CHROMOSOME MICROMANIPULATION

III. Spindle Fiber Tension and the Reorientation of Mal-Oriented Chromosomes

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ABSTRACT

Kinetochore reorientation is the critical process ensuring normal chromosome distribution. Reorientation has been studied in living grasshopper spermatocytes, in which bivalents with both chromosomes oriented to the same pole (unipolar orientation) occur but are unstable: sooner or later one chromosome reorients, the stable, bipolar orientation results, and normal anaphase segregation to opposite poles follows. One possible source of stability in bipolar orientations is the normal spindle forces toward opposite poles, which slightly stretch the bivalent. This tension is lacking in unipolar orientations because all the chromosomal spindle fibers and spindle forces are directed toward one pole. The possible role of tension has been tested directly by micromanipulation of bivalents in unipolar orientation to artificially create the missing tension. Without exception, such bivalents never reorient before the tension is released; a total time "under tension" of over 5 hr has been accumulated in experiments on eight bivalents in eight cells. In control experiments these same bivalents reoriented from a unipolar orientation within 16 min, on the average, in the absence of tension. Controlled reorientation and chromosome segregation can be explained from the results of these and related experiments.

INTRODUCTION

Controlled chromosome distribution to the daughter cells in mitosis depends upon kinetochore orientation and reorientation. Thus, kinetochore orientation (the association of a chromosome with a particular pole via chromosomal spindle fibers) determines the pole to which the chromosome will move in anaphase. But kinetochore *re*orientation is crucial to controlled distribution, because some mal-oriented chromosomes result from the initial orientation process in early prometaphase. In meiosis, for instance, bivalents occur with both half-bivalents oriented to the same pole. This "unipolar" orientation (see Fig. 1) would result in nondisjunction if it persisted; but instead, reorientation occurs, the normal, "bipolar" orientation

(see Fig. 1) results, and orthodox segregation follows (e.g., refs. 10, 1).

The initial orientation process, and hence the flawless bipolar orientation of most chromosomes, is now understood (18). Equally certain, however, is our failure to understand the reorientation of mal-oriented chromosomes. Dietz (5) suggested that reorientation is effective not because a single reorientation certainly leads to bipolar orientation, but rather because only the bipolar orientation is stable, and any other orientation is unstable. That is, reorientation within a small fraction of the total prometaphase time is highly probable. Thus eventually the stable bipolar orientation is reached more or less by chance, and no
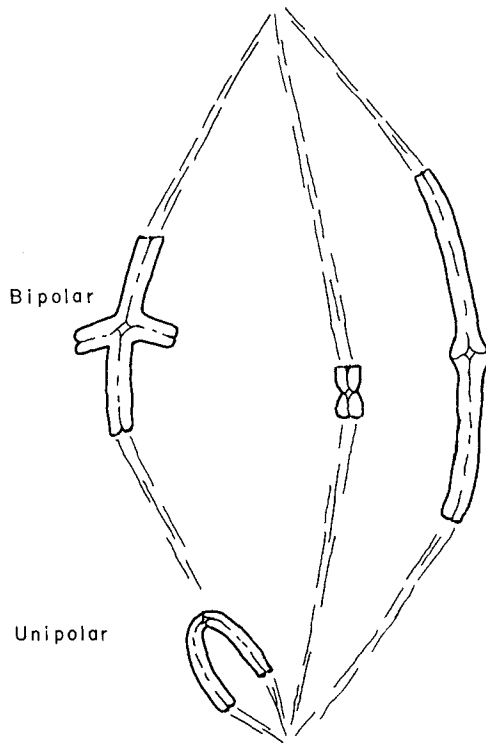
FIGURE 1 Semi-diagrammatic representation of orientation at prometaphase I in *Melanoplus*. Four bivalents, each composed of two chromosomes (half-bivalents), are shown. Chromosomal spindle fibers, represented by broken lines, run between the kinetochore of each half-bivalent and the pole to which that half-bivalent is oriented. Three bivalents (above) are shown in bipolar orientation, in each, the partner half-bivalents are oriented to opposite poles. One bivalent (below) is shown in unipolar orientation; both half-bivalents are oriented to the lower pole. The kinetochore appears as if it were terminal in all *Melanoplus* chromosomes.

further changes in orientation occur. This is exactly what is seen in living cells (1). Therefore the origin of differences in orientation stability is the key to understanding controlled chromosome distribution in eucaryotic cells.

Possible sources of differential orientation stability are readily suggested from the numerous other differences between mal-oriented and appropriately oriented chromosomes. Spindle fiber tension is one such difference: only in bipolar orientation is a chromosome or bivalent subjected to forces toward opposite poles. The presence of these opposed forces is readily recognized from the straightening or even stretching of the chromosomal material between the kinetochores oriented to

opposite poles, and the absence of similar deformation in mal-oriented chromosomes is equally obvious. "Spindle fiber tension" signifies only that the tension forces are transmitted to chromosomes by chromosomal spindle fibers (e.g. ref. 19); spindle fiber *production* of the forces need not be assumed. Dietz (5, see p. 432) was the first to suggest that spindle tension might explain differences in orientation stability; he tentatively considered its attractiveness on theoretical grounds. We report here a direct test of the role of tension by micromanipulation experiments on living spermatocytes. A straightforward explanation of kinetochore reorientation is suggested by the results of these experiments.

