Elements of Error Correction in Mitosis: Microtubule Capture, Release, and Tension
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Abstract. The correction of certain errors in mitosis requires capture and release: new kinetochore microtubules must be captured and old, misdirected ones must be released. We studied capture and release in living grasshopper spermatocytes. Capture is remarkably efficient over a broad range in the angle at which a microtubule encounters a kinetochore. However, capture is inefficient when kinetochores point directly away from the source of properly directed microtubules. Capture in that situation is required for correction of the most common error; microtubule–kinetochore encounters are improbable and capture occurs only once every 8 min, on average.

Release from the improper attachment caused by misdirected microtubules allows kinetochore movement and the completion of error correction. We tugged on kinetochores with a micromanipulation needle and found they are free to move less than one time in two. Thus error correction depends on two improbable events, capture and release, and they must happen by chance to coincide. In spermatocytes this will occur only once every 18 min, on average, but a leisurely cell cycle provides ample time.

Capture and release generate only change, not perfection. Tension from mitotic forces brings change to a halt by stabilizing the one correct attachment of chromosomes to the spindle. We show that tension directly affects stability, rather than merely constraining kinetochore position. This implies that chromosomes are attached to the spindle by tension-sensitive linkers whose stability is necessary for proper chromosome distribution but whose loss is necessary for the correction of errors.

Accurate chromosome segregation in mitosis and meiosis begins with chance encounters. Microtubules growing from a spindle pole may happen to encounter a chromosome's kinetochore and be captured by it. The capture of microtubules by kinetochores has been convincingly demonstrated by correlating chromosome movement in living cells with the presence of astral microtubules at the kinetochore, seen after fixation and immunostaining (Rieder and Alexander, 1990; Merdes and De Mey, 1990). Moreover, microtubule capture has been directly observed by using video-enhanced differential interference contrast (DIC) microscopy (Hayden et al., 1990), though only in one cell. The result of capture is the mechanical attachment of the chromosome to the spindle and the movement of the chromosome toward the pole from which the microtubule grew. If the kinetochore of the partner chromosome captures a microtubule from the opposite pole, all is well (Fig. 1 d), and the partners will move to opposite poles in anaphase. But reliance on chance makes errors inevitable (Nicklas, 1988).

Sources of Change
Microtubule capture and release are necessary for the changes in attachment that lead to error correction. One kinetochore or the other must capture a microtubule from the other spindle pole (Fig. 1 b) and that kinetochore must also be free to move (Fig. 1 c) — it must have been released from the old, improper connection. Otherwise, the mitotic motors will be unable to move the chromosome in the proper direction (toward the upper pole as drawn in Fig. 1) and error correction will not be completed. Release from the old, er-
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Figure 1. The elements of error correction. Kinetochores are depicted as black ovals, microtubules as thin lines, and spindle poles as black circles. (a) A pair of partner chromosomes (a bivalent) in meiosis is shown; both kinetochores are attached to the same pole. Left uncorrected, this would result in the segregation of both chromosomes to the same pole. (b) One kinetochore has captured a microtubule from the opposite pole. (c) That kinetochore was no longer attached to the original (lower) pole, and hence could be moved toward the opposite pole (arrow). (d) Mitotic forces toward opposite poles (arrows) stabilize the new, correct attachment to the spindle; it persists, and accurate chromosome segregation, one chromosome to each daughter cell, is the result. The values for the probability of capture, $P_c$, and of release, $P_r$, are considered in the Discussion. (e) The effect on the configuration in (a) of applying tension with a micromanipulation needle in the direction of the arrow. The configuration is stabilized and the kinetochores point at the pole.

rart connection has generally been assumed to pose no problem, e.g., "after capture, the motors associated with a single microtubule extending in the appropriate direction are sufficient to move the chromosome" (Nicklas, 1988). Recent, direct tests suggest otherwise, however: the old, improper connection may be unstable but often it tethers the kinetochore to the pole so that the kinetochore is not freely movable (Nicklas et al., 1993).

Our goal in this study was a quantitative appraisal of both capture and release. New, direct observations of microtubule capture by kinetochores in living cells are presented. Most encounters of microtubules with kinetochores in living cells pass unseen, however. We used poleward chromosome movement, a consequence of capture, to detect all or most capture events. The probability per unit time of microtubule capture by kinetochores in various positions was determined. We extended the earlier tests of release from an improper attachment and determined the probability that a kinetochore is free to move at any given time. The result is a quantitative picture of error correction in mitosis as a process requiring the chance coincidence of two unlikely events, capture and release.

The Source of Stability

Change must cease when the proper attachment is reached. It is tension that distinguishes the proper attachment (Nicklas and Koch, 1969). Connection of partner kinetochores to opposite poles leads to forces toward opposite poles (Fig. 1 d) and a stable attachment. Conversely, tension is absent in improper attachments (Fig. 1 a), and the instability that leads to error correction is the result. The identification of tension as the element that confers stability came from experiments in which unstable attachments such as those in Fig. 1 a were artificially stabilized by the tension that results when a micromanipulation needle pulls the chromosome toward the opposite pole (Fig. 1 e). The exact role of tension is ambiguous in these old experiments, however. Stability might come directly from the tension itself or indirectly, from an effect of tension on kinetochore position. In these experiments, the applied tension causes the kinetochores to point directly toward the pole to which they are both attached and directly away from the opposite pole (Fig. 1 e). Hence, the kinetochores are in a good position to capture microtubules from the nearby pole, but they are in a poor position to capture those from the opposite pole. Thus, position by itself favors stable attachment. The role of tension is equally ambiguous in error correction in normal, unmanipulated cells. Thus, in properly attached chromosomes, the normal forces that act toward opposite poles produce tension, but they also cause the kinetochores to point directly opposite poles (Fig. 1 d). So is it the tension itself or its effect on kinetochore position that is decisive?

