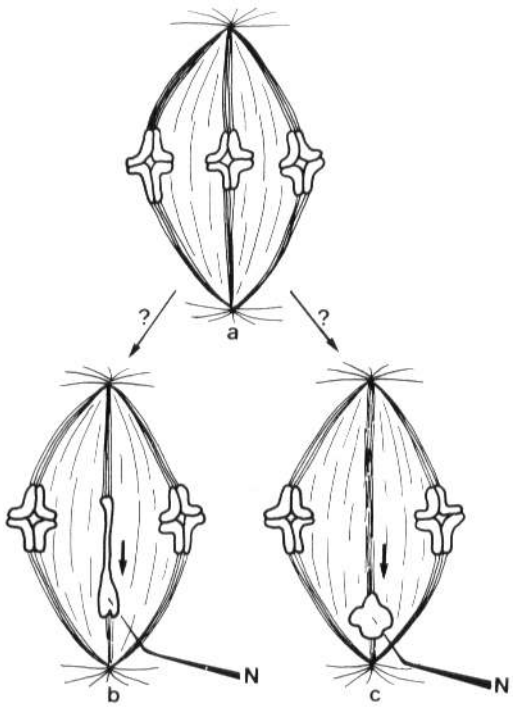

FIGURE 1 Diagram of micromanipulation showing three chromosomes and their associated spindle fibers (*a*). Fig. 1 *b* and *c* represent the two alternative responses to pulling a bivalent toward a spindle pole. In Fig. 1 *b* the bivalent stretches, but the kinetochores remain fixed. In Fig. 1 *c* the entire bivalent is displaced in the direction of the pull. *N*, microneedle. Heavy arrows indicate direction of pull.

pulling the chromosome toward a spindle pole. Before manipulation, the birefringent retardation of the chromosomal fiber was measured at the kinetochore of the chromosome which was to be operated upon. In early division stages, before chromosomal fibers were discernible, the retardation of the spindle adjacent to the kinetochore was measured. The operation was carried out with phase-contrast optics. Observations before and after manipulation were made with either phase-contrast optics to determine chromosome position, or polarization optics to observe spindle fiber morphology and retardation.

Spindle birefringence was altered experimentally with either colcemid or vinblastine, as described above, and chromosome anchorage within the cell was tested. Except for nonirradiated drug-treated cells, all the cells considered in this report completed a normal anaphase subsequent to manipulation. The culture temperature ranged from 22°–25°C.

RESULTS

Prometaphase Manipulations

In prometaphase, before birefringent chromosomal fibers develop, a bivalent can be displaced by pulling it toward either spindle pole. This result was consistently observed in a total of 10 *Trimeratropis* and 11 *Nephrotoma* spermatocytes. In early prometaphase a single poleward pull moves a bivalent the entire distance to the spindle pole (Fig. 2). The same manipulation in mid to late prometaphase displaces a bivalent a variable distance (Figs. 3 and 4) with the magnitude of displacement decreasing as the cell approaches metaphase.

Although chromosomes from both *Trimeratropis* and *Nephrotoma* spermatocytes respond identically to prometaphase manipulations, the behavior of chromosomes after manipulation differs significantly between the two species. When a chromosome is experimentally displaced toward a spindle pole in *Trimeratropis* spermatocytes, it immediately begins to migrate back to the equator with a velocity ranging between 0.8 and 2 μm/min. In *Nephrotoma* spermatocytes, however, a manipulated bivalent shows no directed movement toward the spindle equator. Instead, the chromosome wanders about its new location, eventually migrating to the metaphase plate along with the nonmanipulated chromosomes. Examples of the postmanipulation movements of chromosomes from the *Trimeratropis* spermatocyte shown in Fig. 2 and the *Nephrotoma* spermatocyte shown in Fig. 4 are presented graphically in Figs. 5 and 6, respectively.

The behavior of manipulated bivalents after poleward displacement in prometaphase reflects the normal pattern of prometaphase chromosome movement in nonmanipulated spermatocytes from these two species. In *Trimeratropis* spermatocytes the chromosomes immediately begin to migrate toward the spindle equator after nuclear membrane breakdown. Time-lapse movies of this process show that within 20-30 min of nuclear dissolution the chromosomes take up an approximately equatorial position, which they maintain for the remainder of prometaphase. As the cell approaches anaphase and birefringent chromosomal fibers develop, the chromosomes become more precisely aligned in the plane of the metaphase plate. The univalent X chromosome is an exception to this general pattern and wanders back and forth between the spindle poles until the onset of anaphase (25).

In *Nephrotoma* spermatocytes, on the other hand, prometaphase chromosome movements are uncoordinated and highly variable. An individual bivalent may slowly drift toward the equator or