MATERIAL AND METHODS

The grasshopper *Melanoplus differentialis* from a laboratory colony (see ref. 17) was used in these studies. Spermatocyte culture, micromanipulation, cinematographic recording, and analysis were carried out as described previously (19). Briefly, the cells were cultured (temperature range: 24° to 26°C) in a modified Ringer's solution. Bivalents in the first meiotic division were manipulated with the Ellis (6) piezoelectric micromanipulator equipped with a glass microneedle about 0.1 μ in diameter at the tip. The cells were observed and cinemicrography was carried out on a Zeiss inverted microscope with a 1.25 N.A. oil immersion, phase contrast objective.

All 20 cells considered in this report completed anaphase following micromanipulation; anaphase was normal in all cells, with the exception of experimentally induced nondisjunction of one bivalent in six cells. No exceptional results were found in the cells which failed to divide. The general influence of micromanipulations on spermatocytes has been considered earlier (18, 19). Here also, differential viability of micromanipulated and adjacent control cells has never been observed, nor have nonspecific effects on chromosome behavior.

RESULTS

The experiments to be described are possible because unipolar orientation of bivalents (see Fig. 1) in grasshopper spermatocytes is easily induced by micromanipulation (18). Reorientation to the normal bipolar orientation (see Fig. 1) follows within minutes and is identical with naturally occurring reorientation. In the present study, unipolar orientation to a given pole was always tested directly by placing a microneedle at the closed end of the bivalent and moving the needle toward the opposite pole. If the bivalent is unipolar, then the kinetochores will remain in position while the rest

of the bivalent is slightly stretched (see Fig. 4 for an example, and Nicklas and Staehly, ref. 19, on the related test for bipolar orientation). Where no further operations intervene, such bivalents always move as expected—toward the pole to which both half-bivalents are oriented (e.g., Figs. 2 and 3 between 5.4 and 16.5 min).

Tension Experiments

If natural spindle tension toward opposite poles makes bipolar orientation stable, then artificial tension should stabilize unipolar orientations. This is the rationale for "tension experiments", which are feasible because a bivalent in unipolar orien-
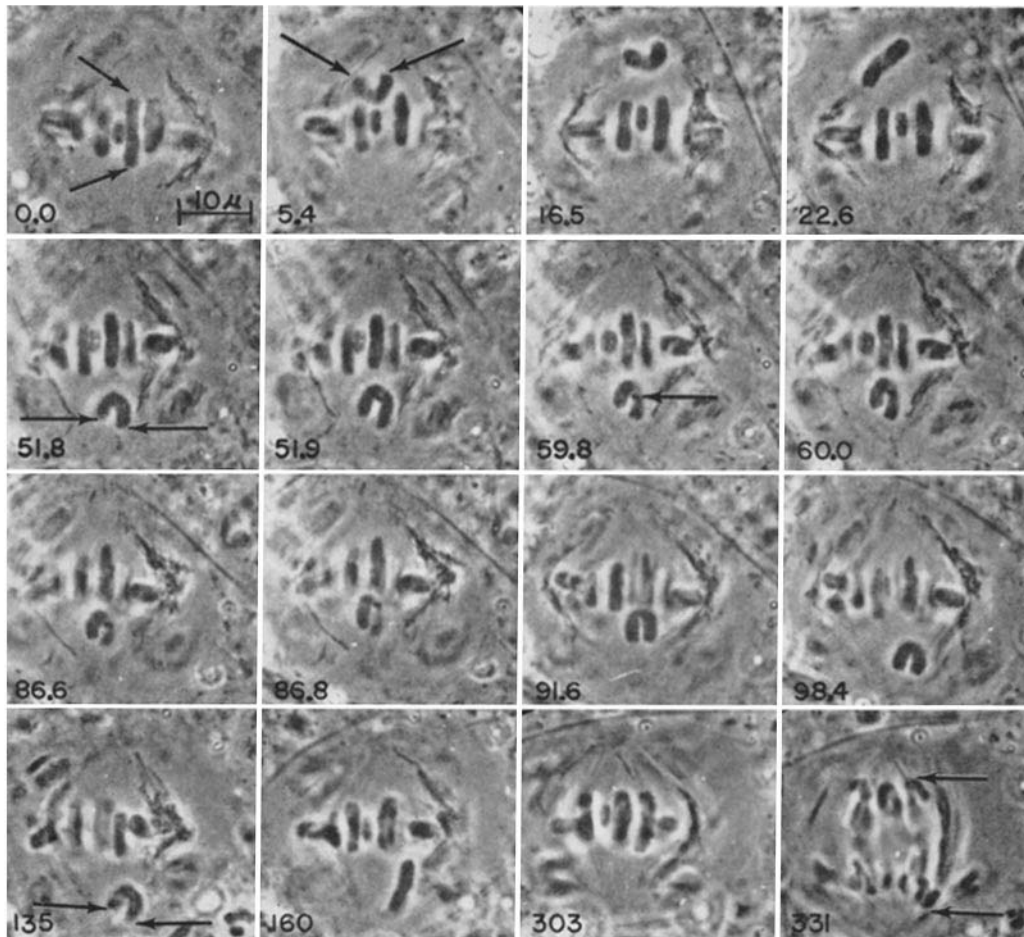