We have now tested the effect of tension in a situation in which any effect on position can be distinguished from the effect of tension itself. We find that tension stabilizes attachments even when the position of the kinetochores favors instability. Evidently it is tension itself that confers stability, which has implications for the molecular biology of error correction.

Terminology

The old term "reorientation", meaning a change in the attachment of a kinetochore from one pole to another, is useful as a one-word designation for the whole process of error correction. We use "mitosis" in the generic sense, to refer to chromosome movement and distribution in both somatic-cell mitosis and in meiosis. The attachment error in question here (both partner chromosomes attached to the same spindle pole) occurs commonly in meiosis and is also seen in somatic mitosis (Ault and Rieder, 1992). A different error is probably more frequent in somatic mitosis (one partner is attached, the other is not). A comparison of errors and error correction in mitosis as contrasted with meiosis is in preparation.

Materials and Methods

Materials

Spermatocytes from laboratory colonies of the grasshoppers Melanoplus sanguinipes (Fabricius) and Chortophaga australior (Rehn and Hebard) were cultured as previously described (Nicklas et al., 1979) at a temperature of 22.5–25°C.
Figure 2. Microtubule capture and kinetochore attachment in a living cell. The time in minutes is given on each image. Shortly after nuclear envelope breakdown, microtubules (arrows) grew downward from a pole (out of sight) (0.0–3.2 min). One microtubule (or a very few) contacted a kinetochore (3.4 min, arrowhead), was captured, and the chromosome was hoisted upward on a changeable array of microtubules (3.5–6.9 min). More microtubules were captured (11.8 min) and the two kinetochores of the bivalent became attached by microtubules to opposite spindle poles (15.9 min). Video-enhanced polarization optics. Bar, 10 μm.

Video-enhanced Polarization Microscopy

Chortophaga spermatocytes are large and optically clear, making them favorable objects for observations of microtubules in living cells. These cells were observed by high extinction/high resolution polarization microscopy as described by Inoue (1986, 1988). An Ellis optical fiber light scrambler (Technical Video, Woods Hole, MA) was used to provide uniform, high-intensity illumination. Optical components (Nikon Inc., Melville, NY) selected for freedom from strain were used: a rectified achromatic-aplanatic condenser used at 1.3 NA and a 1.4 NA/60× plan apochromatic objective. Video images from a Newvicon camera (model 70, Dage-MTI; Michigan City, IN) were acquired and processed with an Image 1 system (Universal Imaging Corp., West Chester, PA). The images were stored as they were acquired, either on an optical disk recorder (model 3038; Panasonic Video Systems, Secaucus, NJ) or on a computer hard disk. Storage to the hard disk avoids the digital/analog and analog/digital conversions associated with storage on the optical disk recorder, conversions that degrade image quality somewhat. Later, the stored images were retrieved and processed: noise was reduced by averaging, haze was removed by unsharp masking, and contrast was enhanced.

Micromanipulation Experiments

Living Melanoplus spermatocytes were viewed by phase contrast microscopy. Micromanipulation was performed and chromosome movement was analyzed as previously described (Nicklas et al., 1979 and references therein), except that the results were recorded on an optical disk recorder (model 2021; Panasonic Video Systems, Secaucus, NJ) rather than on movie film.

We detached chromosomes from the spindle by pulling on them with a micromanipulation needle (e.g., Nicklas and Kubai, 1985). After detached chromosomes are released from the micromanipulation needle, they remain motionless for some time and then begin to move again. We used the beginning of renewed movement as a sign that a kinetochore had captured a microtubule, and recorded the time in minutes that elapsed before one of the two kinetochores in a chromosome moved. Sometimes only one of the two kinetochores is of interest, e.g., the upper kinetochore of a chromosome in a vertical position or the kinetochore facing the spindle of a chromosome placed far out in the cytoplasm (see Fig. 5). In these cases, the time until the kinetochore of interest moves obviously reflects events only at that one kinetochore, it is a "per kinetochore" time. The situation is a little less obvious when both kinetochores of a chromosome face in the same direction, as when the chromosome is bent into a U shape or is in a horizontal position (see Fig. 5). Here, the two kinetochores are equivalent, and so we recorded the time before movement for whichever kinetochore moved first. Naturally, the probability of microtubule capture at either one of two kinetochores is twice as great as for only one kinetochore. Consequently, when two kinetochores are watched, the time before capture and movement is only half as long, on average, as when only one kinetochore is of interest. Hence, the observed time before movement of either one of two kineto-
chores must be multiplied by two to put it on a "per kinetochore" basis, to permit comparison with the values for kinetochores in configurations in which only one kinetochore is watched.

