Directional Instability of Kinetochore Motility during Chromosome Congression and Segregation in Mitotic Newt Lung Cells: A Push-Pull Mechanism

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Abstract. Most models of mitotic congression and segregation assume that only poleward pulling forces occur at kinetochores. However, there are reports for several different cell types that both mono-oriented and bi-oriented chromosomes oscillate toward and away from the pole throughout mitosis. We used new methods of high resolution video microscopy and computer-assisted tracking techniques to measure the positions over time of individual kinetochores with respect to their poles during mitosis in living newt lung cells. The results show that kinetochores oscillate throughout mitosis when they are tethered to spindle poles by attachment to the plus-ends of kinetochore microtubules (kMTs). Oscillations were not sinusoidal. Instead, kinetochores abruptly (as quick as 6 s or less) switched between persistent (~1.5 min average duration) phases of poleward (P) and away from the pole (AP) movement. This kinetochore "directional instability" was a property of motility at the plus-ends of kMTs since fluorescent marks on the lattice of kMTs have previously been observed to exhibit only relatively slow P movement. Each P and AP phase consisted of one or a few constant velocity domains (~1.7 μm/min average velocity). Velocities of P and AP phases were similar from prometaphase through mid-anaphase. Kinetochores occasionally switched to an indeterminant (N) phase of no or confused motion, which was usually brief compared to the durations of P and AP phases. Net chromosome displacements that occurred during congression to the equator or poleward movement during anaphase were primarily generated by differences in the durations and not the velocities of P and AP movements. Careful analysis of centromere deformation showed that kinetochore P movement produced pulling forces while kinetochore AP movement produced pushing forces. These data show that kinetochore directional instability is fundamental to the processes of chromosome congression and segregation. We argue that tension at the kinetochore attachment site is a key factor which controls the switching between P and AP phases of kinetochore motion.

KINETOCHORES in association with kinetochore microtubules (kMTs)[1] are responsible for the attachment of chromosomes to spindle poles, chromosome congression to the spindle equator during metaphase, and segregation to the spindle poles during anaphase in mitotic animal cells (for review see Salmon, 1989*b*; Rieder, 1991; McIntosh and Pfarr, 1991). Kinetochores become tethered to polar microtubules (MTs) by capturing and stabilizing the dynamically instable MT plus-ends (Pickett-Heaps et al., 1982; Rieder, 1982; Hayden et al., 1990; Merdes and De Mey, 1990; Rieder, 1990). The number of kMT attachment sites is genetically determined with sister kinetochores having a similar number of attachment sites (Rieder, 1990). Many, if not all, kMTs extend from their nucleation sites at the spindle pole to the kinetochore where they embed end-on (Rieder, 1981; Witt et al., 1981; MacDonald et al., 1992). In early prometaphase, a chromosome may associate to the lateral face of a polar MT and rapidly glide toward the spindle pole (Pickett-Heaps et al., 1982; Rieder et al., 1986; Merdes and De Mey, 1990; Rieder et al., 1990; Alexander and Rieder, 1991). Typically, however, chromosomes become "mono-oriented" and move poleward when polar MTs associate end-on with one kinetochore. The distal kinetochore of a mono-oriented chromosome faces away from the pole and has no MTs associated with it (Rieder et al., 1986). When the distal kinetochore captures MTs nucleated from the opposite pole, the chromosome becomes "bi-oriented" and it congresses toward the spindle equator. At the onset of anaphase, sister chromatids split and move poleward as their kMTs shorten.

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1. *Abbreviations used in this paper*: AP, away from pole; DIC, differential interference contrast; kMTs, kinetochore microtubules; MTs, microtubules; P, poleward; SFM, Single Frame Movement.

Recent evidence shows that in vertebrate cells most kinetochore movement is tightly coupled to the plus-end assembly dynamics of kMTs (Mitchison and Kirschner, 1985; Mitchison, 1988; Salmon, 1989*b*; Wise et al., 1991; Cassimeris and Salmon, 1991; Mitchison and Salmon, 1992). Most kinetochore poleward (P) movement coincides with kMT plus-end depolymerization while all away from the pole (AP) movement coincides with kMT plus-end polymerization (Mitchison, 1989; Mitchison and Salmon, 1992). Force production for kinetochore motility may be produced by MT motors (Rieder et al., 1990; Steuer et al., 1990; Pfarr et al., 1990; Hyman and Mitchison, 1991; Hyman et al., 1992; Yen et al., 1992) and/or driven by the energetics of plus-end assembly dynamics (Inoué and Sato, 1967; Koshland et al., 1988; Hotani and Miyamoto, 1990; Coue et al., 1991; Lombillo, V. A., R. J. Stewart, L. S. B. Goldstein, and J. R. McIntosh. 1992. *Mol. Biol. Cell.* 3:166a). The movement of kMTs with respect to their poles (poleward flux) appears to be only a minor contributor to kinetochore movement during mitosis in animal cells (Nicklas, 1989; Gorbsky et al., 1987; Mitchison and Sawin, 1990; Mitchison and Salmon, 1992).

Traditionally, chromosome congression to the spindle equator has been thought to be produced by a force balance mechanism involving a tug-of-war between sister kinetochore pulling forces, where pulling force increases with distance from the pole as originally proposed by Ostergren (Ostergren, 1950; Hays et al., 1982; Hays and Salmon, 1990). With the recognition that poleward force production is mainly generated at kinetochores and not along the length of kinetochore fibers (Mitchison et al., 1986; Gorbsky et al., 1987; Nicklas, 1989; Wise et al., 1991; Mitchison and Sawin, 1990; Mitchison and Salmon, 1992), two classes of congression models have emerged in recent years. One model (Kinetochore Motor-Polar Ejection model) proposes that the strength of the kinetochore pulling force is independent of kinetochore-to-pole distance and that the polar MT arrays associated with each half-spindle push or "eject" chromosome arms away from the poles. This ejection force is postulated to be dependent on MT density, both of which increase closer to the poles (Rieder et al., 1986; Salmon, 1989*a*,*b*; Rieder, 1990; Theurkauf and Hawley, 1992; Leslie, 1992). The second model envisions a "smart" kinetochore which is capable of producing both P pulling as well as AP pushing forces (Mitchison, 1989). Smart kinetochores are proposed to be capable of sensing their positions within the spindle, congressing to the equator upon becoming bi-oriented and moving to the poles after sister chromosome separation (Mitchison, 1988, 1989).

It is well established that chromosomes are pulled poleward only at their kinetochores, but there is only limited evidence that kinetochores may be capable of pushing the chromosome away from the pole. Hyman and Mitchison (1991) reported that kinetochores in vitro produce MT gliding over their surfaces toward the MT minus-end (P direction) when unphosphorylated and MT gliding toward the MT plus-end (AP direction) when the kinetochore is phosphorylated. In a wide variety of living cells, both mono-oriented and bi-oriented chromosomes have been shown to exhibit low amplitude oscillations, continuously moving toward and away from the spindle poles in both meiosis and mitosis (Seto et al., 1969; Molé-Bajer et al., 1975; Roos, 1976; Tippit et al., 1980; Bajer, 1982; Pickett-Heaps et al., 1982; Rieder et al., 1986; Fuge, 1987, 1989; Wise et al., 1991). According to the Kinetochore Motor-Polar Ejection model, these chromosome oscillations could be produced by changes in either the strength of kinetochore pulling forces or the strength of the ejection forces exerted on the chromosome arms. An alternative model was proposed by Bajer (1982) based on his observations of the oscillations of mono-oriented chromosomes in newt lung cells. He suggested that kinetochores were capable of both pulling and pushing on the centromere. However, the effects of kinetochore pushing were difficult to observe in Bajer's pioneering data because of resolution limitations in the instrumentation available and because, in most examples, the chromosome arms were ejected away from the pole at the same rate as the AP motion of the centromere.

We have applied new high resolution video microscopy methods and semiautomatic tracking techniques to investigate the motion of individual kinetochores and the deformation of centromeres for both mono- and bi-oriented chromosomes during the various stages of mitotic chromosome movement in newt lung cells. We were able to obtain clear images of kinetochores, kMT fibers, and centrosomes within the same field of view and to track changes in the distance between kinetochores and centrosomes at high temporal and spatial resolution (2 s and 50–200 nm). Newt lung cells provide an excellent model system for this study because the typical pole-to-pole distance in mitosis (40–50 μm) requires extended kinetochore excursions during congression to the metaphase plate and segregation to the poles during anaphase (Rieder and Hard, 1990). In addition, newt lung cells do not round up during mitosis, but remain flat and optically clear. Finally, previous studies using newt lung cells have allowed us to differentiate kinetochore movement at kMT plus-ends from kinetochore movement which resulted from the poleward flux of kMTs (Mitchison and Salmon, 1992). The data showed that tethered kinetochores on both mono- and bi-oriented chromosomes abruptly switch between constant velocity phases of P and AP movement at kMT plus-ends, motility we call "kinetochore directional instability." This term was derived from both the dynamic instability of plus-end MT assembly (Mitchison and Kirschner, 1984; Horio and Hotani, 1986; Walker et al., 1988*b*) and the directional instability exhibited for MTs on surfaces coated with both dynein (minus-end directed) and kinesin (plus-end directed) (Vale et al., 1993). Analysis of centromere deformation during P and AP motions showed that kinetochores produce pulling forces on the chromosome during P motility and pushing forces during AP motility. These results provide support for smart kinetochore motor mechanisms and are used to develop a new view concerning the mechanism of chromosome congression and segregation.

Materials and Methods

Tissue Culture

Newt lung cultures were prepared as previously described (Rieder et al., 1986; Rieder and Hard, 1990). Briefly, Oregon newts (Taricha granulosa) were obtained from Charles Sullivan (Nashville, TN). Using sterile technique, newt lungs were dissected from the animal, minced, washed three times in calcium-free/magnesium-free Hanks Ringer solution for amphibians, trypsinized for 5 min, and then placed in L-15 medium supplemented with FBS and antibiotics. Lung tissue explants were cultured overnight, placed in Rose chambers, and incubated 10–15 d at room temperature

(22–26°C). Before observation, the chambers were disassembled and the coverslips inverted onto glass slides with Scotch double-stick tape spacers. Fresh medium was added and the slide-coverslip chamber was then sealed with VALAP (1:1:1 of vaseline, lanolin, and paraffin).

Microscopy

Differential interference contrast (DIC) images were obtained using either Nikon 60×/1.4 NA or 100×/1.4 NA Planapochromat objectives and matching 1.4 NA condensers and Wollaston prisms. A Nikon FXA stand, Nikon 1.4 NA DIC condenser and DIC prisms, and a 100 W Quartz Halogen illuminator were used for low magnification images in which the whole spindle was contained in the video image. A 100 W Hg illuminator and an Ellis fiber optic scrambler (Ellis, G. W. 1985. *J. Cell Biol.* 101:83a) were used to generate the brighter light intensities needed at the higher magnifications required to see individual kinetochores within the chromosome centromere regions. Our highest resolution images were obtained using either a very stable optical bench microscope (Walker et al., 1990) or a Zeiss Universal stand on a vibration isolation table, a Nikon 100×/1.4 NA Planapochromat objective, a Zeiss 1.4 NA condenser, and Zeiss DIC Wollaston prisms ("Zikon optics"). All the microscopes we used in this study were equipped with a Zeiss heat-reflecting filter, a Zeiss heat-cut filter, and an Omega (Omega Optics, Burlington, VT) green interference filter (540-nm center wavelength, 20-nm bandwidth) before the condenser lens. Diffusion and neutral density filters were removed from the optical path to maximize light intensity to the camera.