FIGURE 2 Prints from the cinématographic record of a tension experiment. The time in minutes is given on each print for comparison with the graph (Fig. 3). The pairs of arrows on several prints indicate the positions of the bivalent's kinetochores. The micromanipulation needle is rarely visible in still photographs, but its tip can be seen as a dark spot on the 51.9 through 86.8 min prints (see the arrow on the 59.8 min print). The bivalent, shown before manipulation on the 0.0 min print, was detached and unipolar orientation induced (5.4 min). A control experiment was then run with the needle close to, but not stretching, the bivalent; the bivalent promptly reoriented (16.5, 22.6 min). The bivalent was later detached again and unipolar orientation induced a second time (51.8 min). Tension toward the upper pole was then applied with the needle; note the slight stretching of the bivalent at 51.9 min. Tension was maintained for 39.7 min with periodic readjustments (compare 59.8 with 60.0 and 86.6 with 86.8 min). Reorientation did not occur during tension (59.8 to 91.6 min). A second control experiment (135 min) was followed by reorientation (160 min), congression (303 min), and normal anaphase (331 min). × 975.

tation is anchored to one pole by its chromosomal spindle fibers, and hence an artificial tension toward the opposite pole can be applied with a micromanipulation needle. The necessary controls, in which no tension is applied to the unipolar bivalent, can be done on the same bivalent before, and sometimes also after, a tension experiment.

A typical experiment is shown photographically in Fig. 2 and in graphical form in Fig. 3. The 0.0 min print (Fig. 2) shows the cell before the start of experimentation. A control experiment was then done as follows: the lower half-bivalent was detached and swung toward the upper pole; it soon oriented to that pole, producing unipolar orientation of the bivalent (5.4 min print). The needle was immediately placed within the U-shaped bivalent, nearly touching the bivalent at its closed end. The needle was moved as necessary to keep it in this position as the bivalent moved toward the pole (5.4 to 16.5) and reoriented (16.5 to 22.6 min), thus restoring the original bipolar orientation (Fig. 2, 22.6 min). The bivalent was then detached from the spindle again and unipolar orientation was in-

duced a second time, this time toward the lower pole (51.8 min print). Then a tension experiment was begun by placing the needle at the closed end of the bivalent and moving the needle toward the upper pole (51.9 min). The tension thus created was evident from the increase in bivalent length (compare the 51.8 and 51.9 min prints). The position of the needle was adjusted repeatedly to maintain this slight tension, always gaged by the deformation of the bivalent when the tension is applied. "Before" and "after" pictures of such readjustments are exemplified by two pairs of prints: 59.8 and 60.0 min, and 86.6 and 86.8 min.

Tension was maintained for 39.7 min. The bivalent did not reorient in this interval. After removal of the needle (91.6 min print, Fig. 2) the bivalent moved toward the lower pole (98.4 min print, Fig. 2; 92 to 130 min, Fig. 3). The bivalent did not reorient for 38.2 min after tension was released; it was then detached, unipolarity was induced a third time (135 min, Fig. 2), and the needle was placed in the control position. Reorientation occurred after 15.7 min; a later stage is shown in
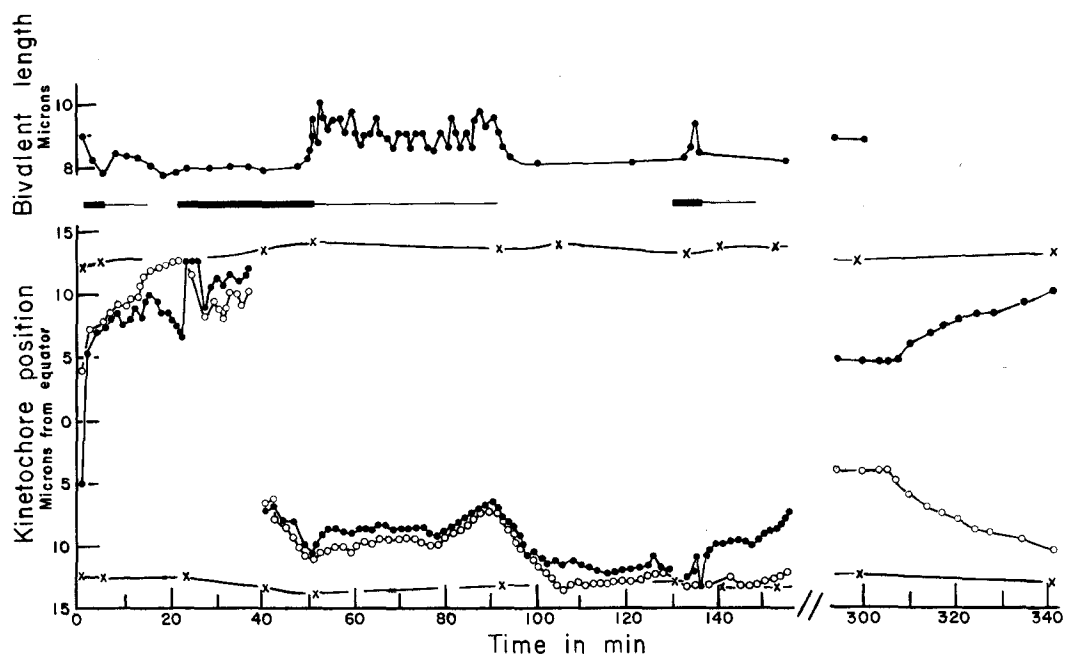

