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Kinetochore microtubules and chromosome movement during prometaphase in *Drosophila melanogaster* spermatocytes studied in life and with the electron microscope

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Abstract. Prometaphase I chromosome behavior was examined in wild-type *Drosophila melanogaster* primary spermatocytes. Cine analysis of live cells reveals that bivalents exhibit complex motions that include (1) transient bipolar orientations, (2) simultaneous reorientation of homologous kinetochores, (3) movements not parallel to the spindle axis, and (4) movement along the nuclear membrane. – Kinetochores and kinetochore microtubule have been analyzed for bivalents previously studied in life. The results suggest that most chromosome motions (complex though they may be) can be explained by poleward forces acting on or through kinetochore microtubules that span the distance between the kinetochore and the vicinity of a pole. The results also suggest that the majority of short kinetochore microtubules may be remnants of previous microtubule-mediated associations between a kinetochore and a pole.

Introduction

Although molecular mechanisms of chromosome movement during mitosis and meiosis are unknown, it is likely that microtubules, a major component of the spindle apparatus, are at least necessary if not sufficient for directed movement to occur during prometaphase. A direct correlation of microtubules and movement comes from combining micromanipulation experiments with electron microscopy (Nicklas et al. 1979; Nicklas and Kubai, personal communication). If a grasshopper bivalent (or chromosome) is detached from and located at a position away from the spindle, the kinetochores are initially devoid of all microtubules. Eventually, the bivalent will return to the spindle. In every case where the kinetochores have been examined, initial movement of the bivalent is correlated with the presence of at least one new microtubule at the kinetochore. The newly formed kinetochore microtubules always lie in the direction towards which the bivalent moves. Initially, bivalents or chromosomes usually move toward the center of the spindle and display microtubules positioned perpendicular to the kinetochore and directed toward both poles (Nicklas et al. 1979).

Microtubule dispositions at the kinetochore of *Drosophila melanogaster* bivalents at early prometaphase I are very complex (Church and Lin 1982). A dyad kinetochore can establish microtubule connections with one or both poles resulting in bivalents with monopolar connections, bipolar connections, and both intermediates. Furthermore, kinetochore position is not always correlated with microtubule disposition; a kinetochore that is clearly facing one pole can have microtubules directed towards the opposite pole as well as the facing pole. Some indication of the magnitude of apparent bivalent malorientations in *D. melanogaster* has been provided by Ault and Lin (1984). From an ultrastructural analysis, it was determined that over 50% of the autosomal bivalents were maloriented during early prometaphase I.

Although there are no strictly comparable observations in the literature, the observations on *D. melanogaster* appear to be at odds with what has been learned from cine analysis of prometaphase I in other organisms. There, most bivalents achieve bipolar orientation immediately. Those that do not show monopolar orientations that are soon resolved by kinetochore reorientation for (for review see Nicklas 1971)

The ultrastructural studies of *D. melanogaster*, in view of what has been learned by cine analysis in other organisms, have suggested that either (1) the majority of the kinetochore microtubules at prometaphase I have little to do with chromosome movement or (2) prometaphase I chromosome behavior in *D. melanogaster* is more complex than has been previously described. To distinguish between these possibilities, we investigated prometaphase I chromosome movements in living wild-type *D. melanogaster* primary spermatocytes and examined microtubules at the kinetochores of bivalents previously studied in life. We conclude that the chromosome movements during prometaphase I of meiosis in *D. melanogaster* males are more complex than previously described and that there is a remarkable correlation between the patterns of movement and microtubule disposition at the kinetochore. In all cases the bivalent maneuvers can be explained by poleward forces acting asynchronously on kinetochore microtubules that span the distance from kinetochore to the pole. The observations also suggest that the majority of short kinetochore microtubules are remnants from previous associations of the kinetochore and the pole.

Materials and methods

Live-cell analysis. All observations were made on primary spermatocytes from Oregon R wild-type flies. Culture chambers were the same as those described by Nicklas and

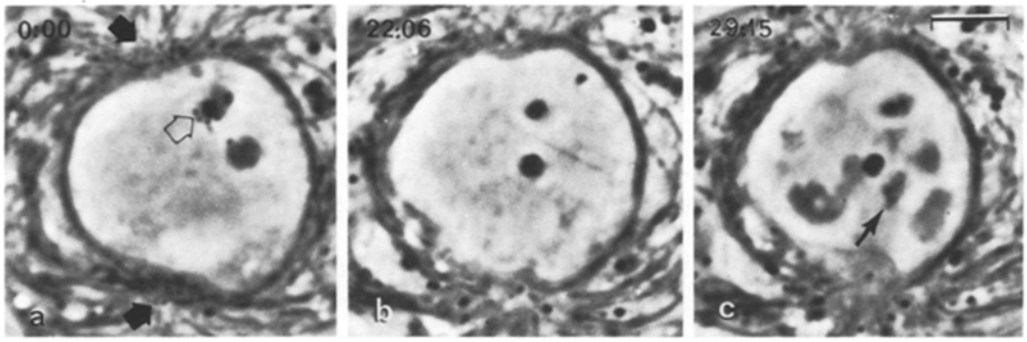

Fig. 1a–c. Cell about to enter prometaphase I. Note the grapelike cluster of small nucleolarlike bodies (*open arrow*, **a**), which fuse to form nucleoli (**b**). The poles can be identified (*closed arrows*, **a**). Phase-dense bodies appear (**c**) and the chromosomes become visible (*arrow*, **c**). Note that the nuclear periphery is distorted at the polar regions. Bar represents 5 μm