Video Equipment

Video images were generated by a C2400 Hamamatsu newvicon video camera using only its analog controls to adjust image contrast. Uneven illumination (shading) was a severe problem when using the Nikon 100×/1.4 NA objective with the Zeiss DIC optics, but this was again corrected for by the C2400 camera controller. Two methods were used to record the raw video data. In one method, cells were continuously illuminated for short durations and the kinetochore movements recorded in real-time using a Mitsubishi BV-1000 Super-VHS video recorder. These real-time recordings provided excellent short "windows" of active kinetochore movements. Selected sequences were time lapsed (one frame every 2 s) into a Panasonic TQ2028F Optical Memory Disc Recorder (OMDR) for analysis (see below). Cells judged to be photo-damaged were not used. Alternatively, long term recordings of mitosis were obtained by time lapsing directly into the OMDR at 2-s intervals. A shuttering mechanism was used to block the light between exposures.

Tracking

A semi-automatic tracking program was developed to accurately track objects displayed on a video monitor using a Max Video digital image processor (DATACUBE, Peabody, MA) and custom software (Salmon et al., 1991). Briefly, the program generated two cursors which overlaid the 512 × 512 pixel digitized images played back from the OMDR. The operator moved each cursor, typically an 8 × 8 pixel array, over the object (typically, one centriole of the centrosome and one kinetochore region of a chromosome) to be tracked. The gray values were then stored as a template and used to automatically search each consecutive image. If the computer could not find a match above a threshold correlation coefficient, the program paused so that the operator could update the template, manually move the cursor to the object's position or advance the OMDR image (in the case of out of focus images) before continuing automatic tracking. The accuracy of the tracking program was determined by repetitively measuring the distance between stationary camera face-plate dirt particles recorded onto an S-VHS tape recorder and transferred to the OMDR.

Analysis

Each tracking file contained the time interval, OMDR frame number, X and Y coordinates in pixels, and Pearson's correlation values for each image tracked. These files were then transferred to an in-house motion analysis program, Single Frame Movement (SFM). SFM enabled the operator to convert pixel coordinates to distance in microns (based on measurements obtained with a microscope stage micrometer), generate distance vs time plots and perform regression analysis on the resulting data to obtain velocity and duration values. For graphing and further analyses, SFM files were transcribed into ASCII files and imported into spreadsheet and graphing programs on a Macintosh Quadra 700 (Apple Co.).

Phase Determination

Kinetochore motions were defined as AP and P movement if movement occurred unidirectionally for greater than 0.5 μm displacement. Rapid and extended kinetochore movements away from the pole (toward the MT plus-end) were easily identified as AP movement and those towards the pole (minus-end) as P movement. These persistent phases comprised the bulk of kinetochore oscillations and net movement. Slow kinetochore velocities (<0.5 μm/min) were initially categorized as N movements (indeterminate or confused). However, after multiplying each N category velocity by the corresponding duration of movement, slow movement resulting in a net kinetochore displacement of over 0.5 μm was recategorized as AP or P, depending on the direction moved.

Results

Imaging and Tracking

High resolution VE-DIC microscopy methods (Salmon et al., 1989; Walker et al., 1990) were used to visualize the kinetochores on chromosomes, bundles of kMTs inserting into individual kinetochores, untethered or distal kinetochores on mono-oriented chromosomes, and the centrosome complex at the spindle poles (Fig. 1). Individual kinetochores were more difficult to detect in low magnification images (Fig. 1 *A*), but were distinct at higher magnification (Fig. 1 *B*). The images of tethered kinetochores were about 300-nm wide and higher in contrast than the surrounding centromere chromatin. The bundle of ~15–20 kMTs (Rieder and Hard, 1990) was usually seen to be constant in contrast up to the point where it terminated at a kinetochore (Fig. 1 *B*). kMT bundles became less obvious toward the poles as if the MTs splayed apart toward the pole as observed earlier using polarization (Cassimeris et al., 1988*a*) and EM (Rieder and Hard, 1990) or the bundles became lost in the higher density of MTs near the pole. Tethered kinetochores were seen both at the edge of the centromere region proximal to the pole and embedded up to 400 nm into the centromere constriction (Fig. 1 *B*). The distal, untethered kinetochore of mono-oriented chromosomes could also be seen, but was often above or below the optical section containing the tethered kinetochore (Fig. 1 *C*).

Video images of kinetochore-to-pole motion were time-lapse recorded onto an OMDR at 2-s intervals for frame by frame analysis. We initially tracked the positions of the kinetochores and centrosomes by eye using a computer-driven video cursor overlay technique (Walker et al., 1988*b*). However, we found that human fatigue generated much more scatter in the data than expected from the resolution in our images. We overcame this problem by constructing a semi-automatic tracking device (Salmon et al., 1991) which is based on modifications of a digital image processing scheme developed by Gelles et al. (1988) for tracking particles. We tested the ability of the program to faithfully track kinetochore movements by retracking a single kinetochore oscillation relative to the associated spindle pole. Superimposition of the two tracks, each of which contained 75 data points, showed that only 4 data point pairs differed from each other by more than 50 nm, the greatest difference was 200 nm (data not shown). The accuracy of the tracking program was further determined by repeatedly tracking two stationary objects at 2-s intervals for 3.5 and 6.5 min (data not shown). The accuracy at both low and high magnification was always at or below +/− 1 pixel (SD of 0.01 and 0.012, respectively).

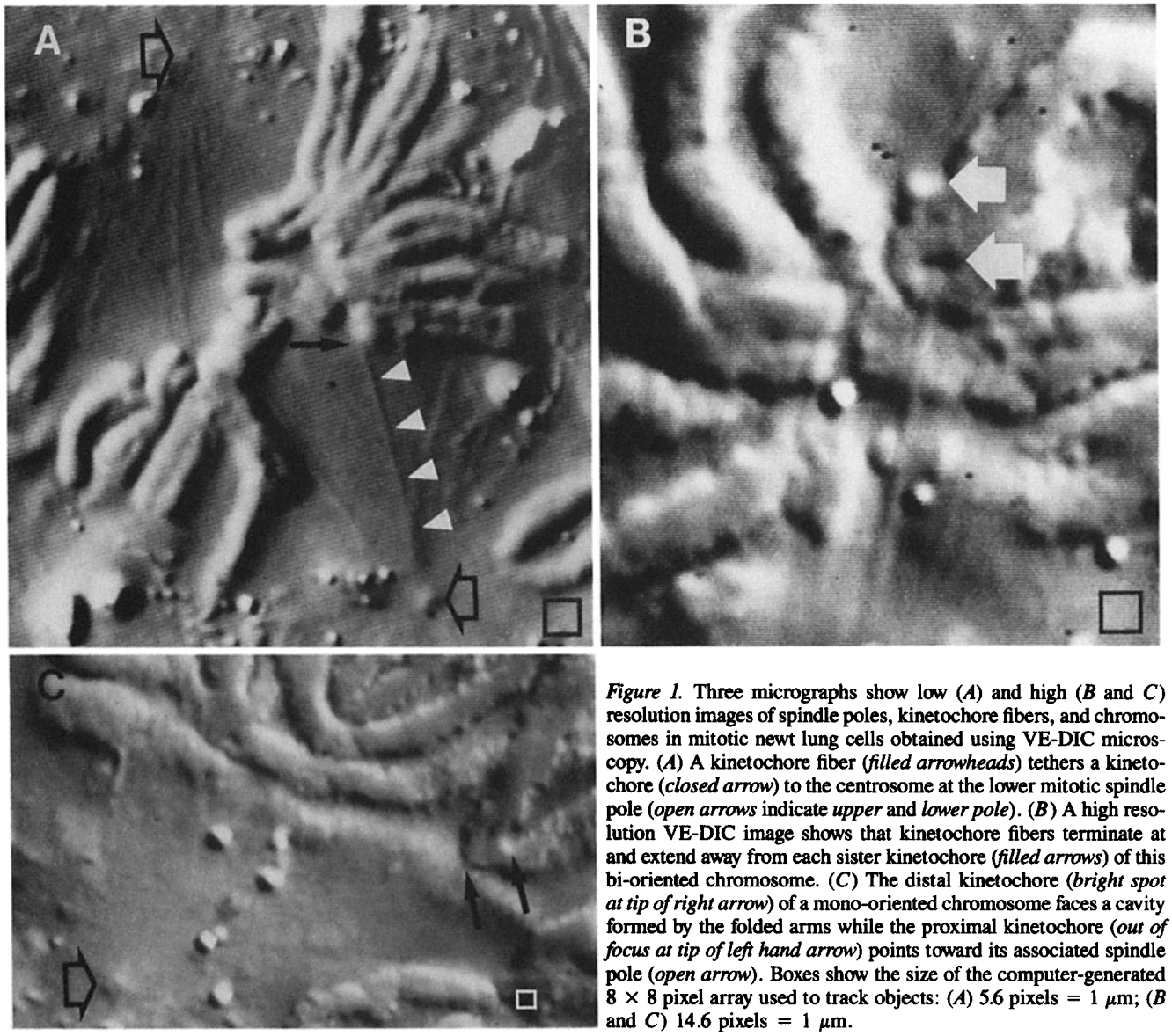

Figure 1. Three micrographs show low (*A*) and high (*B* and *C*) resolution images of spindle poles, kinetochore fibers, and chromosomes in mitotic newt lung cells obtained using VE-DIC microscopy. (*A*) A kinetochore fiber (*filled arrowheads*) tethers a kinetochore (*closed arrow*) to the centrosome at the lower mitotic spindle pole (*open arrows* indicate *upper* and *lower pole*). (*B*) A high resolution VE-DIC image shows that kinetochore fibers terminate at and extend away from each sister kinetochore (*filled arrows*) of this bi-oriented chromosome. (*C*) The distal kinetochore (*bright spot at tip of right arrow*) of a mono-oriented chromosome faces a cavity formed by the folded arms while the proximal kinetochore (*out of focus at tip of left hand arrow*) points toward its associated spindle pole (*open arrow*). Boxes show the size of the computer-generated 8 × 8 pixel array used to track objects: (*A*) 5.6 pixels = 1 μm; (*B* and *C*) 14.6 pixels = 1 μm.

The Stages, Phases, Domains, and Directional Instability of Kinetochore Motility

We organized our kinetic analysis based on the five stages of kinetochore motility which occur during mitosis (Fig. 2 *a*). These stages are gliding (G); mono-oriented (M); congression to the equator after bi-orientation (C); bi-oriented near the equator (B); and anaphase A (A). Except during gliding, kinetochores oscillated toward and away from the centrosome (Fig. 2 *b*).

Gliding was clearly different from the other stages (Rieder et al., 1990) where kinetochores were tethered by MTs embedded end-on into the kinetochore plate (Roos, 1976; Witt et al., 1981; Rieder, 1982). Gliding was not obvious for many chromosomes but was clearly seen for chromosomes distant from the pole and delayed in attaching as described by Rieder et al. (1990). The average distance of gliding for these chromosomes was 10.4 μm (Table I). Though the average P velocity (~18 μm/min) was an order of magnitude faster than the velocities measured during the other stages of kinetochore movement, gliding contributed little with respect to the duration of kinetochore movement throughout mitosis. Furthermore, AP movement, an important aspect to chromosome congression and oscillations, was not observed during gliding (Fig. 2).