Results

Capture

Watching Kinetochores Capture Microtubules in Living Cells. In the early stages of spindle formation, microtubule capture by kinetochores is sometimes visible by video-enhanced polarization microscopy, as in Fig. 2. One spindle pole lies right at the bottom of the figure while the other lies straight above, out of sight. Just after the dissolution of the nuclear envelope, microtubules, seen as thin, dark lines, invade the clear nuclear space from above (Fig. 2, arrows, 0.0 min image). These are probably single microtubules, not groups, since at this stage, only single microtubules are seen by electron microscopy (our unpublished observations). However, direct comparisons between polarization microscope and electron microscope images have not been made, so we only assume that these lines represent one or a very few microtubules. 3 min after the dissolution of the nuclear envelope, the growing microtubules reached the vicinity of a chromosome (Fig. 2, 3.2 min image) and contacted its kinetochore (arrowhead, 3.4 min). The chromosome was hoisted upward (Fig. 2, 3.4 to 6.9 min). The microtubule array was continually changing. This is particularly evident at 6.9 min, when the existing kinetochore microtubules (left arrow) were bent after encountering a microtubule lying at an odd angle (right arrow). Gradually, more microtubules were captured (11.8 min), and the two kinetochores became attached to opposite spindle poles by stable arrays of kinetochore microtubules (15.9 min image).

The kinetochore featured in Fig. 2 happened to face quite directly toward a pole (this becomes obvious from 3.8 min onward), so the whole surface of the kinetochore faced the source of growing microtubules. Kinetochores at other angles can also capture microtubules efficiently, as seen in Fig. 3. One chromosome lies at the periphery of the cell (Fig. 3,
Figure 4. Kinetochores twitch and sustained movement in a living cell. A chromosome was detached and bent into a U-shape with both kinetochores facing the same pole (0.0 min). The kinetochore on the right (arrows) made a short movement, a "twitch," toward the upper pole (0.0–3.0 min) and back toward the original pole (3.0–4.0 min). That kinetochore later made a sustained movement toward the upper pole (7.5–10.4 min). The kinetochore on the left (arrowheads) moved toward the lower pole (0.0–10.4 min). The original, improper attachment was rectified as the kinetochores became attached to opposite poles. Phase contrast optics. Bar, 10 μm.

The Geometry of Capture. We used chromosome movement as an assay for microtubule capture by kinetochores in a variety of positions. A bivalent was detached from the spindle by micromanipulation and placed as desired. Detached chromosomes initially lack kinetochore microtubules (Nicklas and Kubai, 1985)—they must start afresh by capturing new ones, just as unmanipulated chromosomes must do when they first meet the spindle. The advantage of using chromosomes detached by micromanipulation is that we can place them wherever we choose. The capture of new kinetochore microtubules was recognized by the sudden movement of a kinetochore toward a spindle pole. The onset of movement after detachment is invariably associated with the acquisition of a new kinetochore microtubule or two (Nicklas and Kubai, 1985). We designate these movements as “twitches” when they are brief, inconclusive movements toward one pole or the other, and as “sustained movements” when they continue and result in a definitive orientation of the kinetochore. Both sorts of movement are shown in Fig. 4. A bivalent was detached and then bent so that both kinetochores faced the same pole (Fig. 4, 0.0 min image). The kinetochore on the right (Fig. 4, arrows) first twitched toward the upper pole (0.0–3.0 min) and back toward the lower pole (3.0–4.0 min) before beginning a sustained movement toward the upper pole (7.5–10.4 min). Meanwhile, the left-hand kinetochore (Fig. 4, arrowheads) moved toward...
placed within the spindle such as this one, we scored only twitch and one sustained movement. For chromosomes movements toward the farther, upper pole as capture events: one to the lower pole

perpendicular to the spindle axis movements toward the farther pole. These are the events of
quires the capture of microtubules from and movement to-
the chromosome generally lies near one pole, and correction requires the capture of microtubules from and movement toward the farther pole.

Detached chromosomes were placed in a variety of positions (Fig. 5): near one pole with one kinetochore pointed straight toward the far pole (Vertical), with the kinetochores perpendicular to the spindle axis (Horizontal), or with both kinetochores pointed toward the pole (U-Shaped). For contrast with capture within the spindle, detached chromosomes were also placed in the cytoplasm, far from the spindle, with one kinetochore facing the spindle (Fig. 5, Far Out). We determined the elapsed time from release of the chromosome from the micromanipulation needle: (a) until its first twitch toward the farther pole; and (b) until sustained movement began. The values were calculated on a "per kinetochore" basis, the time in minutes before a kinetochore moved (details in Materials and Methods).

The number of experiments in each class varies. The number is large for the U-shaped class because older experiments (Nicklas et al., 1993) were repeated for confirmation. The data in the two samples proved to be statistically indistinguishable and hence they were pooled. The number is fairly large for the horizontal class because the first experiments suggested that capture occurred unexpectedly, so we did more experiments to be sure the first results were typical. They were.

Mean and median values for the onset of movement in detached chromosomes are given in Table I. We will use the means for present purposes, but the median values give the $t_{50}$, the time at which half the kinetochores have moved, which may be useful for kinetic analysis.

Microtubule capture by kinetochores is often swift (Table I). Detached bivalents placed either vertically or horizontally twitch toward the farther pole in under 2 min, and sustained movement begins in less 5 min (Table I). There is no significant difference between vertical and horizontal bivalents in the time required to capture microtubules (t-tests; the hypothesis of equal means is accepted, with $P = 0.78$ for the first twitch and $P = 0.52$ for sustained movement).