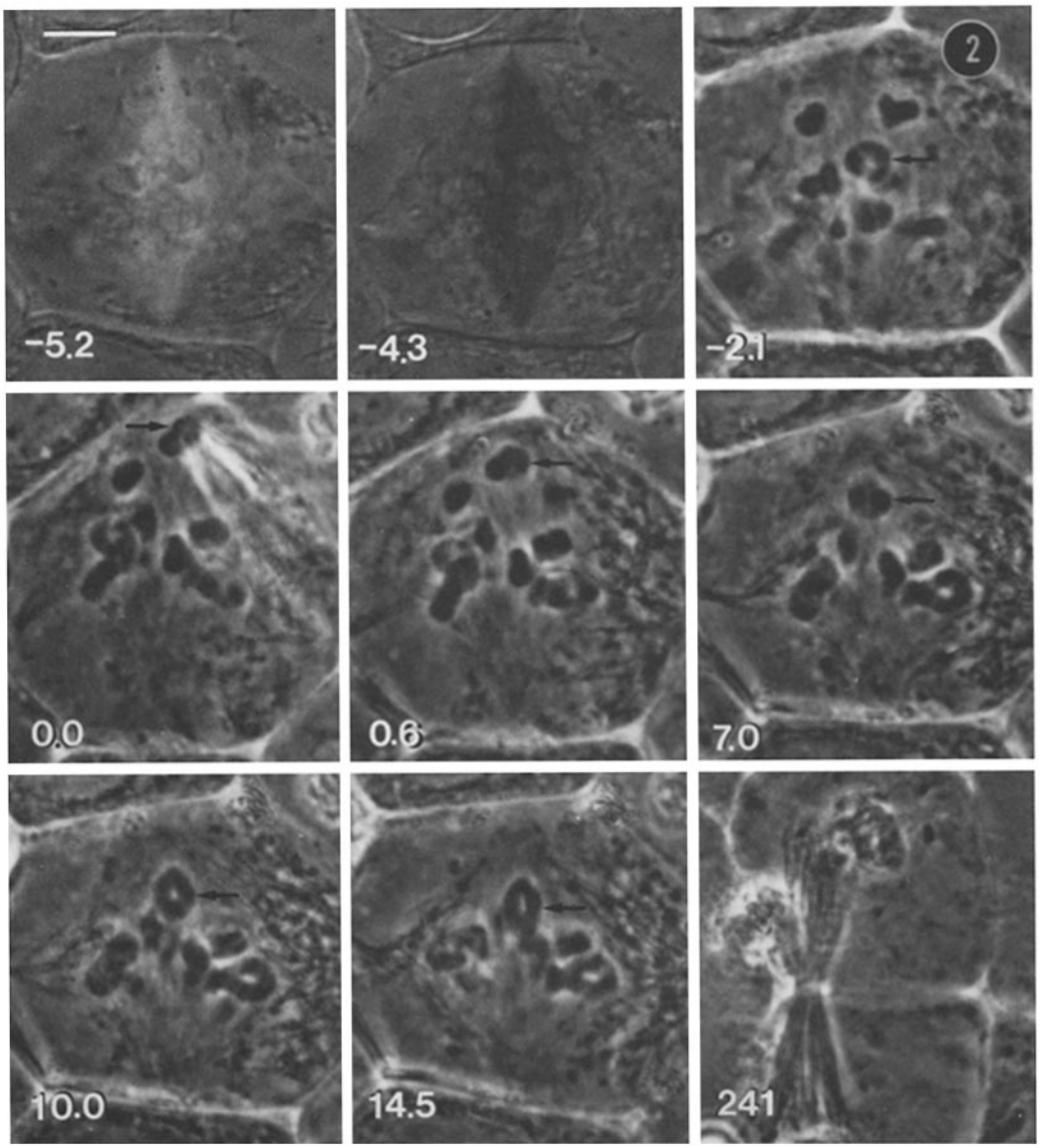

FIGURE 2 Manipulation of a *Trimeratropis* bivalent in early prometaphase. In this and in all subsequent plates the time with respect to the manipulation is given in the lower left of each frame. Arrows indicate the position of the manipulated bivalent. −5.2 and −4.3 min, Bright and dark compensation, polarization optics. Retardation (Γ) = 0.8 nm. −2.1 min, Chromosomes scattered throughout the spindle. 0.0 min, Ring-shaped chromosome displaced to upper spindle pole. Micromanipulation needle appears as white area in upper right of frame. 0.6 min, Needle removed; the manipulated bivalent remains at pole. 7.0–14.5 min, Manipulated bivalent moves toward equator along with nonmanipulated chromosomes. 241 min, Cell cleaved. Bivalent displaced 13 μm by the manipulation. Bar, 10 μm.

may migrate repeatedly back and forth between the spindle poles before becoming aligned on the metaphase plate. This same chromosome behavior has been described by Dietz in spermatocytes from two other species of crane flies (8, 9).

Metaphase Manipulations

In metaphase, when the birefringent chromosomal spindle fibers reach their maximum degree of organization, a bivalent cannot be significantly displaced toward either spindle pole. No exception