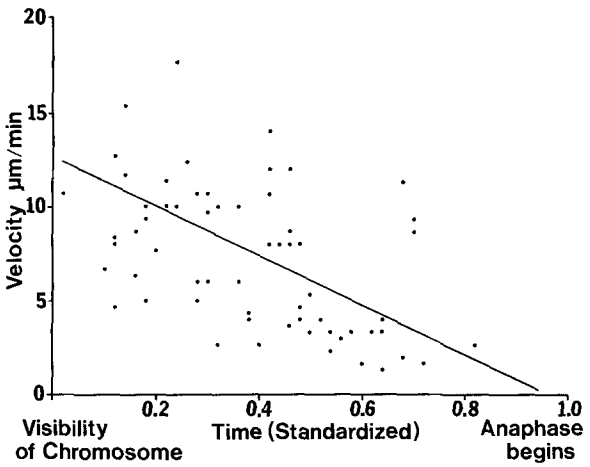

Fig. 2. Observations were standardized by determining the length of the interval from the time the chromosomes became visible to the time when anaphase began for each cell from which measurements were taken. The time from the beginning of the interval to the velocity measurement was determined and divided by the total length of the interval. It is clear that chromosome velocity decreases throughout the interval. $r^2 = 0.324572$, $P = 0.005$ (F test)

Staehly (1967). The chamber was filled with halocarbon oil (Voltalef 10s) and the abdomen of the fly immersed in the oil at the edge of the chamber. The testes were dissected directly into the oil, cleaned of adhering fat, and moved to a clean region of the chamber. Each was cut at the mid-point and the two halves dragged over the surface of the coverslip until emptied. The spermatocytes were viewed with a Zeiss inverted-phase-contrast microscope and events were recorded on Agfa copex Pan Rapid 16 mm cine film. All cells photographed were selected within 15 min after dissection. The temperature during the photographic sessions was 24° ± 1° C. Based on all visible criteria, the cells in culture remain healthy for at least 3 h. Each preparation was discarded after a single cell was filmed and no filming session lasted more than 1.5 h. In those cases where the cell was fixed for electron microscopy, the primary fixative was delivered within 1 min after the last photograph of the cell in life.

Electron microscopy. The general procedures for delivering the fixative to the target cell are found in Niclas et al. (1979). For *D. melanogaster*, microfixation was accomplished with 6% glutaraldehyde in 0.1 M potassium phosphate buffer at pH 7.2. Following microfixation, the coverslip with adhering cells was removed from the culture chamber and immersed in 3% glutaraldehyde (same buffer) for an additional 15 min, rinsed three times in phosphate buffer (10 min each), three times in 0.1 M veronyl acetate buffer at pH 7.2 (5 min each), and stained in 2% osmium tetroxide in veronyl acetate buffer (20 min). Two more buffer rinses followed (5 min each) prior to staining in 0.5% uranyl acetate in veronyl acetate buffer for 1 h. The coverslips were rinsed twice more in buffer followed by two water rinses (5 min each). Dehydration and embedding procedures are described in Nicklas et al. (1979). The target cell was serially sectioned and reconstructed as previously described (Lin et al. 1981; Church and Lin 1982). The computer programs of Moens and Moens (1981) were used to prepare the representative two- and three-dimensional reconstructions. The details of this procedure can be found in Nicklas et al. (1982).

Results

General

Cells about to enter prometaphase I of meiosis had a characteristic morphology. The nucleoplasm was homogeneously light in appearance except for grapelike clusters of small nucleoli (Fig. 1a). The periphery of the nucleus was phase dense and the poles were well defined. Once such a cell was located, within 30 min several dramatic changes occurred. The grapelike clusters fused into one or two large nucleoli (Fig. 1b). and other phase-dense bodies appeared (Fig. 1c). These latter bodies are most likely remnants of the Y chromosome ribonucleoprotein (RNP) loop material that is so prominent during meiotic prophase in *D. melanogaster* males (Tates 1971). The phase-dense material eventually condensed into one to several large masses (see Fig. 4). The nuclear envelope does not break down during meiosis in *Drosophila* males. Rather, the nucleus is surrounded by several membrane layers throughout meiosis I (Tates 1971). The membrane layers did become distorted in appearance at the polar regions (Fig. 1). During this period when dramatic changes were occurring in the spermatocyte nucleus, the chromosomes suddenly became visible (Fig. 1c). With experience, an observer could easily distinguish them from other phase-dense bodies by their characteristic movements.

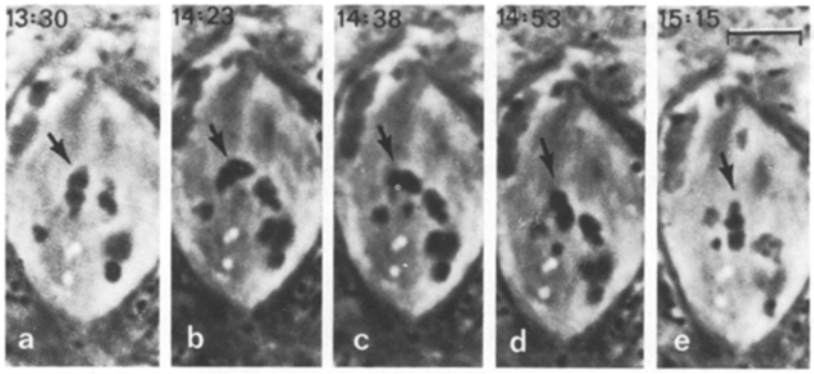

Fig. 3a–e. Bivalent showing transient bipolar orientation (*arrows*). Both kinetochores reorient simultaneously causing the bivalent to rotate 360°. Zero time is the time chromosomes become visible. Bar represents 5 μm