The kinetochores of U-shaped bivalents acquire microtubules toward the farther pole much more slowly than those of horizontal and vertical bivalents (Table I). For U-shaped bivalents, the mean time until the first twitch or sustained movement is four to five times greater than for horizontal or vertical ones. The differences are highly significant statistically (t-tests; the hypothesis of equal means is rejected, with $P = 10^{-4}$-$10^{-9}$).

A final comparison is between kinetochores within the spindle and kinetochores of bivalents placed in the cytoplasm outside the spindle (Fig. 5 and Table I, Far Out). The distance from the kinetochore facing the spindle to either spindle pole (21-44 $\mu$m) was at least as great as for bivalents placed within the spindle (21-32 $\mu$m, the distance from the kinetochore to the farther pole). The far out kinetochores captured microtubules even faster than those within the spindle and sustained movement began in a mean time of less than two minutes, more quickly than any others (t-tests of the hypothesis of equal means for far out versus vertical, horizontal, and U-shaped: $P = 0.02$, $10^{-4}$, and $10^{-10}$, respectively). Even more impressive is the median time: half of the far out kinetochores began sustained movement within 0.2 min after release from the micromanipulation needle, so fast that we did not attempt to determine if twitches can be discerned even sooner.

Microtubule capture by the kinetochores of horizontal bivalents has one remarkable feature: preferential capture of microtubules from the farther pole rather than the nearer one. In the example in Fig. 6, the left-hand kinetochore (arrows) began to move upward, toward the farther pole, only 1.5 min after the micromanipulation needle was removed (0.0-1.5 min images), and the right-hand kinetochore (arrowheads) soon followed (1.5-5.0 min). The left-hand kinetochore, the first to move upward, then reversed its course and moved toward the lower pole (5.0-7.5 min). By this devious route the proper orientation was established (33 min image), and the chromosomes segregated properly in anaphase (46 min). Bivalents placed horizontally on the spindle showed initial movement of both kinetochores to the farther pole in 69% of 26 experiments. The probable cause of this behavior is considered in the Discussion (see Fig. 10 and associated text).

The Frequency of Capture. For U-shaped bivalents, we measured the frequency of all capture events that result in

![Figure 5. Diagrams of the capture experiments. Chromosome with kinetochores depicted as black ovals are shown in the various positions in which capture was studied. The spindles are represented by pairs of curved lines.](jcb.rupress.org)
kinetochore movement toward the farther pole. That is, we counted not just the first twitch and sustained movement as in Table 1, but all the additional twitches as well. The capture rate is particularly interesting in U-shaped bivalents because this is the starting point for error correction (Fig. 1 a) and because capture is difficult since the kinetochores face away from the farther pole.

In 23 experiments, we counted 71 capture events (twitches plus sustained movements) in a total of 273 min, a frequency of 0.26 events/min. Since events occurring at either of a chromosome's two kinetochores were counted, the frequency for any one kinetochore is half as great, 0.13 events/min. The reciprocal of that frequency, 7.7 min/event, is the average time required for the capture of a microtubule emanating from the opposite pole. In other words, there is about one chance in eight that a capture resulting in detectable movement will occur in any given minute.

Release: Tether Tests

A successful change in chromosome attachment to the spindle requires the release of the old attachment as well as the capture of new kinetochore microtubules. The status of the old attachment can be directly tested by gently tugging on a kinetochore with a micromanipulation needle (Nicklas et al., 1993). The test was devised to determine the effects of a drug on chromosome behavior, and results for normal cells were mentioned only in passing and were not illustrated (Nicklas et al., 1993). U-shaped bivalents were made as usual by detaching a bivalent and bending it so that its kinetochores faced the same pole. Kinetochores quickly acquire microtubule attachments to the pole they face (Ault and Nicklas, 1989). Tension was applied toward the opposite pole for 3 min to stabilize the improper attachment and also to verify the connection of both kinetochores to the same pole. After tension was relieved, the chromosome could be treated with a drug that interferes with microtubule dynamics. If the drug caused a successful change in chromosome attachment, the kinetochore facing away from the drug was not tethered to the opposite pole and moved freely by the microtubule network. However, if the drug did not interfere with microtubule dynamics, the kinetochore facing away from the drug was tethered to the same pole and remained immobile.
After release from the micromanipulation needle, one kinetochore or the other eventually began a sustained movement toward the opposite pole. Clearly that kinetochore was no longer attached to the original pole, but what about the other kinetochore? We answered this question by a "tether test": a micromanipulation needle was inserted near the kinetochore and was moved so that the kinetochore was gently tugged toward the opposite pole. Sometimes the kinetochore was freely movable (Fig. 7). If that untethered kinetochore had happened to capture a microtubule at that time, the associated motors could have moved the kinetochore without hindrance. In contrast, however, sometimes the tug of the needle was resisted, and the kinetochore was pulled out (Fig. 8). The kinetochore was still tethered to the pole and presumably would not have been free to move if it had captured a microtubule from the opposite pole. When the tether test was performed after one kinetochore had moved, the other kinetochore was free to move less than half (47%) of the time (Table II).

We have now done more extensive tether tests to discover how an improper attachment changes with time. As earlier, a U-shaped configuration was established and stabilized by three minutes of tension directed toward the opposite pole. Tether tests were performed first on one kinetochore and then on the other, alternately. In one set of experiments, the tests came at 2-min intervals, so that any one kinetochore was tested every 4 min. Tether tests necessarily place the tested kinetochore under tension, if only briefly. When applied more or less continuously, tension stabilizes orientations and keeps kinetochores tethered to a pole (Nicklas and Koch, 1969). Hence we were concerned that testing every 4 min might enhance stability, thus biasing the results by increasing the probability that a kinetochore would be tethered. Therefore we performed a second set of experiments in which the tests came at four min intervals, so that any one kinetochore was tested every 8 min.