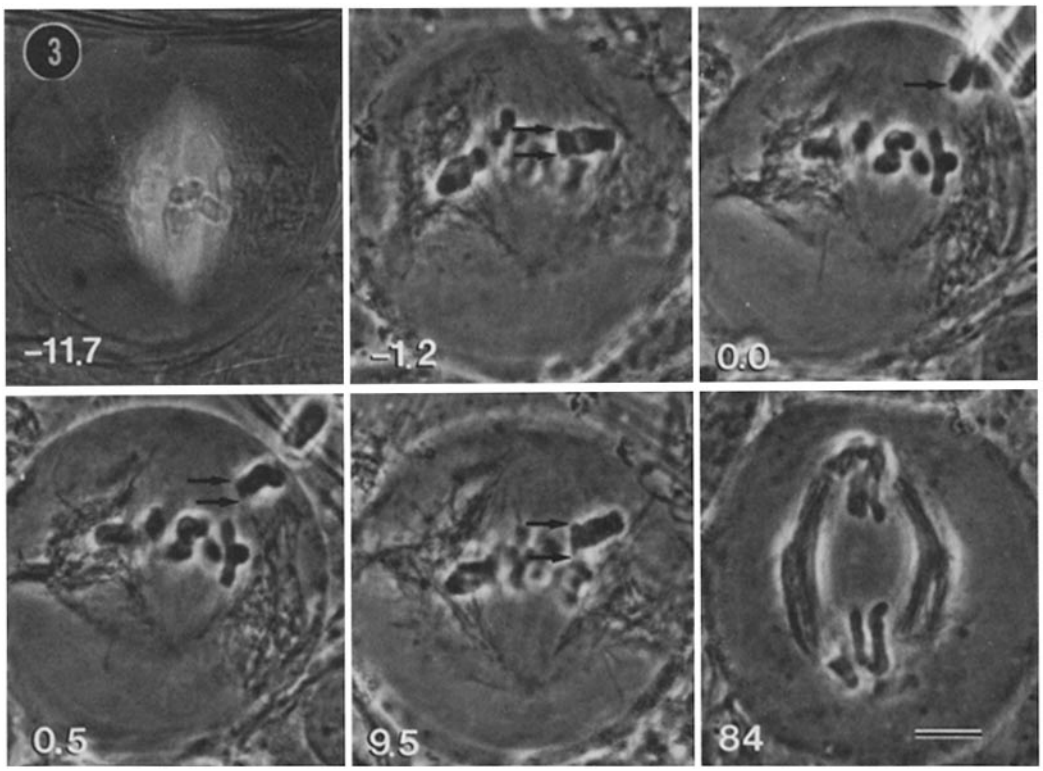

FIGURE 3 Manipulation of a *Trimeratropis* bivalent in late prometaphase. Arrows indicate kinetochore positions of manipulated bivalent. −11.7 min, Polarization optics (Γ = 1.3 nm). −1.2 min, Chromosomes nearly aligned on metaphase plate. 0.0 min, Bivalent pulled toward upper pole. 0.5 min, Needle removed. The manipulated bivalent was displaced 7 μm by the operation. 9.5 min, Bivalent returns to equator. Note that the kinetochore regions appear stretched. 84 min, Telophase. Bar, 10 μm.

to this response was observed in the six *Trimeratropis* and eight *Nephrotoma* spermatocytes studied. In the *Trimeratropis* spermatocyte shown in Fig. 7, distinct birefringent chromosomal fibers are associated with the kinetochores of the manipulated chromosome. Although the manipulation displaces the spindle within the cell and deforms the mitochondrial sheath, the bivalent is pulled <0.5 μm away from its original position on the metaphase plate. Thus, the distance which a bivalent can be displaced toward a spindle pole by a single continuous pull with the microneedle is inversely proportional to the degree of development of the birefringent chromosomal fibers.

Chromosome Manipulation in Colcemid and Vinblastine-Treated Cells

The physical anchorage of chromosomes within the cell has also been studied in spermatocytes whose spindle birefringence has been artificially altered with either colcemid or vinblastine. Spindle birefringence is completely abolished in *Nephrotoma* spermatocytes by treatment with either 1×10^{-6} M colcemid or 5×10^{-6} M vinblastine. Concentrations of 5×10^{-6} M colcemid and 1×10^{-5} M vinblastine are necessary to destroy *Trimeratropis* spindle birefringence. Colcemid-treated *Nephrotoma* spermatocytes show a decreased tolerance to micromanipulation. Only ~10% of the drug-treated cells survive manipulation. In addition, many colcemid-treated *Nephrotoma* cells also lyse spontaneously. No similar drug sensitivity was observed for *Trimeratropis* spermatocytes nor were *Nephrotoma* spermatocytes adversely affected by vinblastine.

When spindle birefringence is abolished with either colcemid or vinblastine, a bivalent shows no evidence of mechanical attachment in response to micromanipulation (Figs. 8 and 9). In cells in which the chromosomes are scattered throughout the cytoplasm, an individual bivalent can be dis-