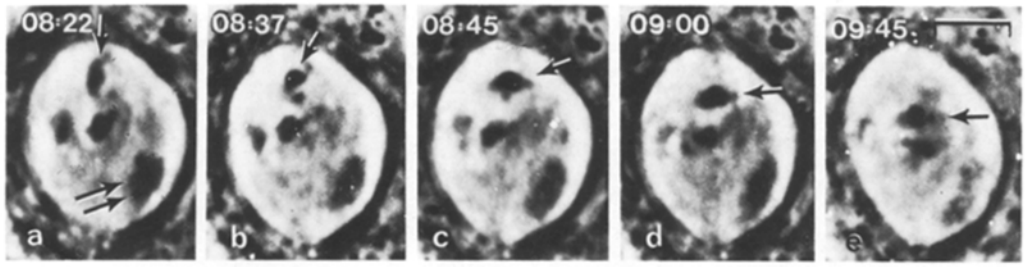

Fig. 4a–e. The bivalent moves even though neither kinetochore is facing a pole (*single arrows*). One kinetochore was initially oriented towards the upper pole (**a**). The bivalent rotates 90° (**b, c**) and moves towards the equator (**d, e**). Condensed Y loop material is also visible (*double arrow*, **a**). Zero time is the time chromosomes become visible. Bar represents 5 μm

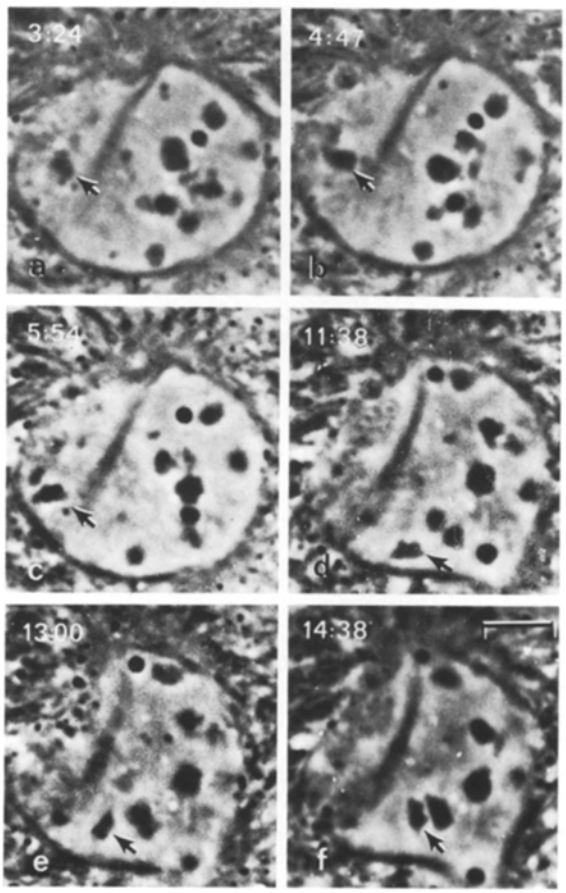

Fig. 5. Shortly after the chromosomes become visible (zero time) the bivalent (*arrows*) moves laterally to the nuclear periphery (**a–c**), moves along the membrane (**c, d**) and returns towards the center of the spindle (**e, f**). Bar represents 5 μm

Chromosome velocity and patterns of movement

From the time that chromosomes became visible to the time anaphase I began required 42 ± 4.26 min ($n = 10$). It was not possible to follow all bivalents within a single cell throughout prometaphase because they were usually at different focal levels. However, by adjusting the focus while filming, a single bivalent could be followed throughout meiosis I. To determine the kinetochore positions on the bivalent, a frame was selected where the kinetochore ends of the bivalent were readily apparent and analysis proceeded frame by frame with forward reverse projection. For this study, only the bivalents *2* and *3* were analyzed.

Chromosome movement began within 5 min after the chromosomes became visible. During approximately the first half of the interval (approximately 20 min) between visibility of the chromosomes and the initiation of anaphase I, the movement was very irregular with much start and stop activity. These saltatory motions ranged in velocity from approximately 2–17 μm/min, a maximum value almost seven times the average anaphase velocity (2.83 μm/min based on measurements from ten cells). A bivalent may move a short distance and then rest for several minutes prior to another change in position. The majority of the unbroken movements were in the range of 1–2 μm although unbroken movements of up to 6.7 μm were recorded. The velocity decreased throughout prometaphase until it approached that observed at anaphase (Fig. 2). During the initial phase, the patterns of movement could be divided roughly into four categories. The first consisted of poleward motion where a kinetochore led the bivalent almost parallel to the spindle axis and towards a pole. The bivalent could appear to be stretched or to have achieved bipolar orientation; however, these orientations never persisted. The most

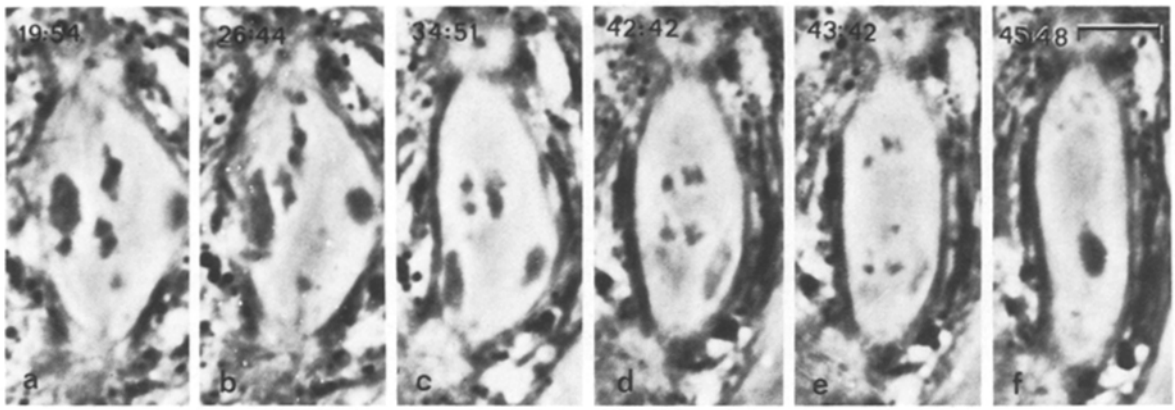

Fig. 6a–f. Cell in which bivalents have achieved persistent bipolar orientation. The bivalents may continue to make poleward motions (**a, b**) but stability of position is eventually achieved (**c**) and disjunction follows (**d–f**). The large masses at the periphery of the nucleus represent condensed Y loop material. Zero time is the time chromosomes become visible. Bar represents 5 μm