Testing a kinetochore every 4 min does indeed increase the fraction of tethered kinetochores (Table II). In the group tested every 4 min, 27% of kinetochores were free to move, but when 8 min elapsed between tests, 41% of the kinetochores were free; the chi-square probability that the 4 and 8 min groups are different is 0.96. To estimate the true fraction of tethered kinetochores, we ignored the results from the 4 min group and averaged the results of the other two data sets (kinetochores tested after one kinetochore moved and kinetochores tested every 8 min, weighted for the number of experiments in each group). On that basis, 43% of kinetochores are free to move at any one time.

Tests repeated every 4 or 8 min reveal that the proportion of tethered kinetochores does not change with the passage of time. It might be expected that the proportion of freely movable kinetochores would increase as time passes and the influence of the stabilizing tension wanes, but that is not the case (Table III). Statistically, tethering shows no correlation with time (the correlation coefficients are a meager 0.11 for

<table>
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<tr>
<th>Experimental set</th>
<th>No. of tests</th>
<th>No. of kinetochores</th>
<th>percent free</th>
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<td>15</td>
<td>15</td>
<td>47</td>
</tr>
<tr>
<td>Tested every 4 min†</td>
<td>49</td>
<td>13</td>
<td>27</td>
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<tr>
<td>Tested every 8 min†</td>
<td>41</td>
<td>17</td>
<td>41</td>
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*From Nicklas et al., 1993.
†A few kinetochores behaved very differently from the others; they remained tethered to a pole indefinitely. Based on Dixon’s statistical test for "outliers," the results for one kinetochore were removed from each data set: in the set tested every 4 min, a kinetochore that was not free after 55 min, and in the set tested every 8 min, a kinetochore that was not free after 60 min; both are outliers at a confidence level >99%.

<table>
<thead>
<tr>
<th>Time of test, minutes after release from tension</th>
<th>Tested every 4 min</th>
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<tr>
<td>Time of test, minutes after release from tension</td>
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<td>100</td>
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Figure 9. Tension stabilizes unstable attachments even when kinetochore position favors instability. A chromosome (double-headed arrow, 0.0 min) was manipulated to produce a U-shaped chromosome with both kinetochores attached to the same (lower) pole. Tension from a micromanipulation needle was applied so that the kinetochore closer to the far pole (21 min image, arrow) was under greater tension than its partner (arrowhead). (The chromosome was not appreciably stretched by the applied force because we wanted to mimic the low tension exerted by the normal mitotic forces; stretching is more obvious in the 40.4 min image.) The kinetochore under less tension lost its old attachment and moved upward (arrowheads, 21-67.9 min) while the kinetochore under greater tension remained stably attached to the lower pole (arrows, 21, 40.4, and 68.0 min images). The chromosomes segregated to opposite poles in anaphase (100 min). Phase contrast optics. Bar, 10 μm.

The effect of tension was tested in a situation in which kinetochore position does not favor stability. A bivalent was detached and bent into a U-shape with both kinetochores facing the same pole. After allowing 1.5 min for the kinetochores to attach to the pole, tension was applied not toward the opposite pole as in earlier experiments (Nicklas and Koch, 1969), but toward the cytoplasm, so that the kinetochores were 45–90° to the spindle axis (Fig. 9, 21 min image). Tension applied in the desired direction causes the spindle to rotate, which we prevented by holding the spindle in place with a second micromanipulation needle. Even so, it is difficult to apply enough force to keep both chromosome arms under tension continuously, with roughly equal tension applied to both kinetochores. For that reason, we did a second series of experiments in which the total applied force was less and most of the force acted on one of the two kinetochores, the one closer to the farther pole (Fig. 9, 21 min image, arrow). The kinetochore under greater tension usually is stable and remains attached to the nearer pole, while its partner, under less tension, is unstable, forms a new attachment to the farther pole and moves to it (Fig. 9).

These are the overall results: (a) Equal tension: seven experiments, 14 kinetochores under tension; in each, a bivalent was kept under tension for 30 min or until one kinetochore or the other reoriented. Only two reorientations (i.e., reattachment and sustained movement to the farther pole) occurred in a total time of 204 min under tension, and these occurred only after a long time, 29 and 29.5 min under tension; and (b) Unequal tension, greater tension on the kinetochore closer to the farther pole: nine experiments; in each, tension was maintained for 30 min or until the kinetochore under greater tension reoriented. Of the nine kinetochores under greater tension (closer to the farther pole), only one reoriented in 248 min under tension. Of the nine kinetochores under less tension (closer to the nearer pole), seven reoriented. Three reciprocal experiments, in which the tension was less on the kinetochore closer to the farther pole, confirmed that tension is necessary for stability: that kinetochore reoriented after 3, 10, or 13 min.

Note that these are imperfect experiments because the tension sometimes lapses and cannot be restored immediately.
For that reason, occasional reorientation of kinetochores "under tension" is not surprising.

For the 23 kinetochores under tension, three reorientations in a total of 452 min were observed, a rate of one reorientation every 151 min. This may be compared with the rate of one reorientation (sustained movement) every 20 min for U-shaped bivalents (Table I) in the absence of tension: tension reduced the reorientation frequency by a factor of 7.5 (151/20). Another computation of the effect of tension is given below.