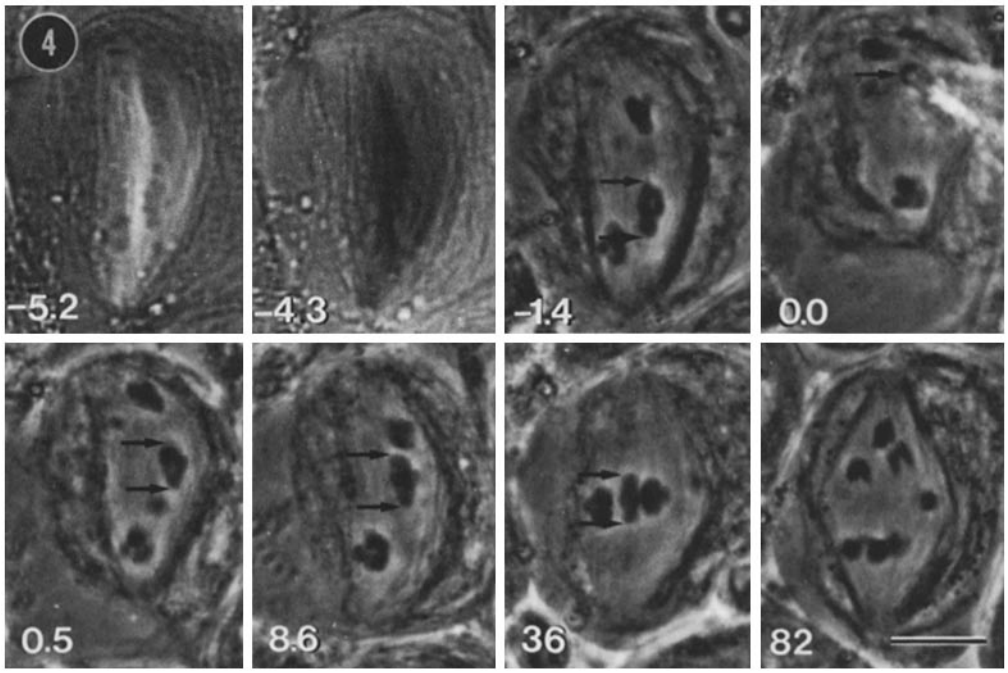

FIGURE 4 Manipulation of a *Nephrotoma* bivalent in early prometaphase. Arrows indicate kinetochore positions of manipulated bivalent. −5.2 and −4.3 min, Bright and dark compensation, polarization optics (Γ = 0.7nm). −1.4 min, Bivalents scattered along interpolar axis. 0.0 min, Bivalent pulled toward upper pole. The spindle is compressed by the operation. 0.5 min, The bivalent was displaced 4 μm by the manipulation and the interpolar distance shortened. 8.6 min, Spindle has recovered its normal morphology. 36 min, Chromosomes aligned on the metaphase plate. 82 min, Anaphase. Bar, 10 μm.

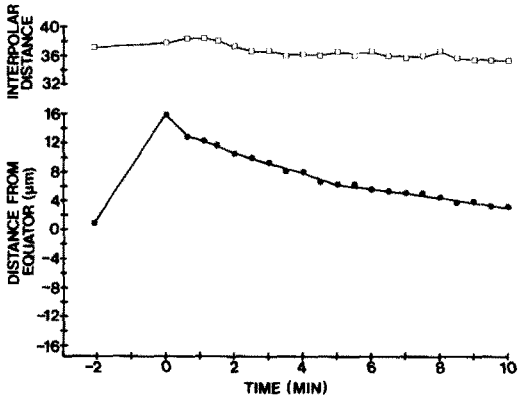

FIGURE 5 Graph of postmanipulation movement of bivalent in *Trimeratropis* spermatocyte shown in Fig. 2. Time is plotted with respect to the manipulation. The equator is taken to be the midpoint between the spindle poles. Closed circles, manipulated bivalent. Open squares, interpolar distance. Average bivalent velocity 1.0 μm/min.

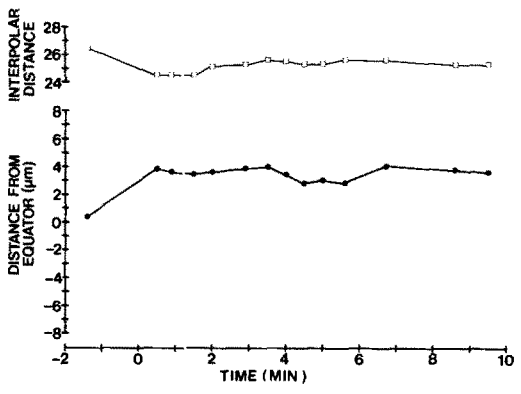

FIGURE 6 Graph of postmanipulation movement of bivalent in *Nephrotoma* spermatocyte shown in Fig. 4. Time is plotted with respect to the manipulation. The equator is taken to be the midpoint between the spindle poles. Closed circles, manipulated bivalent. Open squares, interpolar distance.

placed anywhere within the cell without altering the positions of the other bivalents (Fig. 8). The manipulated bivalent does not move back toward its original position, but together with the non-manipulated chromosomes gradually drifts toward the center of the cell at a rate of ~0.1 μm/min. (Fig. 8, 0.5–21.5 min.).