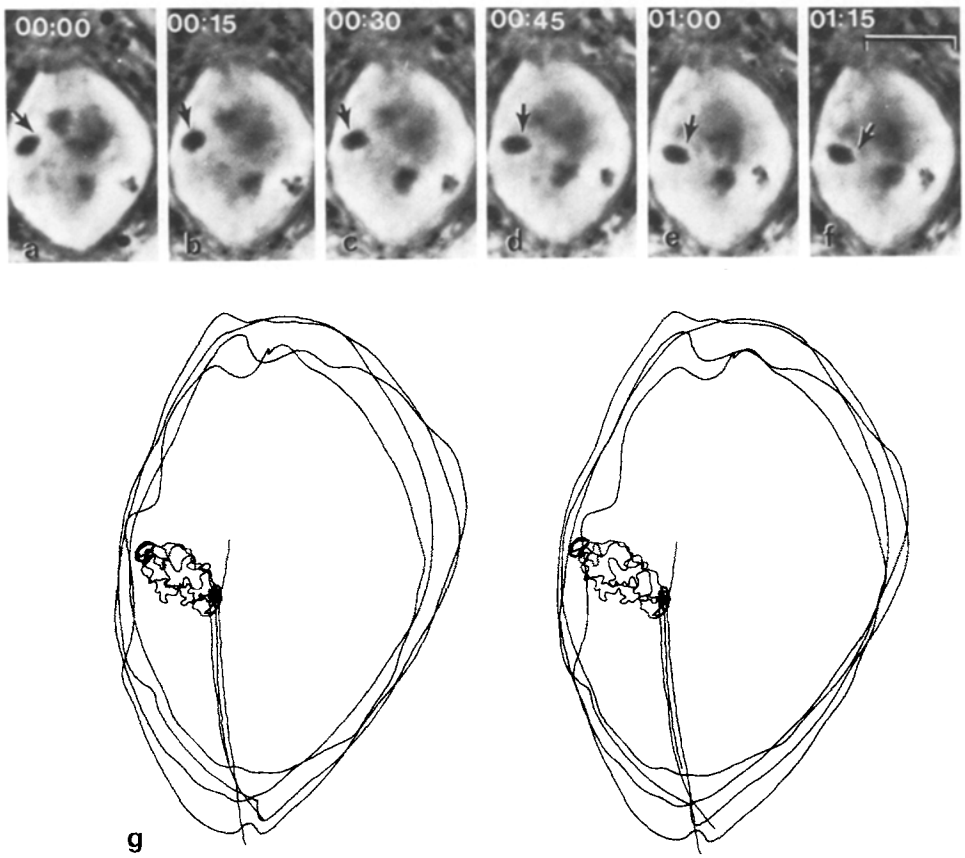

Fig. 7a. The bivalent (*arrows*) initially is tilted towards the upper pole. It changes angles (**b–e**) and points to the lower pole (**f**). The kinetochore displays one short microtubule directed towards the upper pole and two long and one short microtubules directed towards the lower pole (stereopair, reconstructed from electron micrographs of serial sections, **g**). Bar represents 5 μm

dramatic example of their transient nature are those cases where both dyad kinetochores reoriented simultaneously; that is, the bivalent suddenly made a flip flop or U turn (Fig. 3). A second type of poleward motion in includes those cases where the bivalent moved towards a pole (or the equator) but the kinetochores were not facing the poles. Rather, both kinetochores faced 90° to the spindle long axis and yet the bivalent moved (Fig. 4). The third category of movement includes those that are almost perpendicular to the spindle long axis, i.e., lateral motion. This type of motion could be directed toward the center of the spindle or towards the nuclear periphery (Fig. 5a–c). Finally, a kinetochore could move toward the nuclear periphery, and subsequently move along the membrane (Fig. 5c–d) in the general direction of the pole.

After the initial 20 min the bivalents achieved a persistent bipolar orientation (Fig. 6a). They maintained this orientation for approximately 22 min at which time anaphase began. They may or may not have continued to make poleward motions during this period (Fig. 6b), but they always came to a stable position some minutes before anaphase began (Fig. 6c). Stability of orientation and position was always achieved prior to disjunction. Interestingly, stability did not always occur at the spindle equator. The bivalents in 5 of 25 cells were not lined up at the equator when anaphase began.