**Discussion**

We studied the components of error correction—microtubule capture and release and the stabilizing effect of tension. These components will first be discussed separately, followed by an integrated view of their impact on error correction.

**Capture**

**Direct Observations of Capture and Kinetochore Movement as an Assay for Capture.** The capture of microtubules by kinetochores has been observed directly in living cells, but only three times (Hayden et al., 1990; this report, Figs. 1 and 2). The fundamental problem is the shallow depth of focus in high-resolution light microscopy, 0.2 μm for DIC and even less for polarization microscopy (Inoué, 1989). That thickness is only one-fifth the diameter of a grasshopper spermatocyte kinetochore, so at one focal level four out of five encounters of microtubules with kinetochores pass undetected. The few examples so far recorded in living cells are supported by more numerous studies in which a chromosome's behavior was followed in life and its microtubule associations were observed after fixation (Rieder and Alexander, 1990; Merdes and De Mey, 1990). Together, these observations provide some crucial information: (a) capture does occur and by inference is the usual way in which kinetochores acquire microtubules; (b) lateral as well as end-on capture of microtubules occurs; and (c) poleward chromosome movement is invariably associated with the acquisition of one or two kinetochore microtubules (Nicklas and Kubai, 1985; Rieder and Alexander, 1990; Hayden et al., 1990; Merdes and De Mey, 1990; Alexander and Rieder, 1991).

We used poleward kinetochore movement as an assay for capture, an assay that works in the microtubule-dense spindle where direct observations are hopeless. As just noted (point c above), moving kinetochores invariably have acquired kinetochore microtubules. Hence, movement faithfully signals capture events. It is possible, however, that some captures do not result in detectible movement; the attachment may be transitory or the motors may be disengaged. Therefore, our measurements of capture rates from the movement assay may be underestimates, but are reliable as minimum estimates. Clearly, the assay is a measure of the functionally significant capture events, those that can lead to the movement essential for error correction.

**Capture, Geometry and Chromosome Structure.** Detached chromosomes placed in a vertical position (Fig. 5) have one kinetochore that is far from the opposite pole but faces directly toward it. Despite the distance from the source of microtubules, such kinetochores capture microtubules quickly, and show a twitch toward the opposite pole in 1.6 min on average (Table I). Those kinetochores are optimally positioned to capture microtubules end-on, which is the final, stable kinetochore/microtubule arrangement always seen by metaphase (reviewed by Rieder, 1982). Thus, the time of 1.6 min for these kinetochores reflects the efficiency of end-on capture and the density of microtubules from the farther pole. The kinetochores of horizontal bivalents (Fig. 5) lie in the same spindle region and see a similar density of microtubules, but the kinetochores are 90° to the spindle axis. They can capture microtubules only by a lateral interaction of kinetochore and microtubule, not an end-on interaction. Remarkably, they capture microtubules just as quickly as kinetochores that directly face the microtubule supply, as judged by the time of the first twitch as well as the onset of sustained movement (Table I). Lateral interactions between kinetochores and microtubules have been seen before, both in vivo (Nicklas et al., 1979; Nicklas and Kubai, 1985; Rieder and Alexander, 1990) and in vitro (Mitchison and Kirschner, 1985; Hyman and Mitchison, 1991). What we can contribute is the relative efficiency of the process: lateral capture is just as efficient as end-on capture.

Incidentally, chromosomes placed in the cytoplasm, a long way from either spindle pole (Fig. 5, Far Out), begin movement at least as quickly as chromosomes placed at an equal distance from a pole, but within the spindle (Table I). Presumably the concentration of microtubules is as great out in the cytoplasm as is the concentration of equally long microtubules within the spindle. U-shaped bivalents (Fig. 5) have both kinetochores attached to the same pole, the most common attachment error in meiosis. Correction requires the capture of microtubules from the farther pole. Since the kinetochores face more or less directly away from that pole, it is not surprising that capture in this situation is infrequent compared with capture by the kinetochores in other positions: 8 min passes before the first twitch compared to less than 2 min for the others. Evidently, the sluggish pace of capture is due to the improbability of any encounter between a microtubule from the opposite pole and the active surface of the kinetochore. On reflection, it is remarkable that the required encounters ever occur. We have considered the possibility that capture occurs only after the attachment of a U-shaped bivalent relaxes a bit, so that its kinetochores no longer face directly toward the nearer pole. Then the kinetochores are in a somewhat more favorable position to encounter microtubules from the opposite pole. Sometimes this happens, but capture often occurs, and movement toward the opposite pole begins, when a kinetochore faces directly away from that pole, as in Fig. 4, 0 to 3 min. The flexibility of long microtubules may well be a key to the occurrence of such events. Bending may bring a microtubule into contact with an otherwise inaccessible kinetochore. Another key is that even a single event can suffice; capture of a single microtubule can lead to the movement associated with error correction (Nicklas and Kubai, 1985).