Figure 3   Graphical representation of the cell shown in Fig. 2. The heavy horizontal lines beneath the "Bivalent length" plot indicate when an operation to induce unipolarity was in progress; the thin lines indicate, from left to right, the durations of the first control, the tension experiment, and the second control. In the lower graph, the kinetochore positions of the two half-bivalents are indicated by open and closed circles; the positions of the poles are indicated by "X-es". The interval between 157 and 292 min is omitted to reduce the length of the plot.

Fig. 2 at 160 min. The bivalent then returned to the metaphase plate (303 min print), and a normal anaphase followed (331 min print).

The "bivalent length" plot in Fig. 3 provides a general guide to the forces acting on the bivalent. First, it reveals the slight tension normally exerted on bipolar bivalents at prometaphase: contrast the 9 μ length at 0.0 min when the bivalent was oriented normally, with the length of 8 to 8.5 μ when the bivalent had been detached or was unipolar (e.g., from 5 to 45 min, Fig. 3). After the conclusion of the experiments the original length of 9 μ was restored as the bivalent moved back toward the equator and was again under natural forces toward opposite poles (Fig. 3, 300 min). Second, measurements show that artificial tension (Fig. 3, 51 to 92 min) produced, on the average, the same 9μ bivalent length seen in normal bipolar orientation, although the length was far more variable, with a range of 8.5 to 10 μ.

The following general analysis of tension effects is based on experiments on eight bivalents in eight cells, including the cell shown in Fig. 2. Table I gives, for each cell, data in the order the experiments were performed. For the controls and the period after the tension experiment ("post-tension"), the time required for reorientation is given in minutes. Reorientation is recognized by the beginning of rapid motion toward the opposite pole of one or both half-bivalents.

Two new qualitative features emerge when the additional cells are considered. First, a second control experiment was not always performed (see

TABLE I

The Duration of Tension Experiments and the Time Required for Reorientation of Controls without Tension.

Time in minutes. A, anaphase intervened before reorientation; nondisjunction occurred; E, an operation intervened before reorientation. "Post-tension": see text.

Cell number	First control	Tension	Post-tension	Second control
1	11.6	39.7	38.2(E)	15.7
2	1.4	14.8	16.8	
3	19.5	40.0	20.0	22.6
4	13.3	41.5	37.5(A)	
5	11.8	50.3	30.2	
6	5.4	50.0		
7	13.6	46.2	62.0(A)	
8	22.1	29.1	11.3	20.0(A)

TABLE II

Tension Experiments and Controls: Average Time Required for Reorientation.

(Data from Table I)

	Reorientation		No reorientation		Average min per reorientation
	No.	Min per reorientation	No.	total min	
Controls	10	13.7	1	20.0	15.7
Tension	0	—	8	311.6	—
Post-tension	4	19.6	3	137.8	54.0

Table I) because the length of these experiments makes likely the intervention of anaphase or operator fatigue. Second, nondisjunction occurred in cells 4, 7, and 8 (Table I). Not included in the Table (because of the brevity of the tension experiment) is a cell in which anaphase began during the tension experiment itself. Thus, nondisjunction can be a direct consequence of the stable unipolar orientation induced by tension, and probably could be obtained routinely by maintaining tension until anaphase begins.

The general effects of tension become clear when average reorientation times are computed (Table II). Thus for the first and second controls combined, reorientation occurs within 15.7 min, on the average. Yet not a single reorientation occurred during a total of 311.6 min of tension applied to these same bivalents. Tension inhibition of reorientation is confirmed by t-test statistics; here each value for the duration of tension (Table I) is treated as a reorientation time, i.e., as if each experiment were terminated by reorientation (a similar assumption was applied to the second control experiment for cell 8; see Table I). This permits a highly conservative test of the null hypothesis of no increase in reorientation time in the presence of tension. The null hypothesis is rejected ($p > 0.0005$; $t = 5.75$; d.f. $= 17$).

Apparently, the inhibitory effect of tension sometimes persists after tension is released: the average "post-tension" reorientation time is 54.0 min (Table II). Again treating each value in Table I as if reorientation did occur, the null hypothesis of no increase in post-tension reorientation time versus the controls is rejected ($p < 0.01$; $t = 2.92$; d.f. $= 16$). Because in this instance the statistical treatment biases the test in an uncertain direction, certain demonstration of post-tension inhibition of reorientation is not claimed, but the

result does justify considering these reorientations in a category separate from the controls.