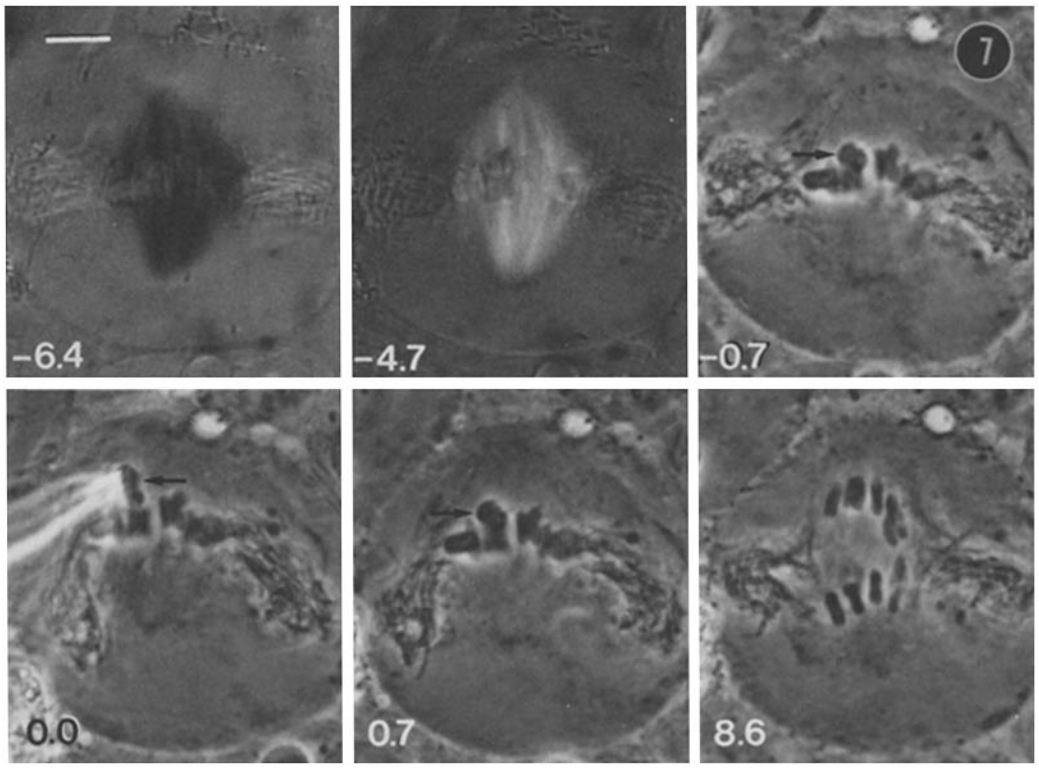

FIGURE 7 Manipulation of a *Trimeratropis* bivalent at metaphase. Arrow indicates manipulated bivalent. −6.4 and −4.7 min, Dark and bright compensation, polarization optics (Γ = 2.0 nm). Well-developed chromosomal fibers are associated with the chromosomes. −0.7 min, Chromosomes aligned on metaphase plate. 0.0 min, Bivalent pulled toward upper pole. 0.7 min, The manipulated bivalent has been displaced <0.5 μm by the operation; however, the entire spindle has been displaced in the direction of the pull, and the mitochondrial sheath has been deformed. 8.6 min, Anaphase. Bar, 10 μm.

In drug-treated *Trimeratropis* and *Nephrotoma* spermatocytes, chromosomes are most often found clumped together in a group. Here the chromosomes appear to be mutually adhesive. Only rarely can an individual bivalent be pulled free from such a cluster of chromosomes. Usually the adhesive forces between the chromosomes are greater than the viscous drag resulting from the attempted manipulation of one of its members, and the cluster moves as a whole (Fig. 9, −2.2–0.6 min).

Colcemid Reversal with 366-nm Light

The irradiation of colcemid-treated *Nephrotoma* spermatocytes with 366-nm light results in the inactivation of the drug and the concomitant reformation of functional birefringent spindles. By combining this method of colcemid reversal with micromanipulation, we have been able to study chromosome anchorage in both the presence and absence of birefringence in the same cell. *Nephrotoma* spermatocytes were used in these experiments because the duration of prometaphase is signficantly shorter than in *Trimeratropis* spermatocytes (~1 ½ vs. ~6 h) and because the effects of colcemid are more easily reversed.

An example of such an experiment is shown in Fig. 9. The colcemid-treated spermatocyte has no detectable birefringence associated with its chromosomes (−5.8 min), which are clumped together in the center of the cell (−2.2 min). All three bivalents are displaced by pulling the central bivalent toward the lower edge of the cell (0.0 and 0.6 min), where they remain until irradiated. Irradiation of the cell for 2 min with 366-nm light results in the formation of a bipolar spindle with distinct birefringent chromosomal fibers (38.3 min). When the bivalent at the far right of the spindle is now pulled toward the lower pole (46.5 min), the entire spindle shifts in the direction of the pull, and the bivalent moves closer to the