Capture occurs over a large range in the angle at which a microtubule encounters a kinetochore. Presumably the molecular nature of the kinetochore/microtubule interaction sets some ultimate limit on this range. At least extreme angles, the chromatin surrounding the kinetochore may act as a shield that determines which microtubules have access to
the capture surface. This seems likely from instances in which the kinetochore protrudes more than is usual from the chromat in (Church and Lin, 1982). Such kinetochores often attach to microtubules from both spindle poles, which is uncommon otherwise. Shielding chromat in is the apparent reason for the sluggish capture of microtubules by U-shaped bivalents: absent a shield, the sides of the kinetochore would be exposed to microtubules and capture should follow quickly. A more striking indicator of how effective chromat in can be as a shield is the behavior of horizontal bivalents. Though close to one pole (Fig. 5), with its very numerous microtubules, horizontal bivalents quickly capture the relatively sparse long microtubules from the opposite pole. In fact, 69% of the time, both kinetochores move first to that pole. This is understandable once the geometry of the situation is considered (Fig. 10). Microtubules from the farther pole can directly contact the kinetochore, while those from the nearer pole cannot, because a chromat in cup partly encloses and shields the kinetochore.

Correcting errors is vital and correction depends on capturing microtubules from the pole behind the kinetochores in a U-shaped bivalent. Why, then, is the kinetochore shielded at all—why is there any impediment to capture? The answer is that greater exposure of the kinetochore leads to a higher frequency of initial errors, e.g., in the protruding kinetochores just mentioned that become attached to both spindle poles (Church and Lin, 1982). Thus, the cell must strike a balance between exposure and shielding that minimizes initial errors and yet permits the correction of those errors that do occur.

**Release from the Tether**

After a new kinetochore microtubule from the proper pole has been acquired, sustained movement toward that pole can occur only if the old, improper attachment has lapsed. Our direct “tether tests” show that, more often than not, a kinetochore is tethered to the original pole. On reflection this is not so surprising. Even though the old connections may be unstable because tension is absent, any that are lost are likely to be quickly replaced by new connections to microtubules from the pole the kinetochores face. The improperly attached kinetochores face a rich supply of microtubules; capturing them maintains the (improper) status quo. It might be expected that an improper attachment would become increasingly likely to lapse as time goes by, i.e., with greater time since stabilizing tension was present. We find, however, that the probability that a kinetochore is free to move does not increase with time. This leads us to view the release from improper attachments as a stochastic process. A group of kinetochore microtubules tethers a kinetochore to a pole; in the absence of tension, individual microtubules in the group come loose at random times. Occasionally, by chance, all the microtubules in the group will happen to be loose, and only then will the kinetochore be free to move. Even when chromosomes are properly attached, kinetochore microtubules are slowly lost and replaced during prometaphase (Gorbsky and Borisy, 1989; Cassimeris et al., 1990; Wise et al., 1991).

**Putting Capture and Release Together: the Overall Probability of Reorientation**

The capture data by themselves reveal that failure in error correction is more frequent than success. For U-shaped bivalents, one detectable capture event occurs every 7.7 min but usually results only in a transitory twitch toward the opposite pole. Sustained movement begins after 19.8 min, on average (Table I). Hence, on average, there are 2.6 capture events (19.8/7.7) for each instance of the sustained movement that heralds the correction of an error. Unyielding attachment to the original pole accounts for most of the aborted capture events, as revealed by the following consideration of the coincidence of capture and release.

Error correction occurs when capture and release happen by chance to coincide. Our data provide values for the capture and release probabilities, $p_c$ and $p_r$ (Fig. 1); $p_c$ is the observed frequency of capture events in U-shaped bivalents, 0.13 events/min, and $p_r$ is the observed fraction of the time that a kinetochore is free to move, 0.43. The probability that capture and release will coincide is the product of the individual probabilities, a meager 0.056/min. The reciprocals of the probabilities are easier to appreciate: in a one minute interval, a kinetochore has one chance in 18 (1/0.056) of capturing a microtubule, and one capture in 2.3 (1/0.43) will happen to occur when the kinetochore is free to move. Hence the overall probability that a kinetochore will both capture a microtubule and be free to move in a given minute is one chance in 18 (8 x 2.3). Thus, these data predict that reorientation, the correction of an error, will require 18 min, on average. This is within 10% of the value actually observed for the mean time before reorientation: 19.8 minutes (Table I, sustained movement). Such close agreement is gratifying considering the many sources of noise and error.
We conclude that capture and release are the major variables that determine the time required for the correction of misdirected attachments in mitosis. Dependence on chance means that correction takes some time. The values in this report are for a single kinetochore, and the reorientation of either of the two kinetochores in an improperly attached bivalent suffices to correct the error. Hence the average time required is half of 19.8 min, or about 10 min. Not surprisingly, ample time is provided for error correction: 10 min is a small fraction of the 4–6 h from spindle formation until anaphase in grasshopper spermatocytes.

**Tension and Stability**

We set out to settle definitively whether the effect of tension on orientational stability is due to the tension itself or to the kinetochore position that tension dictates. Kinetochores were placed under tension but in a position that does not favor stability. The kinetochores lay at an angle that allows the capture of microtubules from either pole. We found that such kinetochores do reorient, but only rarely. Especially telling are experiments in which the two kinetochores of one bivalent were under unequal tension yet lay at about the same angle to the spindle. In the example in Fig. 9 (21 min image), reorientation requires the capture of microtubules from the upper pole. One kinetochore (Fig. 9, arrow, 27 min) is closer to that pole and hence is closer to the supply of the microtubules needed for reorientation. To make the experiment more conclusive, that kinetochore was put under greater tension than its partner. Though in a more favorable position to capture microtubules from the upper pole, the kinetochore under tension reoriented in only one trial out of nine. In contrast, the kinetochore under less tension (Fig. 9, arrowhead, 21–40 min), though farther from the source of microtubules, reoriented in seven out of nine experiments. In these experiments, it is clearly the effect of tension itself rather than kinetochore position that affects stability.