The time required for reorientation in control experiments is so variable that for some purposes more values seemed desirable. Therefore an additional series of 11 control experiments was done on 6 bivalents in 6 cells. In four of these experiments the micromanipulation needle was not present after the test for unipolarity. No differences were observed from the usual control experiment in which reorientation occurs in the presence of the needle. Some of the variation in reorientation time may be due to micromanipulation, but most is probably of natural origin: in nine untreated crane fly spermatocytes each with a unipolar bivalent, the average time required for reorientation was 11 min with a range from 2 min to over 18 min (ref. 1, page 166).

The combined group of 22 controls was used for three analyses. First, the statistical comparison of controls versus tension duration was repeated, using the approach described above for the smaller group of controls. The mean time required for reorientation was 17.2 min for the combined group of controls. Again the null hypothesis of no increase in time required for reorientation was rejected (p < 0.005; t = 3.55; d.f. = 28). Second, the time required for reorientation in the controls was plotted as a function of the time at which reorientation occurred (minutes before anaphase). No effect was observed: in the interval from 5 hr before anaphase until its inception, there is no trend toward more or less rapid reorientation. This rules out a possible complication in interpreting results from separate experiments in one cell which may extend over 3 hr of prometaphase (e.g., the cell in Figs. 2 and 3). Third, we have considered the possible effect of position within the spindle on reorientation: is reorientation more likely near the poles or in the equatorial region? Bivalents in unipolar orientation move promptly to a pole, and since reorientation occurs only after 17 min on the average, reorientation is far more frequent near a pole than elsewhere on the spindle. But the critical datum is reorientation probability as a function of the time actually spent in each spindle region. This was determined by arbitrarily dividing each half of the spindle into a polar third and an equatorial two-thirds, and computing reorientation time for each region. The data are summarized in Table III for the 20 controls in which reorientation occurred. As expected, few, only 6 of 20, reorientations occurred in

Spindle region	Total time in min	Number of reorientations	Time required (min per reorientation)
Equatorial	80.8	6	13.5
Polar	199.3	14	14.2

the equatorial region. However, the time required for reorientation is not different for the two spindle regions, at least for this rather small sample.

Direct Induction of Reorientation

The kinetochores of chromosomes under natural spindle tension are forced to point quite precisely toward a spindle pole. Thus it is possible that this constraint on kinetochore position, rather than the tension itself, produces stable orientation. This possibility has been examined experimentally by determining whether an enforced *change* in kinetochore position leads to a predictable change in orientation. Such an experiment is shown in Fig. 4. The upper half-bivalent (0.0 min print, arrow labeled "1") was first pushed toward the interpolar axis, thus tilting the bivalent. The needle was than placed near the middle of the bivalent and moved toward the upper pole, producing the inverted "J" configuration seen in the 10.5 and 10.7 min prints. Thus the kinetochoric end of half-bivalent number 1 was forced to face the lower pole, but neither half-bivalent was detached from the spindle. This configuration was established at 5.1 min and maintained for 9.0 min; the 14.1 min print shows the bivalent just after the needle was withdrawn. The altered orientation was documented by reinserting the needle and stretching the bivalent toward the upper pole (14.7 min print): the kinetochores of both half-bivalents remained in position and pointed toward the lower pole while the rest of the bivalent was deformed. The needle was then removed from the cell. Both half-bivalents then moved toward the lower pole (15 and 21 min prints), confirming the unipolar orientation. The bivalent then reoriented, returned to the metaphase plate, and divided normally in anaphase (30, 87, and 104 min prints).

Similar experiments have been performed on four additional cells. The results for cell 2 duplicated those in the cell in Fig. 4, but experiments on cell number 3 add final evidence that orienta-
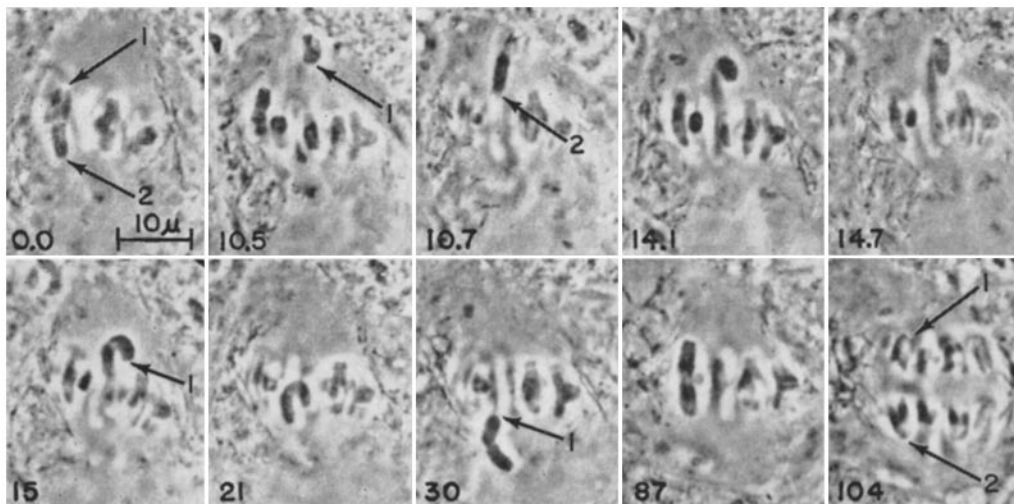
FIGURE 4 Prints from a cinématographic record illustrating direct induction of reorientation. The time in minutes is given on each print. Before the operation (0.0 min), the half-bivalent labeled "1" was oriented to the upper pole, half-bivalent "2" to the lower pole. Without detaching either half-bivalent, needle pressure was applied, and the kinetochoric end of half-bivalent 1 was forced to face the lower pole (10.5, 10.7 min) for 9 min. The needle was then withdrawn (14.1 min) and then reinserted, and the bivalent stretched to demonstrate unipolar orientation (14.7 min). The bivalent then moved toward the lower pole (15, 21 min), the number 1 half-bivalent reoriented (30 min), and normal anaphase followed (104 min). × 1000.