Our high resolution measurements showed that tethered kinetochore oscillations were not sinusoidal as reported by others (Bajer, 1982; Fuge, 1987, 1989), but appeared as a saw-toothed pattern consisting of abrupt switches between constant velocity phases of P and AP movement (Figs. 2 *b* and 4, *A–D*). Though P and AP phases often consisted of a single constant velocity domain, two or three discrete velocity domains were also observed within either P or AP phases as diagrammed in Fig. 3. Occasionally, kinetochores appeared to pause in their motion or exhibit short, unmeasurable erratic movement. These indeterminate (N) states interrupted otherwise continuous P or AP phases or occurred

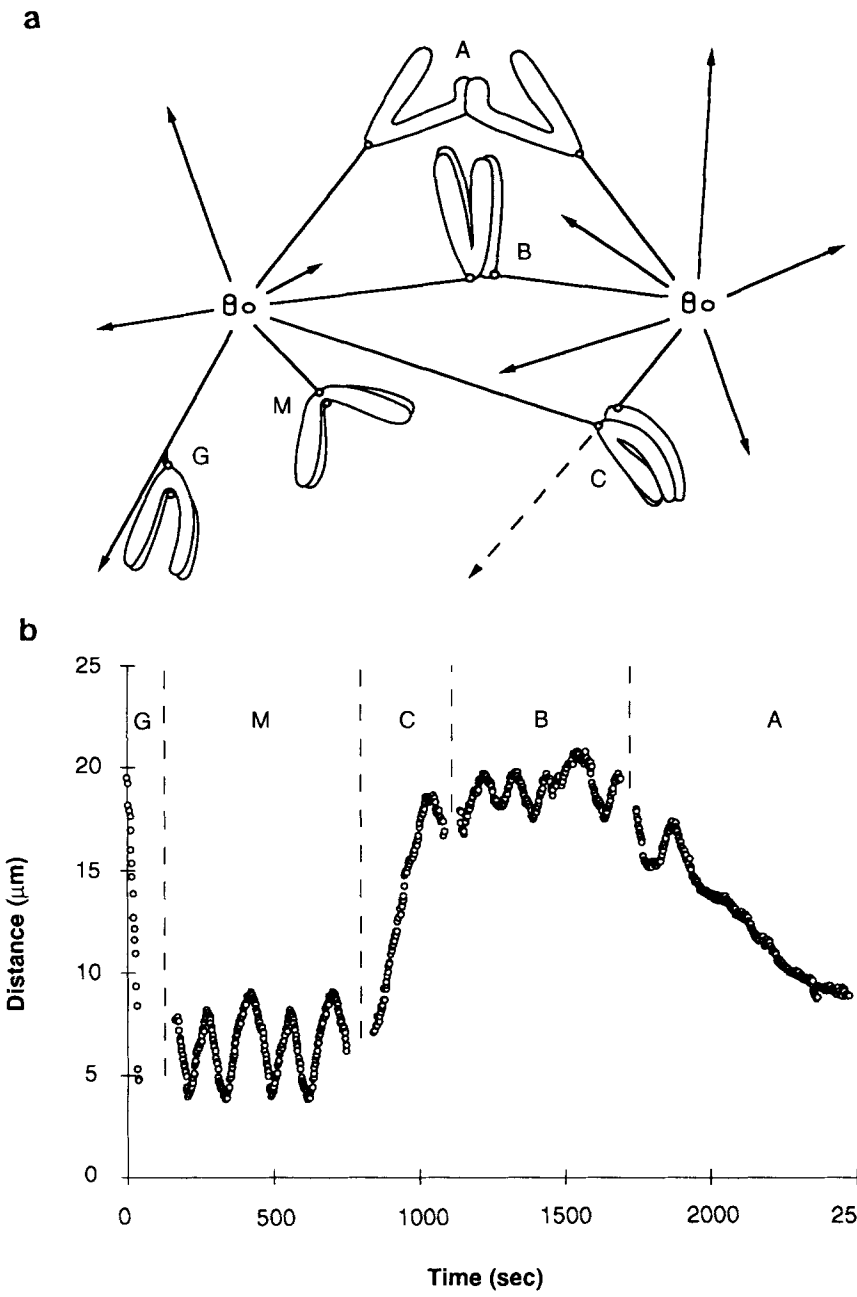

Figure 2. (*a*) Schematic illustrating the five stages of chromosome movement during mitosis in newt lung cells: *G*, gliding; *M*, mono-oriented; *C*, "congression" of a bi-oriented chromosome towards the equator; *B*, bi-oriented near the equator; and *A*, anaphase A poleward movement. (*b*) Distance vs time plot of the movements a single kinetochore may exhibit throughout mitosis. Note that the history plot was generated by concatenating several files of actual kinetochore movements onto one time axis and aligning the resulting plots to represent the stages of movement one kinetochore could make throughout mitosis.

Table I. Kinetochore Phase Velocities, Durations, and Excursions

					Anaphase	
	Gliding	Mono-oriented	Congression	Bi-oriented	Fast	Slow
Poleward (P)						
V μm/min ± SD	17.9 ± 5.20 (6)	1.40 ± 0.33 (21)	1.75 ± 0.64 (11)	1.98 ± 1.06 (37)	1.84 ± 0.89 (35)	0.52 ± 0.25 (9)
t minutes ± SD	0.58 ± 0.22	1.47 ± 0.68	0.64 ± 0.61	1.25 ± 1.03	1.28 ± 0.82	6.26 ± 4.73
D μm	10.3	2.1	1.1	2.5	2.4	3.3
Away (AP)						
V μm/min ± SD	N/A	1.24 ± 0.42 (24)	2.08 ± 1.02 (18)	1.98 ± 1.42 (35)	1.68 ± 0.86 (25)	N/A
t minutes ± SD	N/A	1.69 ± 0.75	2.30 ± 1.66	1.22 ± 0.99	0.64 ± 0.31	N/A
D μm	N/A	2.1	4.8	2.4	1.1	N/A
Indeterminate (N)						
t minutes ± SD	0.75 ± 0.00	0.39 ± 0.33	0.61 ± 0.45	1.32 ± 1.99	0.70 ± 0.53	N/A
% of total time	15.6	8.3	7.2	10.8	4.4	N/A

Phase velocities, durations, and excursions of kinetochore movements throughout mitosis. Average kinetochore velocities (*V*) were obtained by performing regression analyses on distance vs time plots of kinetochore movement, relative to the associated spindle pole. Durations (*t*) were measured directly for each phase. The average distances (*D*) traveled during AP and P phases were calculated by taking the product $t \times V$. This table represents >360 min of data analyzed at 2-s intervals for the five categories of kinetochore movement. Indeterminate kinetochore movement (*N*) indicates very short-lived movements or long-lived periods where the direction of movement (AP or P) could not be determined. Phase values for gliding, mono-oriented, congression, bi-oriented, and anaphase are based on 6 chromosomes in 3 cells, 6 chromosomes in 4 cells, 8 chromosomes in 7 cells, 11 chromosomes in 7 cells, and 15 chromosomes in 6 cells, respectively. Sample size for each kinetochore AP and P movement is noted in parentheses after each velocity.

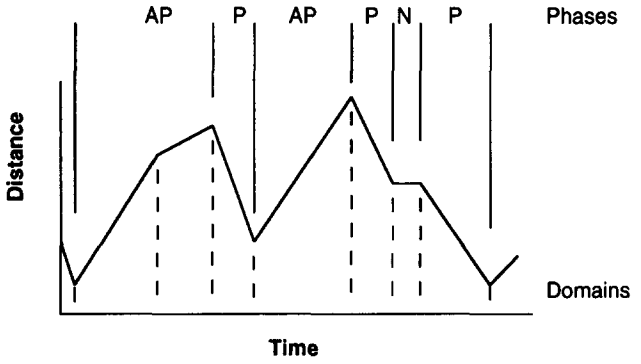

Figure 3. Schematic illustrating hypothetical phases and domains of kinetochore motility relative to the spindle pole. AP, P, and N phases (*solid vertical lines above plot*) indicate persistent AP, P, or N movement regardless of velocity. Switches between AP and P phases are typically abrupt. Different constant velocity domains can occur within an AP or P phase (*dashed vertical lines below plot*). The indeterminate stage (*N*) represents either extremely short movements or longer periods where the direction of kinetochore movement (*AP* or *P*) cannot be determined.

during transitions between P and AP phases. N states accounted for 11% or less of the total duration of mitosis (Table I).

Changes in Chromosome Position Were Achieved Mainly by Changes in Phase Durations

Kinetochores of both mono-oriented chromosomes near the pole and bi-oriented chromosomes near the equator went through many cycles of P and AP phases throughout mitosis, but the chromosomes in each case remained on average a constant distance away from the pole (typically 5–10 μm for mono-oriented chromosomes and 15–25 μm for bi-oriented chromosomes (Fig. 4, *A* and *C*). For mono-oriented chromosomes, both P and AP phases produced kinetochore displacements of ~2.1 μm toward and away from the pole, respectively (Table I). For proximal kinetochores on bi-oriented chromosomes near the equator, P phases produced, on average, 2.5-μm displacements while AP phases produced 2.4-μm displacements (Table I). The average net displacement was only 0.1 μm in the AP direction per cycle. The balance between P and AP phase velocities and durations is evident in both the distance vs time plots (Fig. 4, *A* and *C*) and in the symmetry about the x-axes in the corresponding domain velocity vs duration histograms (Fig. 5).