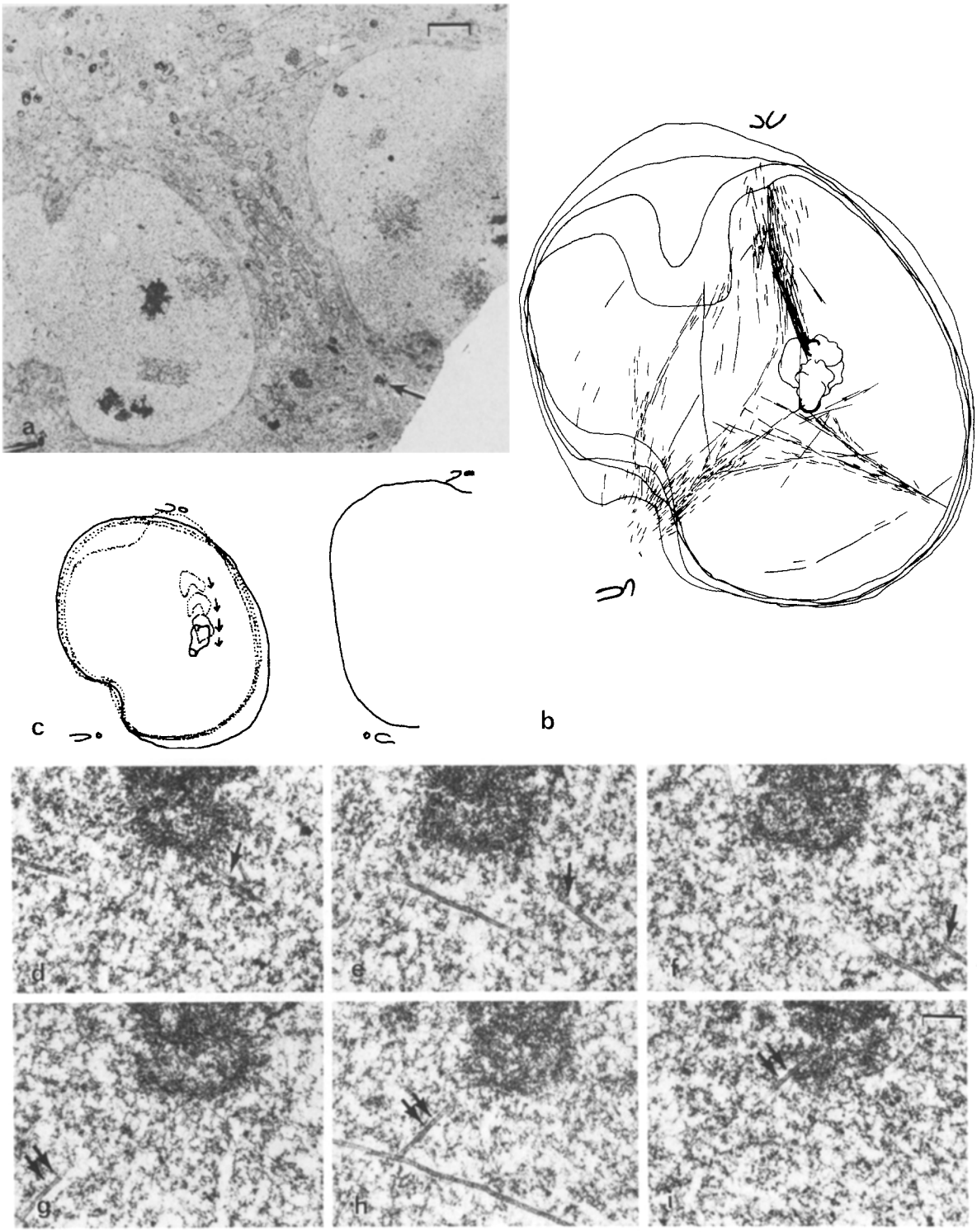

Fig. 8a. Low-magnification electron micrograph of a binucleate cell. The polar region of one nucleus (*arrow*) is participating in the formation of a tripolar spindle in the second nucleus. Bar represents 2 μm. **b** Two-dimensional reconstruction of tripolar spindle. The cine record (**c**) shows that a bivalent is moving in a path that is about equidistant to two of the poles (*arrows* **c**). **d–i** The leading kinetochore displayed only three kinetochore microtubules. Note the angles of two microtubules in the serially sectioned kinetochore (*single* and *double arrows*). Each was directerd to a different pole and each was traced to the membrane layers near the polar regions. The trailing kinetochore displayed two kinetochore-to-pole microtubules in addition to six short grazing microtubules each with two free ends. Bar represents 0.2 μm