tion can be altered. Here the half-bivalent whose orientation was altered retained the new orientation while the other half-bivalent reoriented; thus, the half-bivalents segregated to different poles than they would have in the absence of experimental intervention. In the fourth and fifth cells of this experimental series, however, reorientation could not be induced in this fashion. No particular significance can be attached to these failures because the appropriate kinetochore position simply could not be produced and sustained. It is not known whether this represents important biological variation or insignificant technical failure. The positive result bears emphasis: whenever a half-bivalent's kinetochores can be forced to face the opposite pole for five minutes or longer, reorientation to that pole invariably follows. This orientation change is induced directly from one pole to the other without first detaching the bivalent from the spindle.

The experiment in a sixth cell merits separate treatment. The general experimental procedure duplicated that described for the cell in Fig. 4, but after tilting the bivalent on the spindle, much less force was applied toward the upper pole. This

force was sufficient to swing the kinetochoric end of the upper half-bivalent nearly perpendicular to the spindle's axis (but not sufficient to make that end face the lower pole as in Fig. 4). The bivalent was held in this position for 40 min; after 20 min the upper half-bivalent had amphioriented (one chromatid oriented to one pole, the other to the opposite pole, as in mitosis). This orientation persisted, and in anaphase the lower half-bivalent moved normally to the lower pole, while the two chromatids of the upper half-bivalent separated and moved to opposite poles in late anaphase (this orientation and segregation pattern has been seen in earlier studies, e.g., Fig. 11 in ref. 18). This and related experiments with similar results were planned to test the prediction that decreasing the natural spindle tension on bivalents in bipolar orientation should lead to reorientation. In the experiment just described, the upper half-bivalent was relieved of natural spindle tension toward the lower pole and, consonant with the tension hypothesis of orientation stability, it was this half-bivalent which reoriented. But the outcome is ambiguous: the needle position which relieves tension also swings the kinetochoric end of the

upper half-bivalent nearly perpendicular to the interpolar axis. Therefore, the observed amphiorientation might be due to direct induction, and the experiment is described in this section for that reason. Parenthetically, our numerous other attempts to induce reorientation by relieving tension should be noted. These have all failed, usually because natural spindle readjustments rapidly restore tension following needle-induced relaxation.

DISCUSSION

We conclude first that applied tension certainly inhibits the reorientation of bivalents in unipolar orientation. This is most dramatically evidenced by the total of over 5 hr with tension without a single reorientation, while in the absence of tension the same bivalents reoriented from unipolar orientation within 16 min on the average. Second, we conclude that this result is relevant to normal reorientation processes. Thus, the applied tension evidently approximates that produced by the spindle on bivalents in bipolar orientation as judged by the similar length increases; by this criterion artificial tension is more variable than, but does not often exceed, natural tension (Fig. 3). Also, unnatural factors other than tension, such as the presence of the needle near the bivalent, are duplicated in the controls, which reorient normally. Therefore, these experiments provide strong evidence that the differences in orientation stability which make possible controlled chromosome distribution arise from differences in spindle tension. Mal-oriented bivalents will reorient until bipolar orientation is achieved more or less by chance. They are then subjected to forces toward opposite poles normally associated only with bipolarity and which somehow confer stability.

Tension effectively stabilizes orientation even in cells where orientation is abnormally unstable. Dr. S. Alan Henderson has discovered that exposure to 10°C before examination at room temperature produces very frequent mal-orientation in *Melanoplus* spermatocytes, and the mal-oriented bivalents orient and reorient several times before achieving a stable, bipolar orientation. Unipolar orientations in cold treated cells are as clearly stabilized by artificial tension as the cells studied in this report, but the effect of tension is more striking because of the frequent reorientations immediately before and after the experiment (7).

We have identified spindle tension as the source of orientation stability, but is tension itself or some

secondary effect of tension the immediate cause of stability? One secondary effect is altered position on the spindle: bivalents in unipolar orientation come to lie close to a pole, and either natural or artificial tension leads to a location nearer the equator. For instance, in Fig. 3 compare the distance from the pole in the presence of artificial tension (55 to 90 min) with that in the absence of tension (50 and 110 min). Position probably can be rejected as a significant influence, however. As argued earlier (18), polar position is at best an ancillary stimulus to reorientation. For the present (rather small) sample, reorientation is not more frequent near a pole than closer to the equator when computed on a minutes per reorientation basis (see Table III and associated text). The secondary effect of tension that cannot be eliminated as a possible cause of stability is its effect on the axial position of kinetochores. Thus tension forces the kinetochores into alignment with the pole-to-pole axis, with each kinetochore pointing directly at a pole, as long as tension is maintained and the spindle fibers are intact. The "direct induction of reorientation" experiments (see Fig. 4) suggest that this might indeed preclude reorientation, for changed orientation is readily induced by forcing a kinetochore to face a different pole. Thus, we are left with the alternatives that tension may act through influencing kinetochore position and/or directly by increasing spindle fiber stability.