Kinetochores oscillated during congression to the equator and segregation during anaphase A, but the durations of the P and AP phases were distinctly different. The data in Table I and Figs. 4 and 5 show that kinetochores proximal to the pole achieved net displacement toward the equator during congression by prolonged AP phases of motion compared to the duration of P phases (2.3 vs 0.64 min, respectively). Conversely, kinetochores achieved a net displacement towards their poles during anaphase by prolonged P phases of motion, compared to AP phases (1.28 vs 0.64 min, respectively). These asymmetries during congression and anaphase A in the frequency and durations of P and AP movement become obvious when plotted as domain velocity vs duration histograms (Fig. 5). Thus, the processes of both chromo-

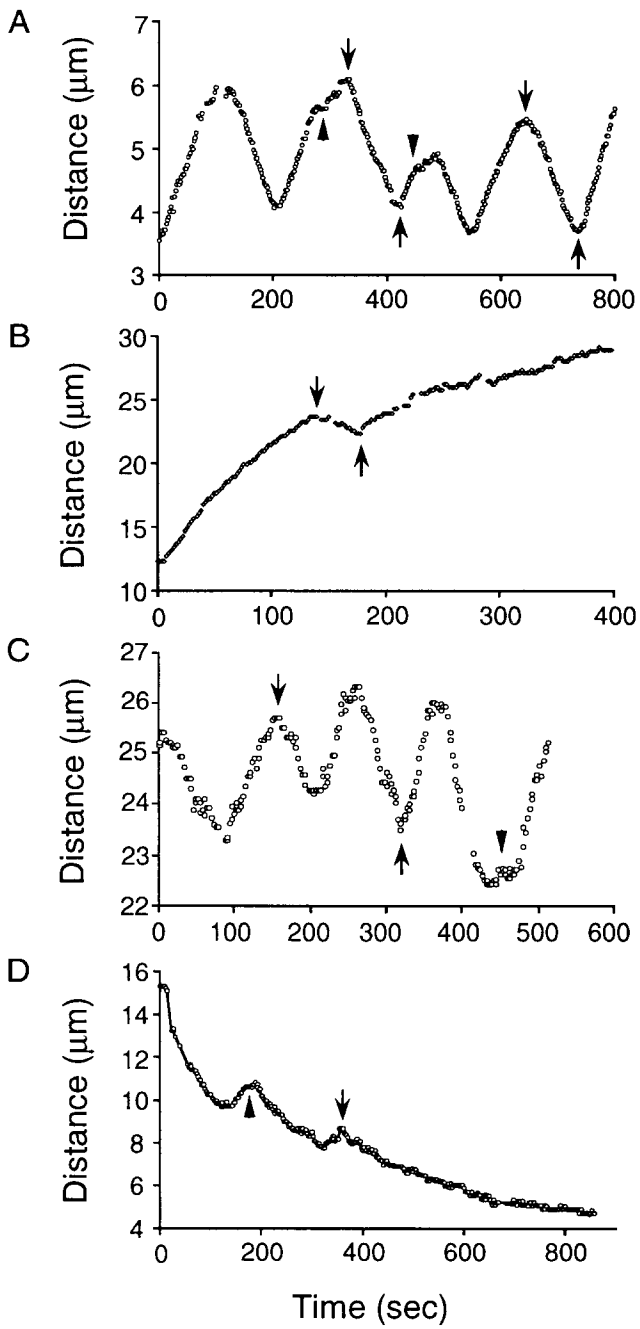

Figure 4. Examples of kinetochore directional instability during the different stages of chromosome movement during mitosis. (*A*) Mono-oriented chromosome: abrupt transitions between persistent P and AP movements predominate (*arrows*), though short pauses are observed (not shown). Discrete velocity shifts occasionally occur before switching between P or AP phases (*arrowheads*). (*B*) Chromosome congression toward the equator after bi-orientation: the proximal kinetochore persists in prolonged AP motion. Pauses and reversals (P movements) often occur (*arrow*), but are short-lived. Discrete velocity shifts in AP movement occur (compare the first 140 s to the final 225 s). (*C*) Bi-oriented chromosome near the equator: kinetochore directional instability has the same features observed for mono-oriented chromosomes. Note that the N state can interrupt P or AP phases or occur during phase transitions. Longer-lived pauses, not observed for mono-oriented chromosomes, are occasionally seen in kinetochore movements of bi-oriented chromosomes (not shown). (*D*) Anaphase A: kinetochore P movement during anaphase is composed of a fast initial phase

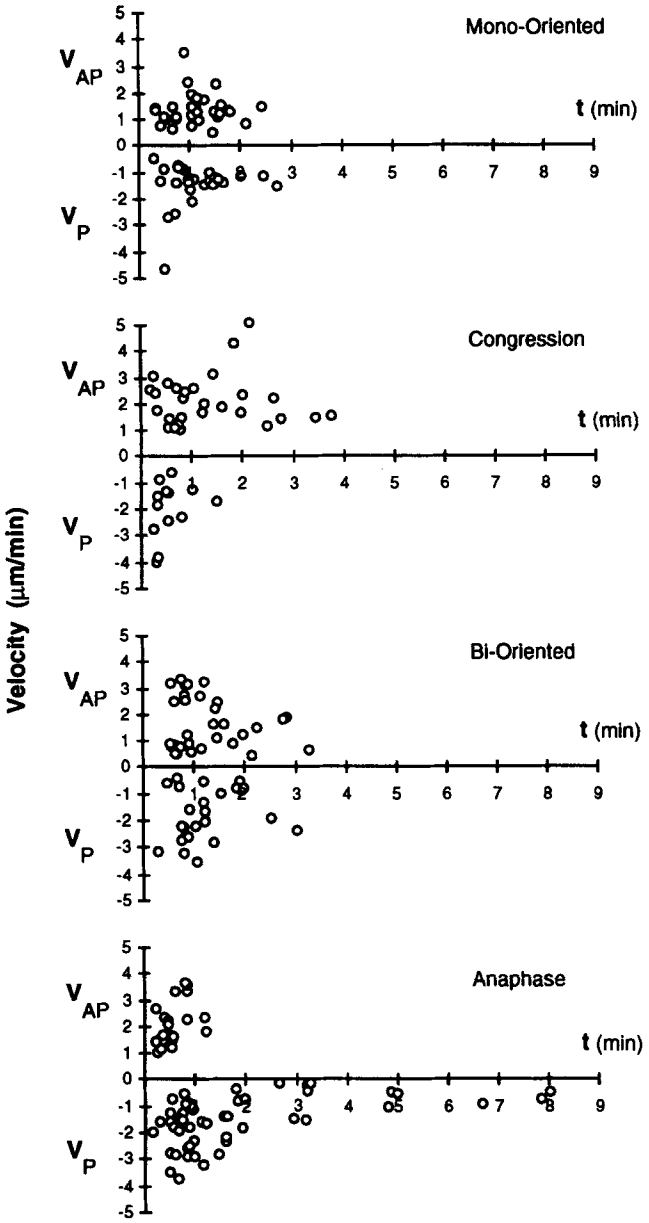

Figure 5. Histograms of domain velocities plotted against the domain durations during kinetochore P and AP motility for four stages of chromosome movement. The range of P and AP domain velocities and durations are similar for mono-oriented chromosomes and bi-oriented chromosomes near the equator. Proximal kinetochores on congressing chromosomes have fewer P movements and have AP domains of longer durations than the domains of either mono- or bi-oriented chromosomes. Kinetochores during anaphase exhibit short-lived AP durations and prolonged P motion. Kinetochores during the latter half of anaphase A exhibit a novel category of very long-lived and slow P movements. Average domains per phase before and after anaphase onset was 1.2 for both P and AP phases.

(0–100 s) and a prolonged slow phase (final 400 s). Switches to AP movement and pauses are frequent and can occur both during the fast phase and early in the slow phase.

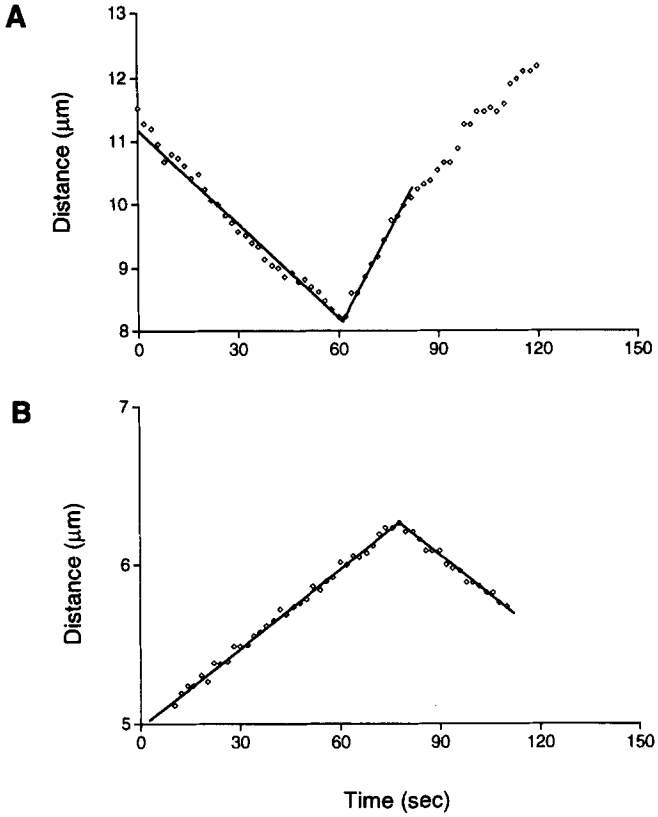

Figure 6. Distance vs time plots with exploded time axes show that the minimum time required for kinetochore switching may be less than our sample frequency (2 s). Least square regression lines were used to determine the theoretical switch point. (*A*) A switch from P to AP; (*B*) A switch from AP to P.

some congression and segregation directly depend on the mechanism(s) which regulate switching between P and AP phases of kinetochore motility since the average velocities of P and AP motion were similar during all stages of tethered kinetochore motility (Table I).

Kinetochore Directional Instability Usually Involved Abrupt Switching between Phases

Newt kinetochores contain, on average, ~15–20 MTs (Rieder and Hard, 1990), which need to switch cooperatively between shortening and growth phases in order for a kinetochore to abruptly switch between P and AP phases. To determine the minimum time required for kinetochores to switch between P and AP phases, we used our analysis program to enlarge the time period encompassing selected switch events. Least square regression lines were fitted to apposing constant velocity domains defining a switch event and the intersection was used to identify the theoretical switch point. We then counted the number of data points which fell below or above the theoretical switch point. One such P to AP switch event occurred exactly at the intersection of the regression lines (Fig. 6 *A*) with the switch point occurring below the 2-s temporal resolution of our measurement system. Similarly, we found AP to P switch events which coincided with the intersecting least square regression lines (Fig. 6 *B*). Thus, assuming constant velocity domains, the

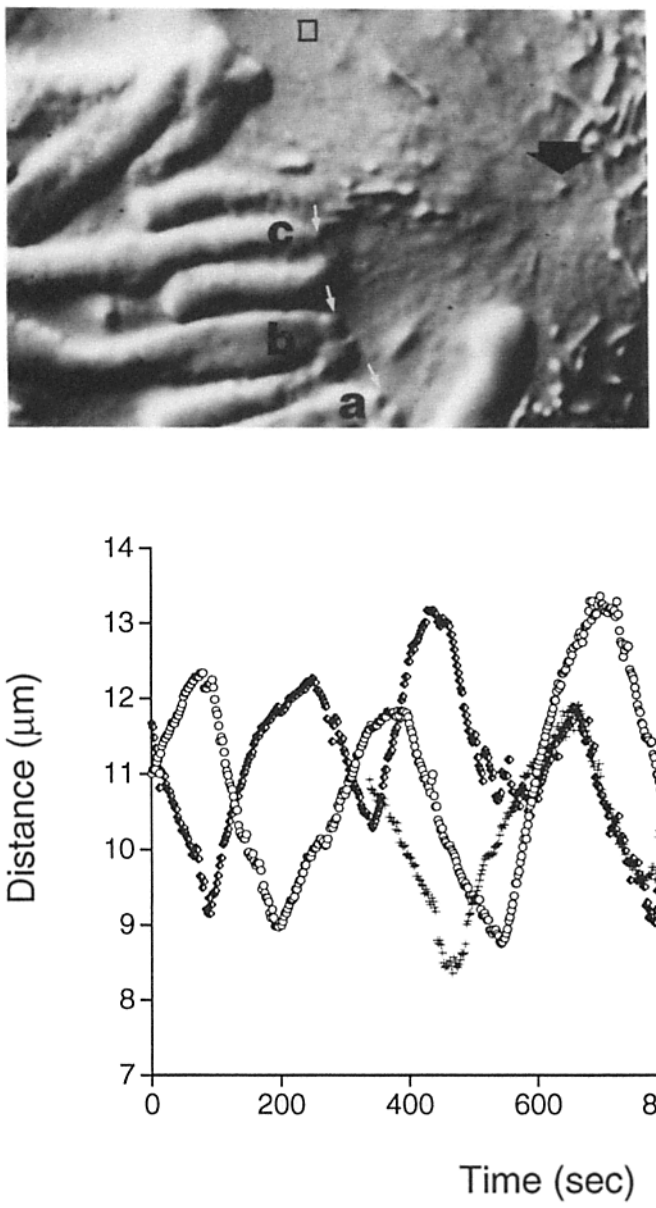

Figure 7. Kinetochore oscillations of adjacent mono-oriented chromosomes tethered to one pole show that each kinetochore acts autonomously, independent of the directional instability of neighboring kinetochores. (*A*) Micrograph of a VE-DIC image showing three mono-oriented chromosomes (*a*, *b*, and *c*) tethered to the same spindle pole (*large closed arrow*). Closest apposition for the pairs of kinetochores was 3 μm (*a* and *b*) and 3.2 μm (*b* and *c*). The kinetochore (*small closed arrow*) of each chromosome was tracked relative to one of the centrioles at the pole (*large black arrow*). The box represents the size of the 8 × 8 pixel array used to track each object (19.5 pixels = 1 μm). (*B*) Distance vs time plots for the three kinetochores shown in *A* were superimposed and analyzed to determine (*a*) if a switch in one kinetochore induced a switch in an adjacent kinetochore, and (*b*) the percent time adjacent kinetochores spent in coordinated movement (see text and Table II).