The quantitative impact of tension is revealed by the capture and tether experiments. For a kinetochore at 45–90° to the spindle axis, the capture of a microtubule from the opposite pole would be expected roughly once every 5 min (Table 1; 5 min is the average of the “first twitch” time for horizontal bivalents and U-shaped bivalents). If the kinetochore were free to move 43% of the time, as are chromosomes that are not under tension, we would expect reorientation in 12 min on average (5 min/0.43). In fact, we found only one reorientation in a total of 248 min. Thus, tension enhanced the stability by a factor of 21 times (we might expect to see 248/12 = 21 reorientations but only one was seen).

So now we know that tension itself increases stability. But how? What does tension affect and how does it affect it? Our new view of error correction is that capture and release must occur simultaneously, and even in the absence of tension, both are improbable events. Hence if tension made either capture or release only a bit less probable it would effectively prevent reorientation.

Consider capture first. Tension might inhibit capture by enhancing the stability of existing kinetochore microtubules. For instance, tension might prevent the detachment of stabilizing caps from the ends of kinetochore microtubules. In that case, when a kinetochore is under tension, all available sites for microtubule capture might already be occupied by stabilized kinetochore microtubules and the kinetochore would therefore be unable to capture new microtubules.

While such an effect of tension on capture is possible, an effect on release is certain. To see this, we need only look at the experiments: kinetochores under tension obviously remain tethered to the poles. The very fact that tension can be applied reveals a sufficiently firm anchorage to preclude movement toward the opposite pole. By design, we pull on these kinetochores with as much force as the mitotic motors can muster (judged from the extent of chromosome stretching) and yet the kinetochores do not move. An additional, clinching point is that the effect of tension on anchorage is directly demonstrated in the tether tests. The test itself, a brief tug toward the opposite pole, causes tension. Even when that brief tension comes as infrequently as once every four minutes, it substantially reduces the probability that a kinetochore will be free to move—from about 43 to 27% of the time.

We conclude that tension prevents reorientation by stabilizing the anchorage of chromosomes to the spindle, making them immovable by the mitotic motors even if microtubules from the opposite pole are captured. Tension might stabilize anchorage by stabilizing the connections of kinetochore microtubules either at the kinetochore or at the pole by by stabilizing the microtubules themselves. The best bet is that tension affects either the kinetochoric or the polar connection. Some evidence favoring a tension-sensitive polar anchorage comes from correlated living cell/electron microscopic studies of reorientation (Nicklas and Kubai, 1985; Ault and Nicklas, 1989). When chromosomes are fixed soon after reorientation begins, one or a few kinetochore microtubules extending toward the pole toward which the kinetochore is moving are invariably seen. In addition, kinetochore microtubules are found that extend generally toward the original pole but do not point directly at it, as depicted in Fig. 1 c. They appear to be the microtubules that formerly anchored the kinetochore to the pole but which lost their polar anchorage and were easily shifted along with the kinetochore as it moved toward the opposite pole. At the time these observations were made, unstable polar anchorage was not a fashionable proposition. The prevalent idea was that polar microtubules remain attached to their nucleation site in the centrosome, and their persistence or loss depends on whether or not the other end is stabilized (e.g., by attachment to a kinetochore). Now, however, the situation is different. Microtubules grown from centrosomes in *Xenopus* egg cytoplasm have been seen to detach and move away (Belmont et al., 1990), and there is evidence, though less direct, for the release of microtubules from the centrosome in nerve cells (Yu et al., 1993) and in cold-treated fish scale cells (McCleath and Fujiwara, 1990). Also, a flux of microtubule subunits from kinetochore to pole during mitosis implies dynamic, not static, associations at both ends of the microtubule (Mitchison, 1989; Sawin and Mitchison, 1991; Mitchison and Salmon, 1992). A candidate for the dynamic linkage at the pole is the motor protein, Eg5, which could both drive the flux and maintain the polar attachment of microtubules (Sawin et al., 1992). All that need be postulated is that the activity of this molecule or some close associate is sensitive to tension. When the motors are working or when a micro-manipulation needle is pulling, tension is present and the microtubules are connected via the active motors. In the ab-
sence of tension, the motor/microtubule connection is lost, and the microtubule is no longer tethered to the pole. A similar proposal could be made for a tension-sensitive anchorage at the kinetochore. Whether at the pole or at the kinetochore, such molecular anchors make normal chromosome segregation possible but prevent the correction of errors unless they are lost. Adding to the interest in the search for tension-sensitive anchors is the prospect that tension-sensitive proteins regulate motors and/or microtubule assembly at kinetochoric end of kinetochore microtubules (Skibbens et al., 1993; Murray and Mitchison, 1994; Rieder and Salmon, 1994).

In conclusion, error correction is a chancy process, which depends on microtubule capture and release. Our measurements show that capture and release are improbable events. This is probably no accident because capture and release are agents of change, even chaotic change. They are sources of errors as well as of error correction. Tension is the source of order in this world of chance. Tension generated by the agents of change, even chaotic change. They are sources of order in this world of chance. Tension generated by the agents of change, even chaotic change. They are sources of order in this world of chance. Tension generated by the agents of change, even chaotic change. They are sources of order in this world of chance.

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