The exact effect of the *absence* of tension in unstable orientations is equally obscure and this reveals our ignorance of the reorientation process. Reorientation involves both the loss of old spindle attachments and the formation of new ones (e.g., 18, 3). For instance, we do not know whether loss and reformation must occur sequentially or whether some connections to one pole persist while connections to the other pole are forming. If the latter is the case, then constraints on kinetochore position may be very important, but if loss must precede reformation, then attachment stability itself must be most critical. Moreover, if spindle attachments are lacking, a kinetochore orients preferentially to the pole it most nearly "faces" (18), and this makes reorientation indeed mysterious since the reorientation from unipolar to bipolar orientation involves at least a partial turning of a kinetochore to face a new pole. Merely a higher probability of spindle fiber loss will not suffice to explain such a reorientation: we would

predict only repeated loss and reformation of spindle fiber connections to the same pole. We suggest that in addition to enhanced spindle fiber instability, the slight tilting of kinetochores with respect to the spindle axis is also involved. This might lead to some spindle connections to both poles, followed occasionally by motion toward the opposite pole, by increasing concentration of spindle connections toward that pole, and finally by stable bipolar orientation. The "direct induction" experiments are consistent with this suggestion, and the studies of Luykx (16) provide some ultrastructural evidence for bipolar microtubular arrays on single half-bivalents during early prometaphase. More decisive ultrastructural observations, on chromosomes in known stages of reorientation, currently are being obtained in collaboration with Dr. B. R. Brinkley.

Spindle tension may suffice as a general explanation of controlled chromosome reorientation. First, it is clearly applicable to ordinary chromosomes in mitosis. That reorientation occurs in mitosis is suggested by observations on "centrophilic" chromosomes of newt cells in tissue culture (2). Equal distribution of chromosomes in mitosis depends upon orientation of daughter kinetochores to opposite poles and the two daughter chromosomes are mechanically linked together until anaphase. Thus, all relevant mechanical properties are identical with those in meiotic bivalents, and, therefore, we postulate that stable orientation depends upon spindle tension produced following orientation to opposite poles. Reorientation will occur until this orientation is reached. Tension experiments have not been performed on mitotic chromosomes because the short distance between the kinetochores makes difficult both the

operations and the observation of orientation patterns.

Second, meiotic units other than bivalents may be considered. Unpaired chromosomes (univalents) which orient both chromatid kinetochores to the same pole would be expected to reorient frequently, and this is observed with the unpaired x and y chromosomes of several tipulid files (4, 1), and with the x chromosome of *Melanoplus* (17). The former show significantly a transition to stable orientation after orientation of chromatids to opposite poles. Similar behavior when chromosomes, which normally form bivalents, are unpaired can be inferred from studies on fixed cells (reviewed by John and Lewis, ref. 12, pp. 52 ff).

Many alternative explanations of orientation in single chromosomes in mitosis and meiosis are possible but no satisfactory explanation of meiotic multivalent orientation has yet been proposed. The major orientation patterns in such linked associations of three or more chromosomes have conventionally been designated "alternate" or "adjacent"; some examples are diagrammed in Fig. 5. In adjacent orientations, the kinetochores of two or more adjacent chromosomes are oriented to the same pole (e.g. Fig. 5 a and b), while in alternate orientations (Fig. 5 c) there is a strict alternation of one chromosome to one pole, the next to the opposite pole, and so on, producing a characteristic "zig-zag" appearance of the multivalent at metaphase. Without exception, where multivalents persist as part of the normal cytogenetic system, they show alternate orientation in a very high proportion of the metaphase I cells. Our major task is to account for this preferential orientation. The general argument is that adjacent orientation is similar to unipolar orientation in
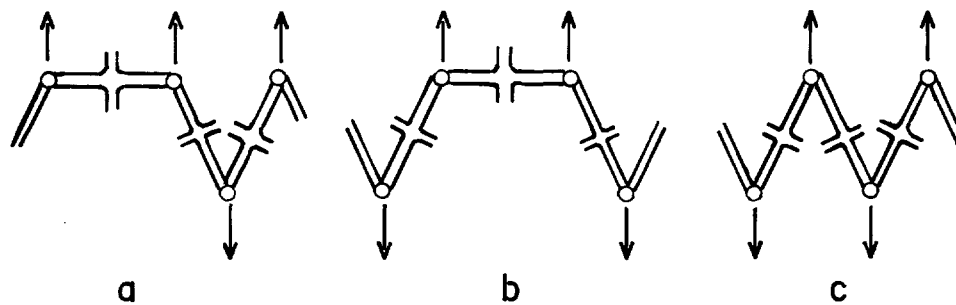

FIGURE 5 Three diagrams of orientation in multivalents, *a* and *b* showing adjacent, *c* showing alternate orientation. Kinetochores are represented by circles; the arrows indicate the direction of orientation. Chromosomes with median kinetochores and one chiasma per arm (as in *Oenothera* and *Rhoeo*) are represented in these examples. The diagrams may be regarded as representing either chains of four chromosomes or sections of still higher multivalents.

bivalents in reducing the tension toward opposite poles on chromosomes so oriented, while alternate orientation leads to uniform forces toward opposite poles on each chromosone in the multivalent, and, therefore, the alternate orientation is uniquely stable.