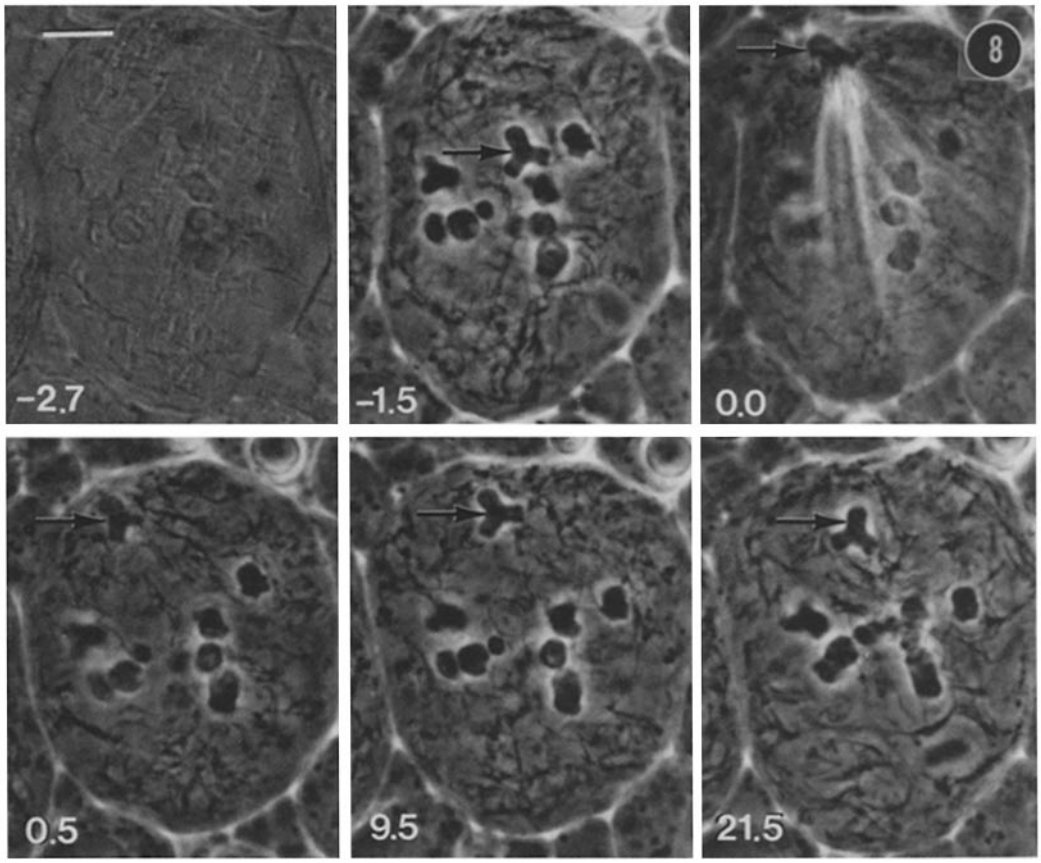

FIGURE 8 Chromosome manipulation in a colcemid-treated *Trimeratropis* spermatocyte. The cell had been in colcemid for ~30 min at the time of manipulation. Arrow indicates manipulated bivalent. −2.7 min, Polarization optics. No spindle birefringence is detectable. −1.5 min, Chromosomes and mitochondria are scattered throughout the cytoplasm. 0.0 min, Bivalent pulled toward upper edge of cell. 0.5 min, Bivalent displaced to cell margin. 9.5-21.5 min, All chromosomes, including manipulated bivalent slowly move toward center of cell. Bar, 10 μm.

interpolar axis. However, the manipulation does not displace the bivalent toward the spindle pole.

DISCUSSION

Birefringent Fibers and Chromosome-Spindle Attachment

The experiments reported here were designed to test the mechanical properties of chromosomal spindle fibers at various times during division by attempting to displace a chromosome along the interpolar spindle axis. The displacement of a chromosome toward either spindle pole would necessitate a change in the length of its chromosomal fibers, thus providing a relative measure of the strength of chromosome-spindle attachment.

The results of these experiments demonstrate a clear correlation between the strength of a chromosome's attachment to the spindle and the degree of development of the birefringent chromosomal fibers. In early prometaphase, before chromosomal fibers are detectable, an individual bivalent can be displaced to a spindle pole by a single continuous pull with the microneedle. Resistance to poleward displacement increases with increased development of the chromosomal fibers. During metaphase, when distinct birefringent fibers are associated with the chromosomes, a bivalent cannot be significantly displaced toward either spindle pole. Instead, the entire spindle shifts as a single body in the direction of the pull.