Table II. Coordination of Kinetochore Movements

A	Adjacent kinetochores		Sister kinetochores	
	Events	%	Events	%
In-phase switches	6	13.6	4	7.7
Out-of-phase switches	4	9.1	20	38.5
Single switch events	34	77.3	28	53.8

B	Adjacent kinetochores		Sister kinetochores	
	(2,422 s analyzed)		(1,870 s analyzed)	
	In-phase	49.5%	In-phase	23.3%
	Out-of-phase	50.5%	Out-of-phase	75.1%

(*A*) Percent of coordinated vs uncoordinated switch events for adjacent kinetochores on mono-oriented chromosomes near the pole and sister kinetochores on bi-oriented chromosomes near the equator. Events were classified as either in-phase, out-of-phase, or single switch events for any 10-s window. (*B*) Percent of time kinetochores of adjacent mono-oriented chromosomes and sister kinetochores of bi-oriented chromosomes exhibited coordinated movement. For adjacent kinetochores of mono-oriented chromosomes, coordinated movement was defined as in-phase if both kinetochores moved in the same phase, either P or AP. For sister kinetochores of bi-oriented chromosomes, coordinated movement was defined as out-of-phase when one sister kinetochore moved P while the other sister moved AP. Net centromere displacement occurred when sister kinetochores moved out-of-phase. The table represents data collected from the movements of three pairs of adjacent and three pairs of sister kinetochores.

~15–20 kMTs appear capable of abruptly switching between P and AP phases in a synchronous manner. Regression line analysis on other switches showed that as much as 6 s was involved in an abrupt switch event (data not shown). This corresponds to only 6–7% of average P or AP phase durations and may involve the difficulty of tracking the kinetochore below the surface of the chromosome (see below).

Kinetochore Directional Instability Was Uncoordinated between Adjacent Mono-oriented Chromosomes

Coordinated movement and switching of tethered kinetochores on adjacent mono-oriented chromosomes would suggest that kinetochore motility is regulated by some diffusable factor within or linked to the mitotic spindle. Evidence for coordinated kinetochore movements has previously been reported for newt lung (Bajer, 1982) and Mesostoma cells (Fuge, 1987, 1989). We readdressed this issue using our semiautomated tracking system to determine the degree of coordinated movement and switching of adjacent kinetochores. We generated kinetic plots from the movements of three adjacent kinetochore pairs on mono-oriented chromosomes. A micrograph of two of the chromosome pairs and the distance vs time plots are shown in Fig. 7. The degree of coordinated switching was determined in two ways. In the first analysis, we asked how frequently switching of one kinetochore resulted in either (*a*) switching of the adjacent kinetochore to the same phase (in-phase); (*b*) switching of the adjacent kinetochore to the opposite phase (out-of-phase); or (*c*) no switch in the adjacent kinetochore (single switch event). As seen in Table II *A*, 77.3% of the switches were single switch events within any given 10-s window while the incidents of in- and out-of-phase switches were quite low (13.6% and 9.1%, respectively). In the second analysis, we calculated the percent of the time adjacent kinetochores exhibited in-phase (coordinated) or out-of-phase (uncoordinated) movement. We found that adjacent kinetochores spent an equal time engaged in in-phase and out-of-phase movement (Table II), suggesting that kinetochores moved randomly relative to one another. Similar results were obtained when adjacent kinetochore oscillations in monopolar spindles were measured (Skibbens, R. V., and E. D. Salmon, unpublished observations). Thus, by both of these criteria, kinetochores on adjacent mono-oriented newt lung chromosomes switch independently and move autonomously.

The Directional Instability of Sister Kinetochores on Bi-oriented Chromosomes near the Equator Was Coordinated

We applied the same methodologies as described above to examine the level of coordinated movement exhibited by sister kinetochores on bi-oriented chromosomes near the equator. Sister kinetochore movements were usually measured relative to the same pole. In one case, the mitotic spindle pole wandered out of the field of view and measurements were made relative to a stationary spot on the face-plate of the camera (Fig. 8). Because sister kinetochores are tethered to opposite spindle poles, out-of-phase movements (one kinetochore moving P while the sister moves AP) resulted in coordinated sister kinetochore movement and centromere displacement. In-phase movements (both kinetochores moving P or AP to their respective poles) resulted in uncoordinated sister kinetochore movement and little centromere displacement.

Sister kinetochores of bi-oriented chromosomes exhibited a high degree of coordinated movement with only occasional

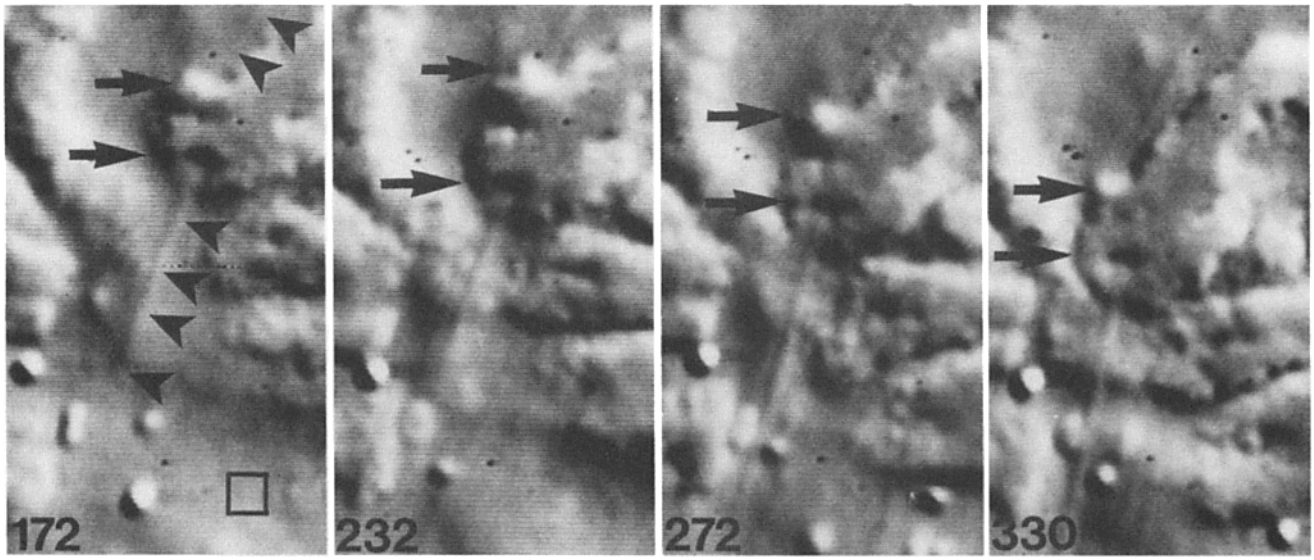

Figure 8. A VE-DIC sequence of a bi-oriented chromosome shows that sister kinetochores can move independently, stretching and compressing the intervening centromere, but that displacement of the centromere involves coordinated movements of sister kinetochores (one in P and the other in AP). Micrographs correspond to four time points in the distance plot in Fig. 9 *A*. A kinetochore fiber (*arrowheads*) extends away from each sister kinetochore. Note that the centromere region becomes compressed (*172 s*) and stretched (*232 s*) though no appreciable centromere displacement occurs. However, net displacement of the centromere region occurs when the upper kinetochore is in AP motion while the lower kinetochore is in P motion (*272–330 s*). Note that the faster AP rate of the upper kinetochore, compared to the P rate of the lower kinetochore, results in a slight compression of the centromere as the centromere moves toward the lower pole.

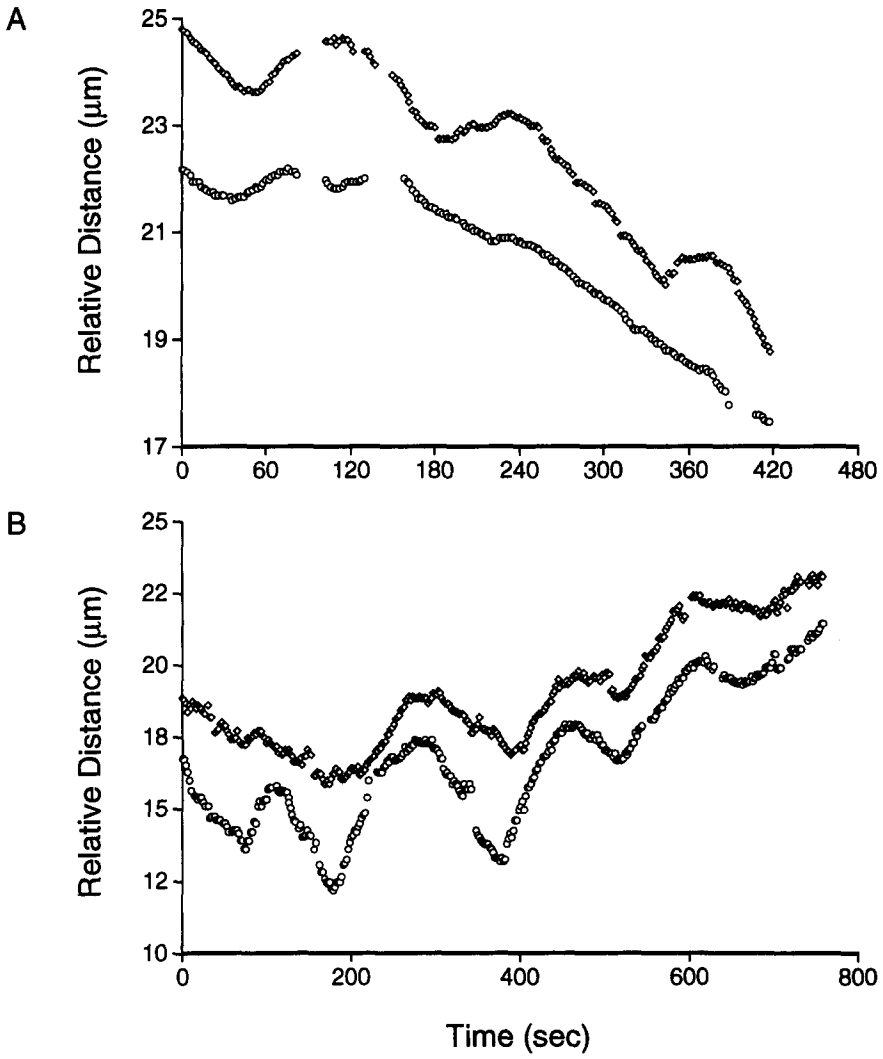

Figure 9. Distance vs time plots for sister kinetochores on bi-oriented chromosomes near the equator. (*A*) The oscillations of both sister kinetochores for the bi-oriented chromosome shown in Fig. 8. In this example, kinetochore movement was measured relative to a stationary mark on the face plate of the camera (not shown) because the pole was not in the field of view. Although uncoordinated movement (both kinetochores in either the P or AP phases) occurred (80–140, 180–230, and 345–375 s), coordinated movement (one in P phase and the other in AP phase) predominated and was responsible for centromere displacement. (*B*) The directional instability of sister kinetochores on a different bi-oriented chromosome (not shown) were tracked relative to one pole. Coordinated sister kinetochore movement was again observed to predominate (no change in length between sister kinetochores), while uncoordinated movements resulted in an increase or decrease in the distance between sister kinetochores with little net centromere displacement.