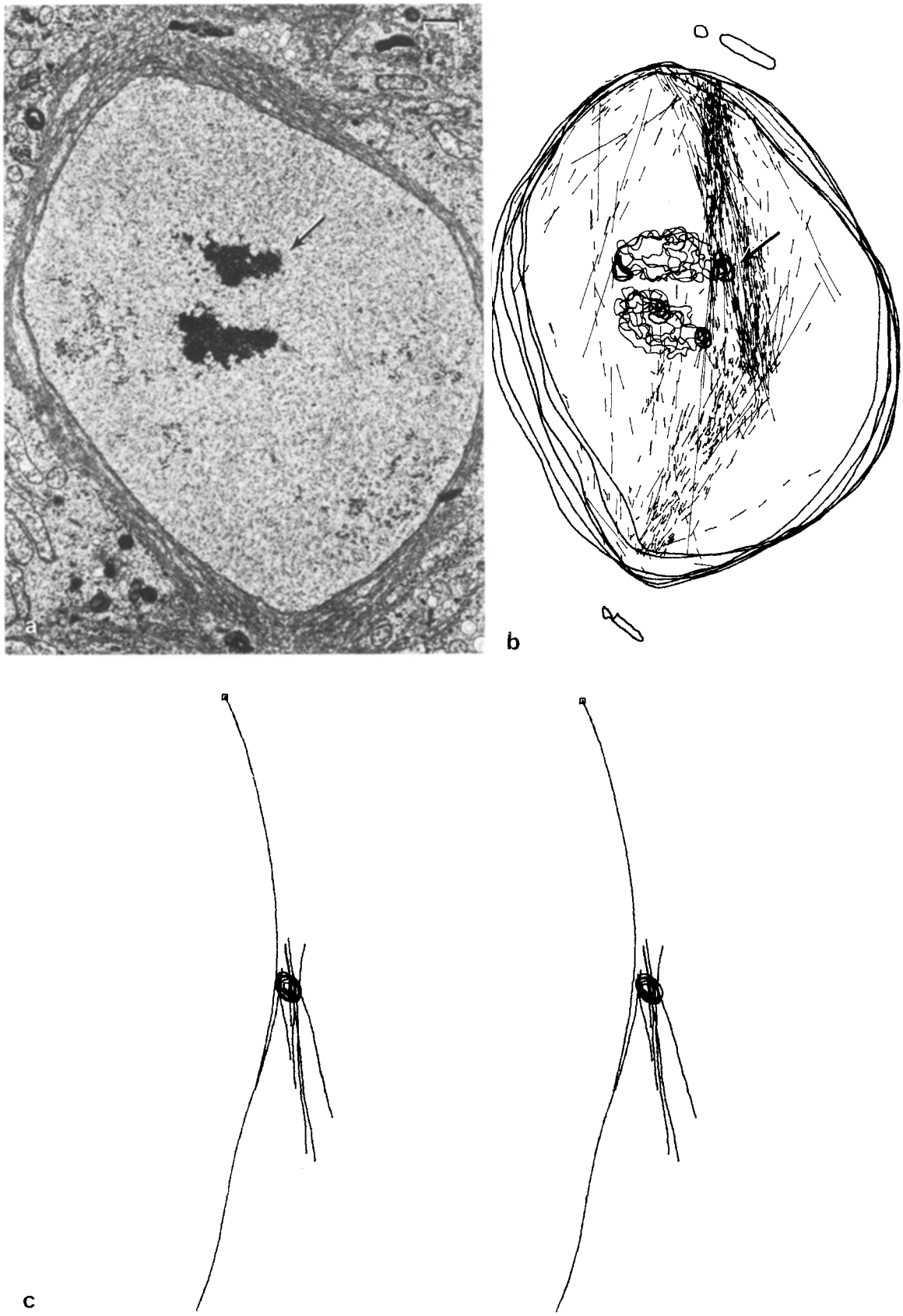

Fig. 9. Electron micrograph (**a**) showing target bivalent (*arrow*) of cell shown in Figure 4 and a two-dimensional reconstruction (**b**) of every other section through the region of the target bivalent. The kinetochore (*arrow* **b**) was surrounded by microtubules. It displayed eight kinetochore microtubules (stereopair in **c**). One long microtubule (*marked*) extended to the membrane periphery near the upper pole and one long microtubule extended to the lower polar region. Six short spearing microtubules were also present. Bar in **a** represents 1.0 μm

Electron microscopy of cells previously studied in life: transient bipolar orientations. A kinetochore in the process of changing positions is illustrated in Figure 7. One kinetochore is positioned near the envelope and the other is tilted toward the upper pole as if force were being directed towards that pole (Fig. 7a). Suddenly, the bivalent changes angles and the kinetochore that initially pointed towards the upper pole is now directed towards the lower pole (Fig. 7b–f). The cell was fixed in this configuration and was examined with the electron microscope (Fig. 7g, h). The kinetochore distal to the nuclear periphery displayed four microtubules, two of which extended from the kinetochore to at least the nuclear membrane positioned near the lower pole (the pole toward which the kinetochore moved). A third microtubule extended towards the lower pole but ended short of the membrane periphery. The forth microtubule was directed towards the upper pole (the pole towards which the kinetochore originally pointed) but ended well short of the membrane. The other kinetochore was devoid of microtubules. The observation suggests that initially, force was directed towards the upper pole through a kinetochore microtubule causing the bivalent to tilt in that direction. Force was then initiated towards the lower pole through kinetochore-to-pole microtubules and was great enough to either break the single microtubule that was previously involved in the force mechanism or alternatively, disrupt lateral associations with other polar microtubules. The above observations suggest a mechanism by which transient bipolar orientations might be explained. Both kinetochores may have microtubule connections with both poles. Such microtubule configurations have been observed (Church and Lin 1982). Asynchronous force transmission in the proper sequence could cause simultaneous reorientation of both kinetochores.

Chromosome movement when the kinetochore does not face a pole. We examined motion of one bivalent under somewhat peculiar circumstances. The cell in question was a binucleate spermatocyte (Fig. 8a). Such cells occurred quite frequently under culture situations. The electron micrographs of this cell showed clearly that a polar region of one nucleus was having an effect on the second nucleus in that a tripolar spindle was being assembled in the second nucleus (Fig. 8b). the cine record of one bivalent is illustrated in Figure 8c. The bivalent is moving in a path that is equidistant to two poles of the tripolar spindle. The leading kinetochore is facing 90° to the spindle long axis established by the two poles. The path of movement suggests that force on the kinetochore is being exerted towards both poles simultaneously. Close examination of the leading kinetochore (Fig. 8d–i) reveals that two long microtubules are present each of which extends from the kinetochore to each of the two poles between which is the path of the movement. A third short grazing microtubule points towards the other pole but ends a short distance from the kinetochore. The trailing kinetochore, which is almost facing the third pole (upper pole in Fig. 8b) but is now moving away from that pole, exhibited two kinetochore-to-pole microtubules in addition to five short microtubules each of which grazed the kinetochore and displayed two free ends. These short grazing microtubules may be remnants of previous association of the kinetochore with the upper pole.

The chromosome movement described above in which a bivalent is moving perpendicular to a spindle long axis appears to be analogous to the lateral chromosome movements that have been previously described for micromanipulated chromosomes and were observed in the cine analysis of live *D. melanogaster* cells. For example, in those cases where a grasshopper bivalent is detached from and moved to a position far from the spindle, the initial motion is usually back towards the center of the spindle. Examined ultrastructurally, the kinetochore displays microtubules pointed in the direction of both poles (Nicklas et al. 1979). The structural evidence from both micromanipulated chromosomes and the cell just described seems to suggest that for lateral movements to occur two events must happen: (1) a functionally single kinetochore must have microtubules directed toward two poles and (2) equal force must be delivered simultaneously along both sets of microtubules.