The same correlation between birefringence and

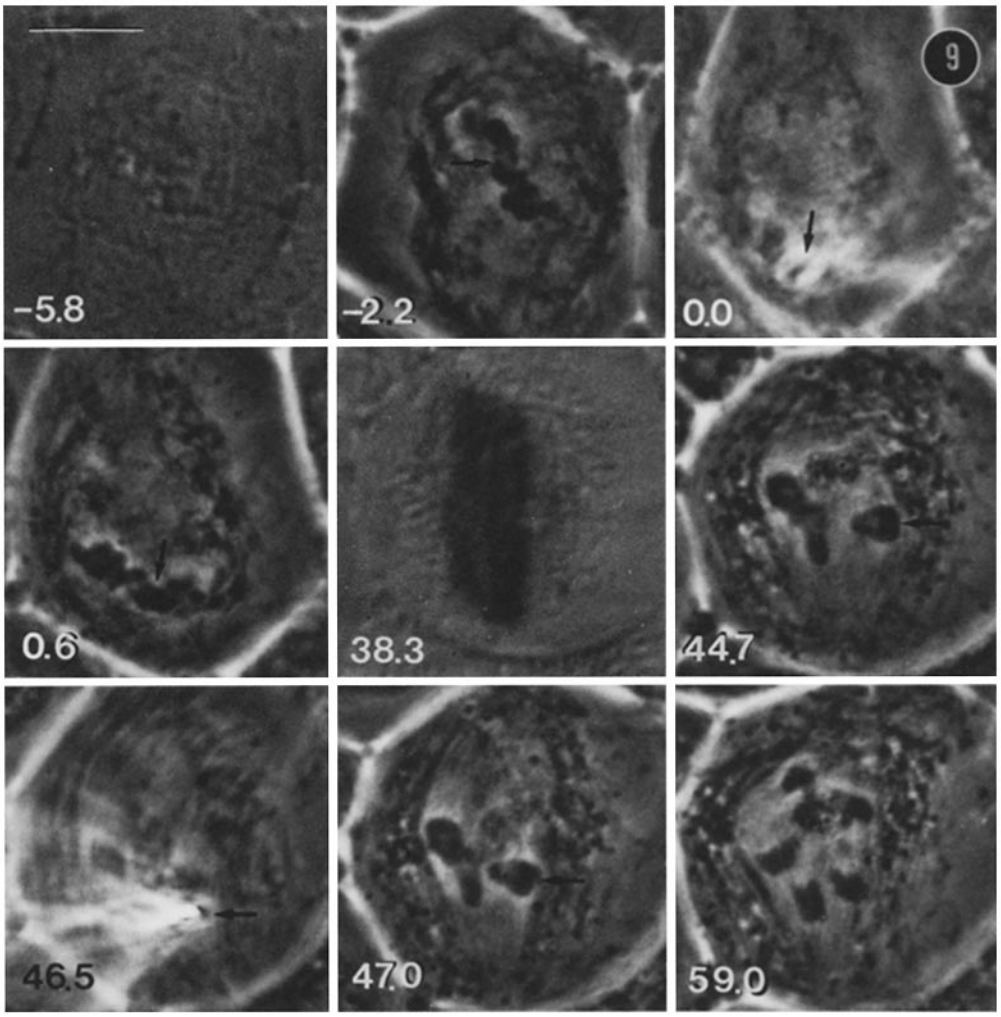

FIGURE 9 Chromosome manipulation and colcemid reversal in a *Nephrotoma* spermatocyte. The cell had been in colcemid for ~40 min at the time of manipulation. Arrow indicates manipulated bivalents. −5.8 min, Polarization optics. No spindle birefringence is detectable. −2.2 min, The three autosomal bivalents are grouped together in the center of the cell. 0.0 min, Bivalent pulled toward lower edge of cell. 0.6 min, All three bivalents displaced in direction of pull. 16 min after the manipulation the cell was irradiated for 2 min with 366-nm light. 38.3 min, Birefringent spindle has reformed. 44.7 min, Three bivalents lie near the metaphase plate. 46.5 min, Bivalent pulled toward lower pole. 47.0 min, Manipulated bivalent has been shifted toward the interpolar spindle axis, but has not been significantly displaced toward the pole. 59.0 min, Anaphase. Bar, 10 μm.

mechanical attachment is also found when spindle birefringence is artificially altered with either colcemid or vinblastine. In the absence of birefringent chromosomal fibers a bivalent can be displaced anywhere within the cell. The photochemical inactivation of colcemid by irradiation with 366-nm light results in the reformation of birefringent chromosomal fibers and the concomitant re-establishment of normal chromosome anchorage to the spindle.

These results demonstrate that the establishment of the mechanical attachment of chromosomes to the spindle parallels the development of birefringent chromosomal fibers. Since birefringent chromosomal fibers have also been shown to have the same spatial distribution as the mechan-

ical attachment fibers (3, 28), these observations strongly suggest that the birefringent chromosomal fibers are the elements which anchor the chromosomes to the spindle. However, the results do not exclude the possibility that a nonbirefringent component of the chromosomal fiber participates in chromosome-spindle attachment. Such an interpretation would require that the nonbirefringent component develop in synchrony with the birefringent fiber, and that it be either sensitive to both colcemid and vinblastine or depend upon the presence of the birefringent fiber for its mechanical integrity. Clearly the most direct interpretation of our results is that the birefringent fiber itself is the attachment element.

Mechanical Attachment and Microtubules

A number of different lines of evidence suggest that microtubules give rise to the observed birefringence of spindle fibers. The distribution and magnitude of spindle birefringence is correlated with the distribution and number of microtubules in a variety of cell types (16, 20, 22, 30, 31). La Fountain (21) had demonstrated that in metaphase, five areas of high microtubule density occur in transverse sections of crane fly spermatocytes, corresponding to the five chromosomal fibers seen with polarization microscopy. This organization of microtubules into chromosomal spindle fibers is clearly seen in high voltage electron micrographs of dividing mammalian tissue culture cells (23, 24). The most direct evidence for the microtubular origin of spindle fiber birefringence comes from the work of Sato et al. (35). They have demonstrated that the birefringence of carefully fixed, isolated spindles measured in imbibing media of refractive indices between 1.33 and 1.67 shows a close fit to a Wiener rodlet form birefringence curve. Since these spindles are composed almost exclusively of microtubules, these results demonstrate that oriented arrays of microtubules give rise to the observed positive form birefringence.

An additional line of evidence which suggests that spindle fiber birefringence results from aligned microtubules comes from immunofluorescent studies of dividing cells. A number of investigators have demonstrated that spindles stained with fluorescent antitubulin show a nearly identical morphology to that seen with polarization microscopy (see, e.g., references 4, 13, 14, and 36). Recent studies using PtK2 cells (29) and mouse 3T3 cells (38) have demonstrated that the pattern of antitubulin staining corresponds to the distribution of microtubules.

The correlation between the formation of birefringent fibers and the establishment of chromosome-spindle attachment therefore suggests that microtubules are the structural elements which anchor chromosomes to the spindle. This hypothesis is supported by the observation that such attachment is abolished by treatment with either colcemid or vinblastine, agents which specifically disrupt microtubules (39). In addition, ultrastructural studies of chromosomes which have been detached from the spindle by micromanipulation show that microtubules are not associated with the kinetochores of detached chromosomes until they reinitiate movement (5, 27). Once a detached chromosome begins to move again, it can be stretched with the microneedle, demonstrating that it has become reattached to the spindle (26).

Birefringent Chromosomal Fibers and Force Transmission

The demonstration that birefringent chromosomal fibers have the same spatial and temporal distribution as the mechanical attachment fibers suggests that the birefringent fibers are the traction elements which transmit the force for anaphase chromosome movement. This conclusion is supported by the observation that the mechanical integrity of the birefringent spindle fibers is necessary for chromosome movement in *Chaetopterus* oocytes (32–34). When these cells are subjected to hydrostatic pressures between 2,500 and 5,500 lb/in^2, spindle birefringence fades gradually, the spindle shortens, and the chromosomes are moved to the cell surface. Both the rate of spindle shortening and the velocity of chromosome movement increase with increasing pressure, up to a maximum of 6,000 lb/in^2. At pressures above 6,000 lb/in^2, the spindle rapidly depolymerizes without shortening, and no chromosome movement results. Similar observations have been made with cold or colchicine as the depolymerizing agent (17, 18). These results suggest that the mechanical integrity of the birefringent spindle fibers is required for the proper transmission of mitotic forces.