periods of uncoordinated movement (Fig. 9). Compared to kinetochores on adjacent mono-oriented chromosomes, sister kinetochores exhibited a fourfold increase in out-of-phase switching, while the percentages of in-phase switches and single switch events decreased significantly (Table II *A*). In addition, we found that sister kinetochores exhibited predominately (75.1%) coordinated movement (Table II *B*). These data show that the autonomous nature of each kinetochore is strongly influenced by the activity of the sister kinetochore.

Kinetochores Pulled on the Chromosome Only during P Motion, Pushed on the Chromosome during AP Motion, and Resisted Movement during the N State

We used the elastic property of chromatin (Nicklas, 1988), specifically the extent of centromere deformation, to infer the state of force produced by kinetochores and exerted on the centromere region for the movements of mono-oriented (Fig. 10 *A*) and bi-oriented (Fig. 10 *B*) chromosomes. In both cases, P forces produced at the kinetochore were expected to pull and stretch the centromere poleward (Fig. 10, *A1* and *B1*). Two possibilities were considered regarding the nature of AP forces. First, if kinetochores could only produce pulling forces, then kinetochore AP movement must be produced by antagonistic forces of greater magnitude than the kinetochore pulling force. For mono-oriented chromosomes, antagonistic forces could be produced by ejection forces which act along the chromosome arms (Fig. 10 *A2*). For bi-oriented chromosomes, the antagonistic force could be produced by either an ejection force acting along the chromosome arms (Fig. 10 *B2*) or by the P movement of the sister kinetochore (Fig. 10 *B3*). The second possibility we considered was that the switch from P to AP movement resulted from kinetochore switching from pulling (P force production) to pushing (AP force production) on the centromere. For mono-oriented chromosomes, a switch from P to AP force production would be detectable by the disappearance of the P phase centromere stretch and the appearance of kinetochore indentation into the centromere region (Fig. 10 *A3*). For bi-oriented chromosomes, a switch from P to AP force production of one of the sister kinetochores would also be detectable by loss of the in-phase P stretch, compression of the centromere region, and net displacement of the chromosome (Fig. 10 *B4*).

We found that the switch from P to AP kinetochore motion

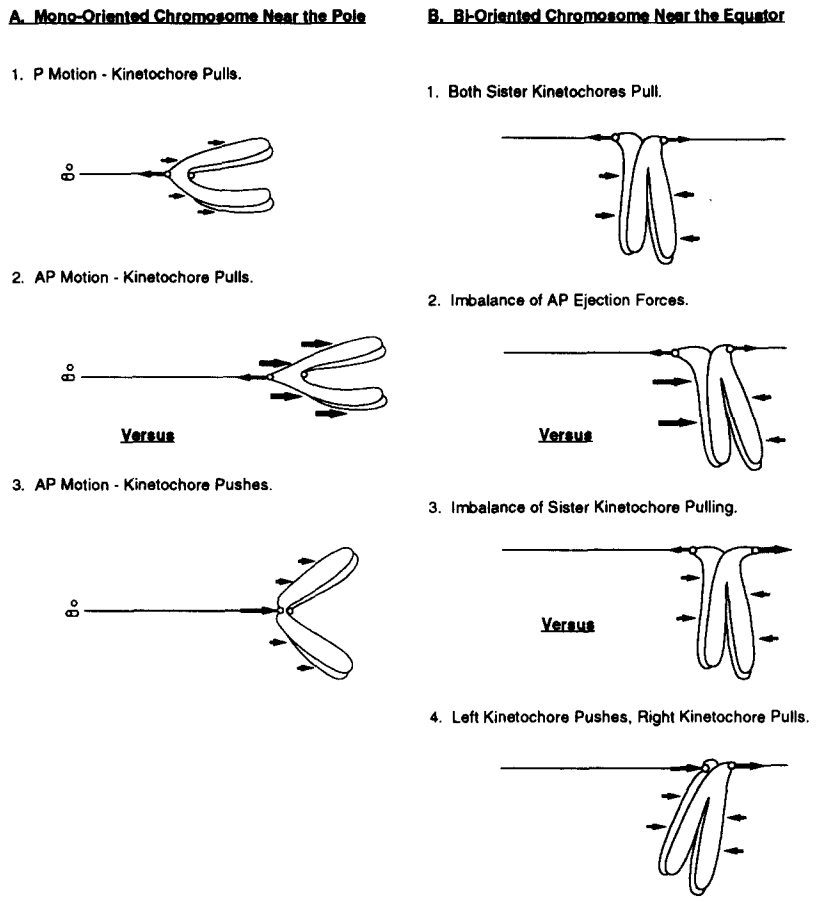

Figure 10. Schematic using the deformation of the centromere to compare the effects of antagonistic P and AP forces proposed for static force balance models (kinetochore always pulling) versus the effects of kinetochore switching between P (*pulling*) and AP (*pushing*) forces. (*A*) A mono-oriented chromosome exhibiting the characteristic V-shape during P movement (*A1*) could move AP either by an increase in the AP ejection forces on the arms which overcome the kinetochore P force (*A2*) or by switching of the kinetochore from P to AP force production (*A3*). (*B*) The centromere of a bi-oriented chromosome is stretched when both sister kinetochores produce P forces (*B1*). The chromosome could move to the right by either an increase in the AP ejection forces associated with the left half-spindle (*B2*), an increase in P forces produced by the right sister kinetochore (*B3*), or switching of the left kinetochore from P to AP force production (*B4*). Spindle poles are removed from *B* for clarity.

was a switch from kinetochore pulling to pushing on the centromere. For mono-oriented (Fig. 11) and early anaphase chromosomes (Fig. 12), the centromere region was distinctly stretched poleward during kinetochore P motion. However, during kinetochore AP motion, the centromere region was not stretched as diagrammed in Fig. 10 *A2*. Instead, the centromere became flattened or punched in as diagrammed in Fig. 10 *A3* and shown in Figs. 11 and 12. Usually, the kinetochore moved AP at a rate similar to the ejection of the arms and the kinetochore only slightly indented the centromere region. Occasionally, however, the kinetochore moved AP faster than the chromosome arms and the kinetochore deformed the chromosome from a "V" into a "W" shape (Fig. 11).

Similar to the results for mono-oriented chromosomes, the centromere region of bi-oriented chromosomes did not appear stretched during AP kinetochore motion as diagrammed in Fig. 10, *B2* and *B3*. Instead, the proximal centromere region was flattened or pushed in as diagrammed in Fig. 10 *B4*. These features of centromere deformation during sister kinetochore movement for a bi-oriented chromosome were evident in both high resolution video sequences (Fig. 8) and sister kinetochore distance plots (Fig. 9). Sister kinetochores on bi-oriented chromosomes occasionally moved independently of one another and exhibited any combination of P, AP, and N phases. For the cell in Figs. 8 and 9, the centromere became maximally compressed (Fig. 8, 172 s) because both kinetochores were previously in AP motion (Fig. 9 *A*, 60–180 s). The centromere appeared maximally stretched (Fig. 8, 232 s) when both sisters persisted in P motion (Fig. 9 *A*, 180–232 s). A natural cleft in the center of the centromeric chromatin became evident during this in-phase sister kinetochore P motion (Fig. 8, 232 s). Little appreciable net displacement of the centromere toward either pole occurred when sister kinetochores exhibited in-phase P or AP movements (Figs. 8 and 9). At ~232 s (Figs. 8 and 9), the upper kinetochore switched to AP motion while the lower kinetochore persisted in P motion. Because the upper kinetochore moved AP at a higher velocity than the P motion of the lower kinetochore, the centromere region became compressed as it moved ~2.5 μm downwards (Fig. 9, 232–340 s). When the upper kinetochore switched back to P motion (340 s), the centromere became stretched again and net displacement stopped (see Fig. 9, *A*). Often, the centromere was neither stretched nor compressed during the out-of-phase sister kinetochore movements which resulted in net centromere displacement (Fig. 9 *B*, 400–440 s, 550–600 s).

Video records also showed that the chromosome arms did not lead the kinetochores during congression toward the equator as diagrammed in Fig. 10 *B2*, but that the arms trailed the sister kinetochore complex toward the spindle equator (Fig. 10 *B4*). Congression towards the equator occurred when the kinetochore proximal to the equator was pulling in the P phase and the kinetochore proximal to the pole was pushing in the AP phase. Furthermore, the centromere did not appear stretched during congression except

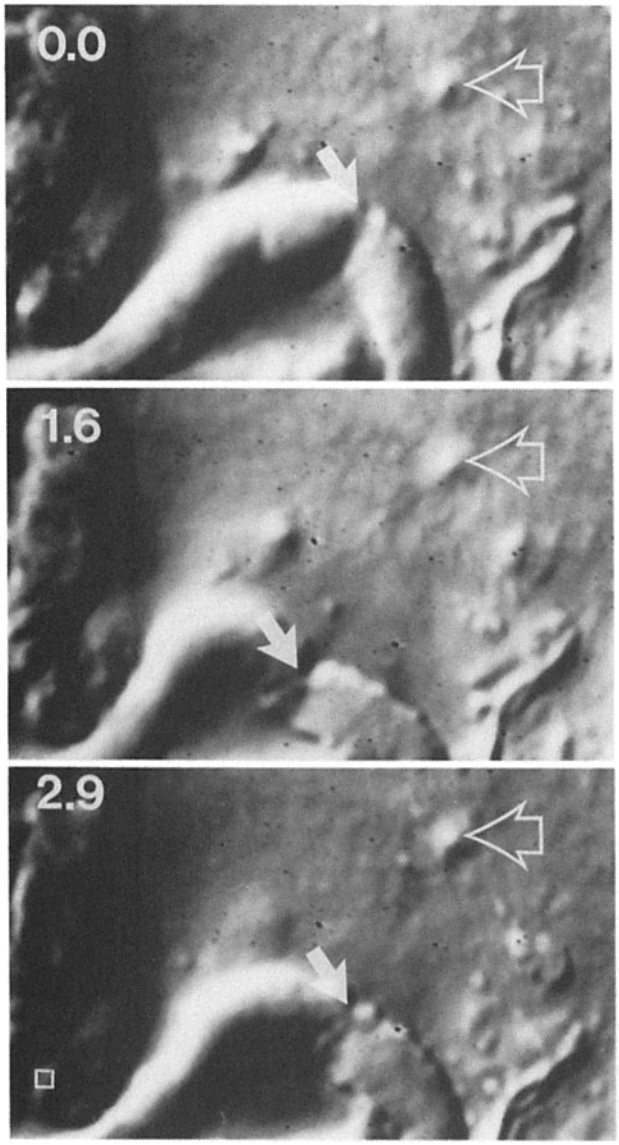

Figure 11. A VE-DIC sequence of a mono-oriented chromosome showing that kinetochore AP motion pushes inward on the centromere. AP kinetochore motion and fiber growth pushed on the centromere region and produced a W shaped chromosome (0.0–1.6 min) while P motion and fiber shortening produced a more V shaped chromosome (2.9 min). This cycle was repeated as the kinetochore oscillated between P and AP phases of instability. Box shows 8 × 8 pixel array (23.5 pixels = 1 μm).