An example of poleward movement where both kinetochores face 90° to the path of movement was previously shown in Fig. 4. In that example, the bivalent was initially situated with one kinetochore near the upper pole and the homologous kinetochore pointing in the opposite direction (Fig. 4a). The bivalent rotated 90° (Fig. 4b–c) and subsequently moved towards the lower pole (Fig. 4c, d) at which time it was fixed for electron microscopy (Fig. 9a–b). Detailed analysis of the electron micrographs revealed that the kinetochore that originally was associated with the upper pole displayed eight microtubules (Fig. 9c). Five microtubules of relatively short length passed through the kinetochore and each had two free ends that were directed towards opposite poles. A sixth short microtubule had one end in the kinetochore and the other end directed towards the pole. These six microtubules were part of a large bundle of microtubules that were members of the upper half-spindle (see Fig. 9b). As previously suggested for the tripolar spindle, it is possible that the short microtubules are remnants that were involved in the previous orientation of the kinetochore towards the upper pole (see Fig. 4a). There were two long microtubules, one of which extended from the upper pole and grazed the kinetochore and another which had one end in the kinetochore and extended almost to the membrane periphery near the pole towards which the bivalent was moving. The second kinetochore was devoid of mictotubules.

The observations reported above were somewhat surprising. We had anticipated that both kinetochores would display microtubule connections with both poles and that would explain the fact that both kinetochores face 90° to the spindle axis (Church and Lin 1982). Given that this was not the case, for this particular bivalent, we suggest that the bivalent moved because force was directed toward the lower pole through or by the long kinetochore microtubule. However, the bivalent was being held in the sideways configuration because the kinetochore still had a connection with the upper pole and was surrounded by microtubules that were members of the upper half-spindle. Here then, microtubules are acting to position the bivalent as well as to move the bivalent.

Kinetochore microtubules. Kinetochore microtubules were examined for a total of six bivalents where the direction of movement had been recorded prior to fixation. Of 12 kinetochores examined, 3 were devoid of microtubules. The remaining 9 displayed a total of 40 kinetochore microtubules. The microtubules either ended in the kinetochore

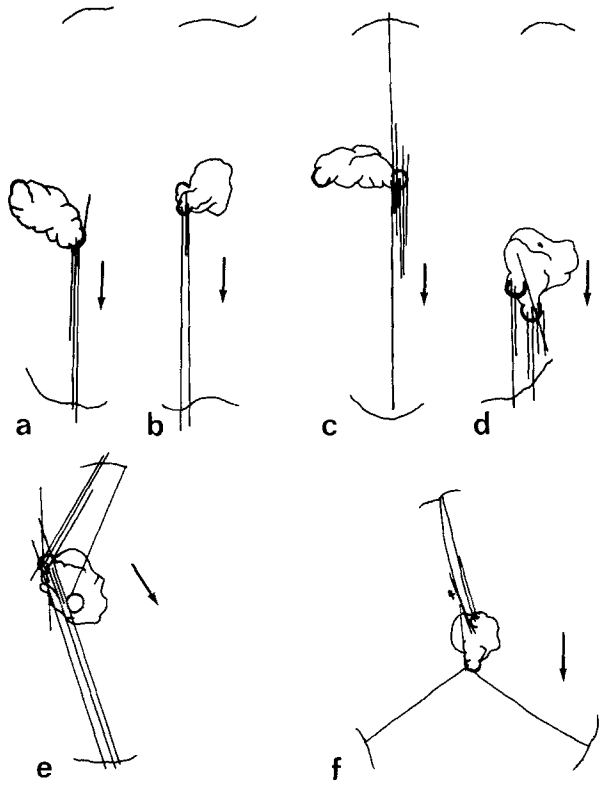

Fig. 10a–f. Diagrammatic representation of all bivalents and all associated kinetochore microtubules. Microtubules that extend from the kinetochore (hemisphere) to the membrane layers near the pole (*lines*) are shown as are the short microtubules and the spearing microtubules (The number 4 in **f** indicates four additional spearing microtubules were observed). Note that the direction of movement (*arrows*) is correlated with the presence of at least one kinetochore microtubule that extends to the membrane layers near the pole with the exception of **c**; there, a kinetochore microtubule comes within 1 μm of the membrane. **f** Bivalent associated with the previously described tripolar spindle

(19) or grazed or passed through (speared) the kinetochore (21). Of those that ended in the kinetochore, 9 could be tracked to the membrane layers in the polar regions of the cell. The others (10) ended short of the membrane layers although many came within 1 μm of the membrane. Of those that speared the kinetochore, 8 extended to the membrane layers and the rest (13) were relatively short pieces. A diagrammatic representation of all microtubules associated with the kinetochores and the direction of movement of the bivalents is illustrated in Figure 10. With but one exception the direction of movement was correlated with the presence of at least one microtubule that extended from the kinetochore (either ended in or speared) to at least the membrane layers near the pole to which the bivalent was moving. Even the one exception (Fig. 10c) displayed a long microtubule pointing in the direction of the movement which ended less than 1 μm of the membrane.

It seems reasonable then that the kinetochore microtubules that span the distance from the kinetochore to near the pole are the microtubules that were "engaged" in the force-producing mechanism at the time the cells were fixed for electron microscopy. Other classes of microtubules were not necessarily correlated with the direction of movement.

Discussion

Chromosome velocity as measured in *D. melanogaster* is within the range reported by others. It is greater than in crane flies (range 0.25–2.0 μm/min; Dietz 1956) but very similar to early prometaphase in *Acheta* (range 5.0–24.0 μm/min; Rickards 1975). The velocities gradually diminish throughout prometaphase until they reach those similar to anaphase (Dietz 1956; Bauer et al. 1961; Rickards 1975; this study). The observation of an overall decrease in speed throughout prometaphase has been used to argue for a role of microtubules as governors or velocity controllers for chromosome movement (Nicklas 1975). Put in terms of numbers of kinetochore microtubules, one might expect that fewer microtubules permit greater velocities and more microtubules lesser velocities. We have established that in *D. melanogaster* spermatocytes there is an overall inverse correlation between numbers of kinetochore microtubules and chromosome velocity. The average number of microtubules per kinetochore in these very early cells was 4.25 ± 2.9 compared to 6.5 ± 4.1 for cells later in prometaphase (Church and Lin 1982) and 13.8 ± 3.9 for cells at anaphase I (Lin et al. 1981).