Trivalents of sex-chromosomes occur widely in animals, and in mantids it is known that their controlled segregation depends upon reorientation of L-shaped adjacent or linear orientations to V-shaped alternate orientations (10). Our tension experiments on bivalents mimic the V-configuration: the ends of the "V" are the kinetochores of the mal-oriented bivalent and the needle at the apex of the "V" assumes the role of the third chromosome's kinetochores (Dr. S. Alan Henderson first suggested this interpretation). The indefinite stability of this artificial trivalent is direct evidence for a role of spindle tension in natural trivalents. We have also attempted to imitate trivalents in adjacent orientation by applying the needle near the middle of a properly oriented bivalent and moving the needle toward a pole (cell 6, direct induction experiments, see p. 46). The prediction is specific: the half-bivalent oriented to that pole should reorient. This is observed, although reorientation to produce a stable V-shaped artificial trivalent has not been produced unless the needle is moved as far as shown in Fig. 4. Thus so far we have reproduced only one possible component of adjacent-trivalent instability which in natural trivalents would be an effect of variable forces at the middle chromosome's kinetochores on the *position* of the kinetochores of the chromosome oriented to the same pole.

Still higher multivalents are part of the normal cytogenetic system in such plant genera as *Oenothera* and *Rhoeo* (reviewed in ref. 12) where chains or rings of up to 14 chromosomes regularly show alternate orientation. Equally regular alternate orientation occurs in the recently discovered sex quadrivalent in the beetle *Cyrsylus volkameriae* (22). The stability of alternate orientation (Fig. 5 c) and the instability of most adjacent orientations in higher multivalents are interpretable as effects of tension or its absence. For instance, if three interstitial chromosomes are oriented to the same pole, the middle chromosome is not subjected to bipolar forces and its reorientation is expected. Similarly, if two chromosomes at one end of a chain multivalent are oriented to the same pole (Fig. 5 a), the reorientation of the end chromosome is predicted. However, one class of adjacent orientations can

not be interpreted at present: adjacent orientation of two interstitial chromosomes, as in Fig. 5 b, appears to produce the force distribution seen in alternate orientation (Fig. 5 c). Quadrivalents or higher multivalents have not yet been studied in living cells; only such studies will permit a final decision on the applicability of the tension hypothesis to multivalent orientation.

The discussion of multivalent orientation can be summarized as follows: we have experimental evidence suggesting a role of tension in trivalents, and many, but not all, other configurations can be understood. An important feature of the interpretation is that no very special features of chromosomes or spindles are invoked to explain regular alternate orientation. Thus, ordinary bivalents in tension experiments behave as if they were part of a trivalent. In nature, newly arisen multivalents frequently show preferential alternate orientation (e.g. 15, 8, 20) as expected from our interpretation. Obviously, however, certain types of chromosome morphology, chiasma distribution, and general reorientation frequency are more or less conducive to regular attainment of alternate orientation. Therefore, it is not surprising that alternate orientation is not universal in multivalents of recent origin (for examples, see especially refs. 9, 1, and the review of John and Lewis, ref. 12), nor is it surprising that selection for alternate orientation is possible, as shown by studies of Thompson (21) and Lawrence (13, 14) on rye. However, it remains to be seen whether or not the tension hypothesis is adequate for the detailed explanation of these situations.

We close by stressing the experimentally verified role of spindle tension in the orientation of bivalents in meiosis and the easy extension of this explanation to mitotic chromosomes. However, the important details may be resolved, we already understand the general cytological basis of controlled reorientation and hence of controlled chromosome distribution. This cytological understanding is the necessary basis for progressing to an explanation of chromosome distribution in molecular terms. The molecular interpretation of tension and orientation stability may seem so mysterious that a possible route to understanding is worth outlining. Micromanipulation clearly shows that each chromosome is individually associated with the spindle, probably by direct connections between its chromosomal spindle fibers and those of the rest of the spindle (19). These connections must produce, or at least transmit, the forces that

move chromosomes. Now in skeletal muscle, mechanical properties vary with functional status: active force production is associated with a resistance to stretch not present in the relaxed state. There is increasingly good reason to think that the resistance to stretch results from formation and/or altered properties of bridges between actin and myosin filaments (reviewed by Huxley and Hanson, ref. 11). No certain analogy between spindle and muscle is, or need be, suggested. Here, muscle merely provides a well-studied example of an association between force production and

mechanical properties that could be related to the stability of the system. This suggests a possible molecular interpretation of one aspect of spindle tension: its direct effect on spindle stability may arise because tension is produced or transmitted by linkages which also determine the structural integrity, the stability, of spindle units.

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