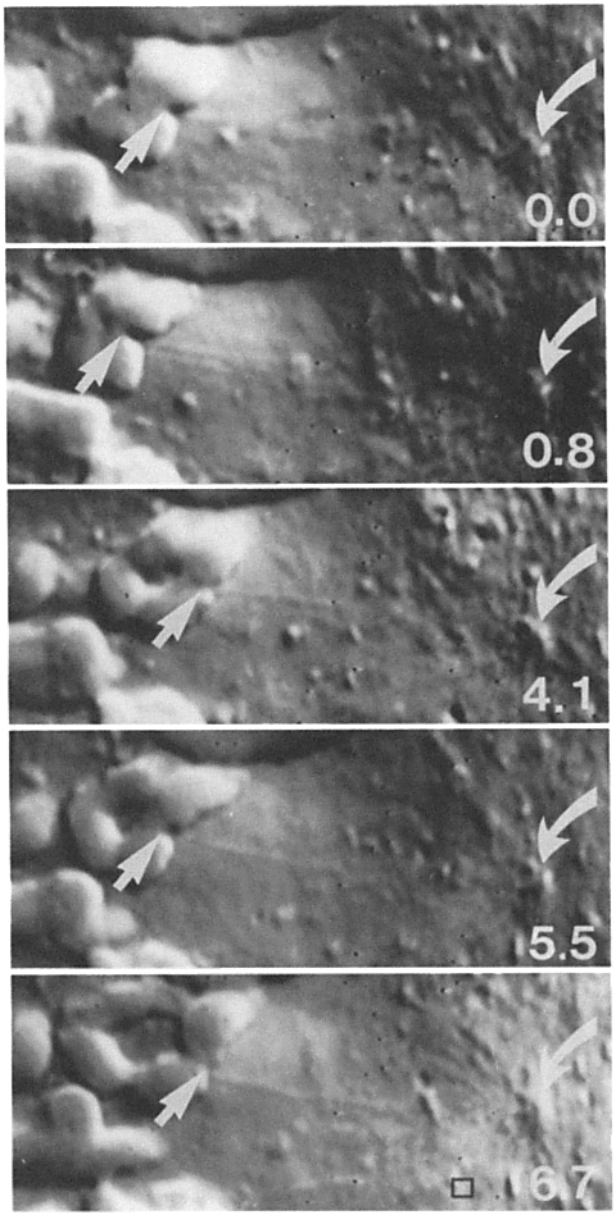

Figure 12. A VE-DIC sequence of bi-oriented chromosome before and after the onset of anaphase which shows centromere deformation during kinetochore P and AP movement. A kinetochore fiber can be seen extending away from each sister kinetochore. AP motion of the kinetochore (*straight arrow*) proximal to the visible pole (*curved arrow*) resulted in a "punching-in" of the centromere region (*0.0–0.8* min) while switching to P movement resulted in the loss of the centromere invagination and stretching of the centromere (*0.8–4.1* min). This chromosome was followed through anaphase to determine if kinetochore fiber pushing forces persisted after sister chromatid separation. During anaphase, punching-in of the centromere was again observed during AP movement of the proximal kinetochore (*4.1–5.5* min), though the sister kinetochore was in P motion and a large space between the separating sister chromosomes was evident. The proximal kinetochore switched back to P movement (*5.5–6.7* min), resulting in loss of the invagination and completion of chromosome segregation. Box shows 8 × 8 pixel array (11.4 pixels = 1 μm).

during brief periods when both sister kinetochores switched to in-phase P phase. Similarly, congression to the spindle equator was stalled when both sister kinetochores switched to in-phase AP motion, compressing the centromere.

Discussion

Fast Kinetochore Poleward Gliding Is Brief and Always Poleward

Rieder et al. (1990) and Merdes and De Mey (1990) have shown that rapid poleward movement of kinetochores can occur before kinetochores attach to MT plus-ends. They

have argued that cytoplasmic dynein, which has been localized to the coronal filaments on the periphery of kinetochores (Steuer et al., 1990; Pfarr et al., 1990; Zinkowski et al., 1991), produces rapid poleward gliding of kinetochores along the lateral face of polar MTs. The velocities of kinetochore gliding measured in our study are similar to those measured previously (Rieder et al., 1990) and are typical of the rates of organelle motility along MTs driven by cytoplasmic dynein (Vallee and Shpetner, 1990). Hyman and Mitchison (1991) have demonstrated in in vitro studies that kinetochores on isolated chromosomes will support plus-end–directed MT gliding over their surfaces in addition to the rapid minus-end–directed gliding thought to be produced by cytoplasmic dynein. The velocity of plus-end–directed gliding measured in their experiments was only several μm/min, much slower than rates typical of either cytoplasmic dynein or conventional kinesin. In our studies, we often observed gliding kinetochores to pause in their poleward motion. However, we never saw any evidence of AP or plus-end–directed motion in newt cells, in contrast to reports for diatoms (Pickett-Heaps, 1982), during the gliding phase. Plus-end–directed gliding in vitro required phosphorylation of the kinetochore in the study performed by Hyman and Mitchison (1991); if a similar mechanism operates in vivo, it is apparently shut off or inaccessible during the gliding phase.

Directional Instability Is a Property of Kinetochores Attached to the Plus-end of kMTs

An important question is how much of the kinetochore P and AP motion and associated centromere pulling and pushing forces are produced at the kMT plus-end attachment sites and how much are produced by the motion of kMTs. The lattice movements of newt lung kMTs have been measured (Mitchison and Salmon, 1992) using photoactivation marking methods (Mitchison, 1989). Mitchison and Salmon (1992) found that during metaphase, there was a slow poleward flux of kMTs, ~0.5 μm/min, which decreased to ~0.3 μm/min by mid-anaphase A. Marks on kinetochore fiber MTs were never seen to move away from the pole. Thus, poleward flux of kMTs accounted for 33% or less of kinetochore P motion and produced none of the kinetochore AP motion (Table I).

In the latter half of Anaphase A, kinetochore P motion is slow (Table I) and occurs at rates similar to the rate of kMT poleward flux (Mitchison and Salmon, 1992). Apparently, kinetochore activity shuts down at this time, blocking kMT plus-end assembly dynamics (Sheldon and Wadsworth, 1992). Thus, kinetochores in late anaphase appear "parked" on the plus-end lattice of kMTs and chromosomes are moved as their kMTs flux poleward.

The Kinetochore Has Four Motility States: P, AP, N, and Park

We conclude from the kinetic and centromere deformation data in this paper and the lattice marking results reported by Mitchison and Salmon (1992) that kinetochores can autonomously exhibit four states of motion at kMT plus-ends analogous to the four gears of an automatic transmission which drive the wheels of a car over the road: P or "Forward" where the kinetochore is actively moving towards the pole at a velocity coupled to the rate of kMT plus-end depolymerization; AP or "Reverse" where the kinetochore is actively moving away from the pole at a velocity coupled to the rate of kMT plus-end polymerization; and a "Park" state where the kinetochore is tightly attached to the kMT plus-ends and moves only as a consequence of the poleward movement of kMTs (flux). The fourth state, "Neutral," occurs when the kinetochore exhibits indeterminant movement but remains actively engaged to the kMT plus-end.

Throughout prometaphase, metaphase, and the first half of anaphase A in newt cells, we found that kinetochores mainly exhibited persistent P or AP states, with the N state comprising a small fraction of the time (Table I). In both the P and AP states, the kinetochore is actively driving the chromosome through the cytoplasm. In the N state there was little or no detectable chromosome motion seen through the cytoplasm. The N state may represent situations where the rate of plus-end kMT growth just matches the rate of poleward flux so that no net translocation of the kinetochore occurs. Another possibility is that within one kinetochore, not all kMTs are in phase; some kMTs may be trying to shorten while others are attempting to elongate.

Directional instability and P force production are a property of kinetochores not shared by other regions of the chromosome. Unlike kinetochore directional instability, acentric chromosome arms are only ejected away from the pole. If the bulk of the chromosome arms are severed from kinetochores by laser microsurgery, the severed arms are ejected AP (Rieder et al., 1986; Ault et al., 1991). The fact that the small centromeric fragments containing kinetochores, severed from the bulk of the chromosome arms, continue to oscillate with kinetics similar to intact chromosomes (Skibbens, R. V., C. L. Rieder, and E. D. Salmon, unpublished results) provides strong evidence that directional instability of P and AP force production is a property unique to kinetochores.

Kinetochore Directional Instability Probably Depends Both on MT Motors and Plus-end Dynamic Instability

Kinetochores, unlike sites along the chromosome arms, have the capability of holding onto the lattice of MT plus-ends as tubulin subunits dissociate from the ends during P motion and associate onto the ends during AP motion. This unique type of attachment is a property of MT motor domains, i.e., the ability to remain attached while translocating along the lattice. Both cytoplasmic dynein and several kinesin-related proteins have been localized to kinetochores (Steuer et al., 1990; Pfarr et al., 1990; Wordeman et al., 1991; Hyman and Mitchison, 1991; Zinkowski et al., 1991; Yen, 1992; Sawin et al., 1992). In addition, there is genetic evidence that a Drosophila kinesin-related protein, nod, is involved in pushing chromosomes away from the pole during first meiosis (Theurkauf and Hawley, 1992). Chandra et al. (1993) have recently shown that a truncated form of the Drosophila ncd motor exhibits attachment and thermal driven bi-directional translocation along the MT lattice, but not ATP-dependent unidirectional motion. Truncation of the protein disrupted ATP-dependent motor activity, but not the ability of the protein to attach to the lattice in a way that allowed for translocation. Thus, independent of their contributions in producing forces for kinetochore motion, MT motor domains are likely to be the key molecular components attaching the kinetochores to the plus-ends of kMTs.