The most detailed studies of the kinds of motions chromosomes exhibit during prometaphase I of meiosis have come from the analysis of the crane fly (Dietz 1956; Bauer et al. 1961) and grasshopper spermatocytes (for review Nicklas 1971). A comparison of *D. melanogaster* with those organisms reveals several types of movements that appear to be unique to *D. melanogaster* or at least have not been described in normal prometaphase without experimental intervention such as micromanipulation or temperature treatment (see Nicklas et al. 1979; Henderson et al. 1970). They include: (1) simultaneous reorientation of both dyad kinetochores (Granholm 1970; this study); (2) movements that are not parallel to the spindle axis, i.e., lateral movements, both towards the center of the spindle and towards the nuclear periphery; and (3) poleward movements of bivalents when neither kinetochore is facing a pole. Finally, the bivalents appear to be delayed in achieving bipolar orientation when compared to other organisms. Most analyses of live cells have led to the conclusion that most bivalents achieve bipolar orientation immediately when prometaphase begins (for review see Nicklas 1971), whereas in *D. melanogaster* bipolar orientation is not achieved for some 20 min after chromosome motion is initiated (roughly half the interval between the time of initial motion to chromosome disjunction).

Some insight into the complex movements exhibited by *D. melanogaster* bivalents most likely resides in the structure of the kinetochore (Granholm 1970; Goldstein 1981). The dyad kinetochore at prometaphase I is composed of two cohesive sister kinetochores (Lin and Church 1982) although the duality of the kinetochore is not visible in electron micrographs at this stage. The kinetochore is very large in relation to the size of the bivalents when compared to crane flies or grasshoppers. It is not recessed in a "cup" of chromatin as occurs in grasshoppers, rather its entire surface is exposed to the spindle. The three layers of the kinetochore (Church and Lin 1982) assume a hemisphere shape at prometaphase and the hemisphere does not develop to a disclike configuration until metaphase (Goldstein 1981). The result of this unusual prometaphase kinetochore topology is that no matter which direction the center of

the kinetochore is facing, the sides of the kinetochore are exposed for interaction with opposite poles (see Fig. 6 in Church and Lin 1982). If kinetochore microtubules originate by capture of polar microtubules (Church and Lin 1982), then the kinetochore is in a convenient configuration to capture microtubules from both poles resulting in unorthodox microtubule arrangements. If, on the other hand, the kinetochore nucleates its own microtubules, the tubules will be in a convenient configuration for lateral interactions with microtubules originating from both poles. The end result is that when prometaphase is initiated all possible kinetochore-microtubule arrangements can and do occur. Given the above, it is perhaps not surprising that the early prometaphase I chromosome movements are somewhat erratic in *D. melanogaster* when compared to other organisms.

The fact that *Drosophila* bivalents do exhibit such complex motions gives us the opportunity to begin to identify those microtubules from the complex array of kinetochore microtubules that might be involved in motile mechanisms of meiosis. What the results seem to say is that the kinetochore microtubules that span the kinetochore-to-pole distance are important. This is certainly not a surprising finding, but direct evidence on this point has been heretofore lacking. If we assume that our fixation techniques are preserving most kinetochore microtubules (a tenuous assumption) then it appears that a single kinetochore-to-pole microtubule can either transmit or exert enough force to visibly move a chromosome (for example see Fig. 10). Also, in early stages of meiotic development the force seems to be in as much disarray as is the developing spindle. It is applied to kinetochores asynchronously, in short spurts of varying degrees and duration, almost in a random fashion as earlier investigators including (Dietz 1956), Bayer (1958), and Tippit et al. 1980) have noted.

The observations also suggest that at any one time there are microtubules at the kinetochore that are not engaged in the force mechanism. One such class of microtubules is most likely the short kinetochore microtubules. The origin of the short microtubules is uncertain. Most of them have two free ends i.e., they spear the kinetochore. We have argued previously (see also Tippit et al. 1980) that such microtubules probably result from capture of polar microtubules. We now suggest that at least some of them are relics of previously captured polar microtubules. The best evidence that this is plausible comes from two cells (Figs. 8, 9) where the cine's analysis suggests that the kinetochores were previously associated with a specific pole and are now moving away from that pole. Both kinetochores displayed short spearing microtubules.

A comparison of the quantitative data from this set of cells with that of a previous study (Church and Lin 1982) reveals that while approximately 75% of the kinetochore microtubules extended from the kinetochore to the pole in the previous study, about 44% fall into that class in the current study. There are several possible reasons for the disparities in the two data sets. For one, it is now clear that the previous study was based on cells in a later stage of prometaphase than those of the current investigation. It may be that the short spearing microtubules (the majority of the short microtubules) are characteristic of early cells where the chromosome activity is more frantic and the microtubules more subject to breakage or disengagement from the pole. We cannot rule out the possibility that they are an artifact of the culture situation or the fixation procedures used for single cell analysis.

All of the observations reported herein are consistent with the notion that the prometaphase chromosome motions (complex though they may be) can be explained by poleward forces acting on long kinetochore microtubules if it is assumed that (1) the force is asynchronous and (2) that the skeletal role of microtubules includes passively positioning chromosomes. The one possible exception may be the membrane-associated movements, which have not yet been analyzed with the electron microscope. It will be interesting to see if such motion is analogous to the prophase chromosome motion described by Rickards (1975) which is apparently accomplished in the absence of intranuclear microtubules or if this motion too can be explained by poleward forces acting on kinetochores through kinetochore-to-pole microtubules.

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