The observations reported here do not support Forer's (12) conclusion that the birefringent chromosomal fibers neither produce nor transmit the force for anaphase chromosome movement. Forer found that the UV-microbeam irradiation of birefringent chromosomal fibers produced a localized

region of reduced birefringence (11). In a number of instances chromosomes whose fibers contained such a lesion were able to undergo apparently normal anaphase movement (12). From this observation, Forer concluded that the mechanical integrity of the birefringent fiber is not required for chromosome movement and, therefore, that these fibers cannot be the traction elements. However, the lesion produced was one of reduced birefringence rather than undetectable birefringence. Thus, it is quite possible that a sufficient number of microtubules remained in the irradiated area to provide mechanical continuity.

The results reported here support the hypothesis that a few microtubules are sufficient to establish mechanical attachment of a chromosome to the spindle. By mid-prometaphase, bivalents show an increased resistance to poleward displacement compared to early prometaphase, yet distinct chromosomal fibers are not detectable. This observation indicates that fewer microtubules than normally occur in the birefringent fibers at metaphase can impart a degree of mechanical attachment and is consistent with the idea that a few microtubules remaining after UV irradiation could provide the necessary mechanical integrity of the birefringent chromosomal fiber to account for Forer's observations.

An additional possibility is that the irradiated area of the fiber loses its optical anisotropy without losing its mechanical integrity. If this were the case, the area of reduced birefringence would represent an area of altered molecular properties rather than an actual mechanical lesion.

In analyzing his data, Forer has assumed that at any time during chromosome movement a given fiber exerts all the force that it can, and that this force is opposed by the chromosome's resistance to movement. Therefore, when UV-microbeam irradiation produced a visible reduction in the organization (birefringence) of the fiber without a corresponding reduction in force (as would be shown under the above assumption by a decrease in chromosome velocity), he concluded that the birefringent chromosomal fiber could not be the site of force production.

An alternative interpretation of Forer's results is possible if one assumes that rather than being force-limited, the motile system is rate-limited. Chromosome velocity would then be controlled by the rate of tubulin depolymerization rather than by the number of microtubules operating (see references 15, 19, and 20). By this mechanism the force available would be greatly in excess of that normally required for chromosome movement, and thus, the velocity of chromosome movement would be unaffected by a reduction in microtubule numbers.

Chromosome Movement in Drug-Treated Cells

Chromosomes in colcemid or vinblastine-treated spermatocytes show no detectable mechanical anchorage within the cell, yet manipulated and nonmanipulated chromosomes alike migrate toward the center of the cell at a rate of ~0.1 μm/min. Once the chromosomes have collected into a mass, individual chromosomes adhere to the others and can no longer be separated from the rest of the group. Manipulation of one chromosome now results in the translation of the entire group.

Although the mechanism of this unusual form of congression is currently unknown, two possible explanations are suggested by previous work. Brinkley and Stubblefield (6) have shown that in dividing Chinese hamster cells which have been treated with low concentrations of colcemid, the centrioles become positioned at the cell center, with the chromosomes arranged about them in a sphere. Microtubules appear to connect the kinetochores of the inner chromatids to the centrioles, while the kinetochores of the outer sister chromatids are devoid of microtubules. A few radially aligned microtubules originating at a centrally located set of centrioles might produce the chromosome movement observed in colcemid and vinblastine-treated spermatocytes. However, the attachment of chromosomes to even a few microtubules would be expected to provide a detectable degree of anchorage, as Nicklas (27) has demonstrated. Since the chromosomes in drug-treated cells show no evidence of attachment, a microtubule-mediated mechanism of movement seems unlikely.

A second, more speculative explanation is suggested by Byers and Porter's (7) study of pigment granule migration in squirrel fish erythrophores. The pigment granules appear to be embedded in an anastomosing network of 30- to 60-Å filaments, termed microtrabeculae. The distribution of the microtrabeculae changes in concert with the granule distribution during pigment aggregation and dispersion, suggesting that it functions in the generation of force for granule movement. Similarly, the chromosomes in spermatocytes treated with

antimitotic drugs may be embedded in a microtrabecular-like network. In the absence of microtubules this network might undergo a gradual contraction, resulting in the observed slow congression of the chromosomes into the center of the cell.

However, the fact that before aggregation the chromosomes are freely moveable without detectable interaction argues against the mediation of any ordinary mechanical traction system and suggests that the mechanical properties of the microtrabecular network, if it is involved, are exceptionally tenuous.

Concluding Remarks

The micromanipulation studies reported here provide direct evidence that the birefringent chromosomal fibers anchor the chromosomes to the spindle and identify these structures as the mechanical attachment fibers which were characterized in the first paper in this series. Previous electron microscope studies as well as our observation that the microtubule-depolymerizing agents colcemid and vinblastine abolish chromosome-spindle attachment, suggest that microtubules are the structural elements which give rise to the observed mechanical properties of the chromosomal fibers. This conclusion is consistent with the observation that the mechanical integrity of microtubules is necessary for force transmission in the spindles of *Chaetopterus* oocytes (33, 34). Our results contradict Forer's (12) conclusion that the birefringent spindle fibers neither generate nor transmit the force for chromosome movement. Instead they suggest that, regardless of the molecular mechanism of mitotic force production, the birefringent chromosomal fibers are the traction element which transmit the force for anaphase chromosome movement.

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