Motor domains probably also contribute to force generation for kinetochore P and AP motion (Hyman and Mitchison, 1991), although there is still no direct evidence of their function in cells. Recently, Vale et al. (1993) have shown that a combination of dynein and conventional kinesin coating a glass surface will produce directional instability in the ATP-dependent gliding of MTs. MTs attached to this lawn of opposing motors were seen to periodically and abruptly switch between dynein- and kinesin-driven constant velocity motions. The MT gliding velocities are 50–100 times faster than the velocities of P and AP kinetochore movement measured during mitosis in newt lung cells. Nevertheless, this observation raises the possibility that autonomous kinetochore directional instability is produced by the spontaneous directional instability of a combination of plus- and minus-end directed motors located at the kinetochore.

As mentioned previously, phosphorylation of kinetochores on isolated chromosomes produces a switch from minus-end–directed MT sliding (P motion) to plus-end–directed sliding (AP motion) (Hyman and Mitchison, 1990). Thus, another important possibility to consider is that phosphorylation increases the probability of switching from P to AP motion. One obvious model for regulating kinetochore motility is based on a gradient of phosphorylation potential, the phosphorylation activity being higher near the poles. In this scheme, a kinetochore far from the pole would be less likely to be phosphorylated and more likely to exhibit P motion. The kinetochore would persist in P motion until it moved up the gradient far enough so that its level of phosphorylation was sufficient to promote switching to AP motion. By such a mechanism, the kinetochore could oscillate between P and AP phases at an average distance from the pole determined by the phosphorylation potentials needed to switch between P and AP motion. This model could explain the oscillations of mono-oriented chromosomes 10 μm or more from their poles in our newt cells. However, it, like any other scalar gradient models of chromosome positioning in the spindle, fails to explain the movements and positions achieved by bi-oriented chromosomes. Sister kinetochores are separated by only 1–2 μm across the centromere region. Because of their close proximity, both sister kinetochores of a bi-oriented chromosome near a pole should be similarly affected by a scalar gradient, particularly considering that the typical pole-to-pole distance in newt lung cells is so large (40–50 μm). However, our data shows that bi-orientation results in active P motion of the kinetochore distal and AP motion of the kinetochore proximal to the pole which moves the centromere toward the equator of the spindle and 10–15 μm further away from the average position a mono-oriented chromosome achieves from the pole. In addition, we have observed that centrophilic mono-oriented chromosomes can reside quite close to the pole for extended periods of time when they are outside of the spindle region (our personal observations). Also, McNeill and Berns (1981) and Rieder et al. (1986) generated mono-oriented chromosomes from bi-oriented chromosomes by laser ablating one of the two sister kinetochores. These chromosomes moved to and remained quite near the pole still tethered to the intact kinetochore.

The major kinetic features of kinetochore directional instability, the persistent P and AP constant velocity phases, and the abrupt switches between these phases, are the major kinetic features of MT-end dynamic instability (Mitchison and Kirschner, 1984; Walker et al., 1988*b*). This similarity suggests that synchronous dynamic instability between the 15–20 plus-ends at the kinetochore attachment sites is responsible, or at least governs, the directional instability of kinetochore motion. The velocities of P and AP kinetochore motion (~1.5 μm/min on average) are, however, an order of magnitude slower than the velocities of the growth phase (14 μm/min on average) and shortening phase (17 μm/min on average) of free plus-end polar MTs (Cassimeris et al., 1988*b*; Hayden et al., 1990). This reduction in velocity is interesting and may occur for several reasons. Possibly not all kMT plus-ends are in the same phase of assembly, i.e., a few are trying to elongate while most are trying to shorten during P motion. In addition, the association and dissociation rate constants could be reduced by diffusional limitations or interactions within the kinetochore attachment sites. If most of the kMTs did not synchronously switch between P and AP phases, the velocity of kinetochore motion would decrease and the oscillations would become dampened. This lack of synchrony may explain why kinetochores in some cell types do not exhibit the large amplitude kinetochore oscillations typical of vertebrate cells.

There is also evidence that the energetics of dynamic instability can contribute to pushing and pulling forces on the centromere. Hotani and Miyamoto (1990) have provided direct evidence that MT ends can push against a boundary while they are growing by tubulin association at the pushing end. Bajer (1982) and Ault et al. (1991) have shown that promoting MT assembly with drugs like taxol induces outward motion of both kinetochores and chromosome arms. In addition, in vitro studies have shown that pulling forces can be generated by depolymerization of MTs attached to the centromere region of isolated chromosomes (Coue et al., 1991) in the absence of ATP to fuel motor activity (Koshland et al., 1988; Coue et al., 1991; Lombillo et al., 1992). In extracts, particles attached to the wall of MTs have been observed to begin moving concurrently with the shortening end of a MT (Coue et al., 1991; Gliksman and Salmon, 1993; and personal observations).

Substantial evidence from living cell experiments shows that factors which specifically alter MT assembly can induce switching between P and AP phases of kinetochore directional instability. Addition of nocodazole to metaphase cells to block MT assembly induces P motion of all kinetochores at typical P velocities with no switches to the AP phase (Cassimeris et al., 1990). Addition of taxol, a MT stabilizing agent, induces persistent AP motion (Ault et al., 1991). Sheldon and Wadsworth (1992) have shown that when mammalian tissue cells are microinjected with tubulin near the spindle equator during early anaphase A, kinetochores switch to AP motion concurrent with kMT elongation at the kinetochore attachment site. The kinetochores switch back to P movement after the local elevation in tubulin concentration decreases by diffusion.

Given the above evidence, it is likely that a switch between kinetochore AP and P motion is fundamentally determined by kMT plus-end switching between the growing and shortening phases of dynamic instability. Growing MT ends are thought to be stabilized by a GTP-tubulin cap (Caplow, 1992). Abolishing the GTP cap on all the plus-ends would switch the kinetochore to the P phase of motion (Mitchison and Kirschner, 1984; Walker et al., 1988*a*, 1991). The

kinetochore would persist in P movement until the ends regained a stabilizing cap. Such a model would explain the effects that nocodazole, taxol, and elevated tubulin concentrations have on inducing kinetochore switching between P and AP movement. Under normal assembly conditions, other factors, such as proteins which activate the hydrolysis of GTP on tubulin (loss of the stabilizing cap) within kinetochore attachment sites could play a key role in regulating the switch from AP to P movement. Alternatively, switching between plus- and minus-end–directed motor activity at the kinetochore attachment site could also influence switching between kMT growing and shortening phases. However, like the phosphorylation model analyzed above, scalar potential gradients do not provide a satisfactory explanation of kinetochore directional instability, nor do they explain the positions achieved by kinetochores during congression and segregation.

Tension at the Kinetochore Is Probably a Key Factor Regulating Switching between P and AP Phases

Tension and compression have previously been postulated to affect MT elongation and shortening, respectively (Hill and Kirschner, 1982). Experiments performed in vivo have shown that an AP force applied to a chromosome arm stretches the chromatin, exerting tension at the kinetochore attachment site and resulting in chromosome AP movement (Nicklas, 1977, 1988). Our evidence that tension is an important switching factor is based on the differences observed between kinetochores of adjacent mono-oriented chromosomes and sister kinetochores on bi-oriented chromosomes. We found that the switching of adjacent kinetochores was random with respect to each other while the switching of sister kinetochores was predominantly cooperative. Although sister kinetochores of bi-oriented chromosomes were occasionally seen to switch independently of each other so that the centromere regions became either stretched (both in P phase) or compressed (both in AP phase), sister kinetochores usually exhibited coordinated and opposite phases of motion with respect to their poles. Based on the extensive studies of Nicklas (Nicklas, 1988), tension generated across the centromere region seems the most likely mechanism generating the cooperativity of out-of-phase motion of sister kinetochores. In addition, we found that chromosome bi-orientation always resulted in immediate tension across the centromere as the distal kinetochore started to move toward the equator (data not shown). When the distal kinetochore started moving toward the equator, the proximal kinetochore switched and persisted in prolonged AP motion. Conversely, at the onset of anaphase and chromosome separation, proximal kinetochores persisted in prolonged P motion, briefly punctuated with short-lived AP phases. In either case, the velocities of AP and P motion were typical of the velocities exhibited by oscillating kinetochores of mono-oriented chromosomes near the pole and bi-oriented chromosomes near the equator (Table I and Figs. 4 and 5). Thus, tension influences only the frequency of kMT switching and not the rate of assembly dynamics. Decreased tension promotes switching to P movement while increased tension promotes switching to AP movement.

A New View of Chromosome Congression and Segregation Based on Kinetochore Directional Instability and Control of Switching by Tension

We summarize our interpretation of the results in this paper with a molecular model of the kinetochore attachment site and a mechanistic model of congression and segregation. (*a*) Kinetochores have the capability of abruptly and autonomously switching between persistent phases of P and AP motion where motion is tightly coupled to the dynamic instability of the plus-ends of kMTs. P motion, produced by kinetochore pulling forces on the centromere, and AP motion, produced by kinetochore pushing forces on the centromere, deform the centromere and drive net chromosome displacement. (*b*) A combination of minus-end–directed or plus-end–directed MT motor domains is probably responsible for persistent attachment to plus-ends. This combination would ensure that the kinetochore attachment site holds onto, or "tracks," the growing and shortening kMT plus-ends similar to the kinetochore model presented by Mitchison and coworkers (Mitchison, 1988; Hyman and Mitchison, 1990). (*c*) Tension at the kinetochore is a key modulator of switching between P and AP phases: high tension increases the probability of switching to AP motion while low tension or compression at the attachment site increases the probability of switching to P motion. Kinetochores persist in P movement under low tension such as that generated by drag forces on the chromosome arms. High tension is produced by either sister kinetochore pulling forces for bi-oriented chromosomes or by the AP ejection forces exerted on the chromosome arms near the pole. (*d*) The strengths of kinetochore P pulling and kinetochore AP pushing forces on the centromere do not depend on distance from the poles since centromere deformations of mono- and bi-oriented chromosomes are similar. (*e*) The AP ejection forces on the arms provide a vectoral control of chromosome positioning, with the ejection force increasing towards the pole due to increased MT density as proposed earlier by Rieder, Salmon, and coworkers (Rieder et al., 1986; Salmon, 1989*a*, *b*; Rieder et al., 1990; Ault et al., 1991). Thus, mono-oriented chromosomes oscillate about average positions many microns from their poles where the kinetochore tension generated by AP ejection forces on the arms is sufficient to trigger a switch to kinetochore AP motion. When the tension drops with outward movement, the probability of the kinetochore switching back to P motion increases. Tension, produced when the distal sister kinetochore orients, biases the proximal kinetochore to switch to AP motion. Bi-oriented chromosomes move to positions near the equator, kinetochores leading the arms, because that is where the effects of opposing ejection forces on sister kinetochore-associated forces are roughly balanced. (*f*) When sisters separate at the onset of anaphase, their kinetochores persist in P motion. AP phases occur probably due to tension generated either by chromosome bridging or ejection forces on their arms. In late anaphase, there is evidence that polar MT dynamics decrease, which would decrease the strength of the ejection forces on the arms and allow the chromosomes to move closer to the pole. Kinetochore directional instability may be turned off in late anaphase (Sheldon and Wadsworth, 1992; Mitchison and Salmon, 1992) and the kinetochore becomes parked on the kMT lattice. Thus, continued kinetochore P movement later

in anaphase A may be solely determined by the rate of kMT poleward flux (Mitchison and Salmon, 1992).

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