TEMPERATURE DEPENDENCE OF ANAPHASE CHROMOSOME VELOCITY AND MICROTUBULE DEPOLYMERIZATION

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ABSTRACT

The time course of chromosome movement and decay of half-spindle birefringence retardation in anaphase have been precisely determined in the endosperm cell of a plant *Tilia americana* and in the egg of an animal *Asterias forbesi*. For each species, the anaphase retardation decay rate constant and chromosome velocity are similar exponential functions of temperature. Over the temperature range at which these cells can complete anaphase, chromosome velocity and retardation rate constant yield a positive linear relationship when plotted against each other. At the higher temperatures where the chromosomes move faster, the spindle retardation decays faster, even though the absolute spindle retardation is greater. Chromosome velocity thus parallels the anaphase spindle retardation decay rate, or rate of spindle microtubule depolymerization, rather than absolute spindle retardation, or the amount of microtubules in the spindle. These observations suggest that a common mechanism exists for mitosis in plant and animal cells. The rate of anaphase chromosome movement is associated with an apparent first-order process of spindle fiber disassembly. This process irreversibly prevents spindle fiber subunits from participating in the polymerization equilibrium and removes microtubular subunits from chromosomal spindle fibers.

Two major events of considerable interest are found in normal anaphase. These are the poleward movement of chromosomes, and the decrease of anaphase spindle birefringence.

Poleward movement of chromosomes in normally dividing plant and animal cells is influenced by the temperature at which the cells are maintained. The studies of Barber (4) with *Tradescantia* stamen hair and petal cells and those of Ris (33) with grasshopper spermatocytes each present chromosome velocities at three temperatures, which reveal a trend that with increasing temperature the rate of chromosome movement increases. Earlier, Bucciante (7) found similar results for the duration of anaphase at different temperatures in cultured chick cells. His data presented as a Krough (27) plot, reciprocal duration of anaphase vs. temperature, showed an exponential-like increase from 20° to 40°C. At still higher temperatures the reciprocal duration of anaphase decreased rapidly.

A decrease of spindle birefringence during anaphase has been observed in various marine eggs (23, 36, 37, 43), tissue culture cells, pollen mother cells of *Lilium* (17), and the endosperm mitosis of *Haemanthus* (2, 21) and *Tilia* (12, 16). This decrease of total spindle birefringence is a common feature of anaphase in dividing plant and animal cells, with the exception of certain insect spermatocytes.

For any given stage of mitosis, temperature can

be used to control the amount of birefringence in the spindle (14, 19, 20, 41). Temperature also affects the rate at which the spindle birefringence changes. As I have briefly reported previously, the rate of birefringence decay seen in anaphase increases with increasing temperature over the normal temperature range for mitosis (12, 14).

The purpose of this investigation is to determine if a correlation exists between anaphase movement of chromosomes and the decay of anaphase spindle birefringence, using temperature as the independent variable, in normally dividing plant and animal cells. Such is predicted by the dynamic equilibrium model proposed by Inoué.

The dynamic equilibrium model of spindle assembly-disassembly (19, 25, 26) states that the movement of chromosomes in anaphase is accomplished by the slow removal of subunits from the chromosomal spindle fibers. The removal of subunits must be maintained at a rate which allows the spindle fibers to reach a new equilibrium position while maintaining mechanical integrity between the kinetochores on the chromosomes and the poles. If the spindle fiber subunits are removed too rapidly, the spindle fibers collapse and the chromosomes do not move. The lability of microtubules required for this model is well established for spindle fibers (20, 25, 26, 31) and nonmitotic microtubular systems (28, 44).

Sato and co-workers (34, 35) have found that the birefringence of spindles isolated from sea star oocytes reveals a close fit to the Wiener rodlet form birefringence curve when measured in imbibing fluids of 1.33–1.67 refractive index range. The rodlet volume fraction value for the best fit Wiener curve is consistent with the volume fraction of microtubules obtained from electron microscopy and hydrodynamic data. These observations indicate that birefringence of the spindle fibers in living cells is attributable to the measures of the concentration of oriented spindle microtubules. The polymerized tubulin content of sea urchin mitotic apparatus, isolated under various conditions, is also directly proportional to the retardation of the in vivo spindle (39, 40).

MATERIALS AND METHODS

Method for Tilia americana Endosperm

The technique and method of preparing *Tilia americana* (American basswood) endosperm cells for observation in polarized light has been previously described (16). The cells, after being mounted on the temperature control slide (22, 24), are held at 22°C until a moderately flattened prometaphase cell is located. The criterion for flattening is that the row of chromosomes be clearly visible (Fig. 1 B, frame A). When a suitable prometaphase cell is chosen, photographs and measurements of spindle retardation are made for a few minutes. The temperature is then rapidly shifted to a higher or lower value and maintained at the new temperature until the cell enters late telophase, when the chromosomes have ceased moving and the cell wall is partially complete.

Method for Asterias forbesi Eggs

Eggs in the first and second mitotic divisions of the sea star *Asterias forbesi* were used to provide a comparison of the anaphase parameters in plant and animal cells. To collect sperm, single arms of a mature male *Asterias* are removed by induced autotomy (13). The testis is removed from the arm by clipping the gonoduct. The isolated testis is stored at 8°C in a covered finger bowl, where "dry" sperm extruded from the testis remains viable for 5–6 days. The technique for obtaining eggs repeatedly from a single female *Asterias* has been previously described (12). The ripe eggs, contained in about 10 ml of filtered seawater are fertilized with two or three drops of 1% solution of dry sperm. Only those batches of eggs which show 98–100% fertilization are used in the experiment. After the percent fertilization is determined by inspection, the number of eggs in the culture dish are reduced until they are no more than one layer deep.

The fertilized *Asterias* eggs are mounted on the temperature control slide in the following manner. A spacer made of a hollowed 16-mm square of filter paper (no. 541 Whatman) is placed on the window of the temperature control slide and saturated with seawater. A small drop (about 5 μl) of the fertilized eggs is placed in the center of the temperature control slide window with a micropipette. An 18-mm square cover slip is placed immediately on the spacer and the drop of eggs, and the completed preparation is carefully sealed with Valap (14, 16) to prevent evaporation of water from the spacer and drop containing the eggs.

Two experimental protocols are used with the fertilized *Asterias* eggs. For observations at temperatures greater than 8°C, the eggs are maintained at a given constant temperature from a few minutes after fertilization to the completion of second cleavage. Eggs treated in this manner comprised the control group. Nuclear membrane breakdown is inhibited in *Asterias* at temperatures below 8°C. Therefore, for observing mitosis at lower temperatures, the fertilized eggs are maintained at optimal temperature (15.5°–17.5°C) until the completion of nuclear membrane breakdown. Then the temperature is rapidly shifted to a lower value and maintained constant until cleavage is complete or until the attempted cleavage furrow regresses. Each procedure resulted in the same response for anaphase in the normal temperature range (8°–23°C) of the animal.

Microscopy

Observations were made with a modified Leitz Ortholux polarizing microscope, using either a specially selected HN-22 Polaroid sheet or a Glan-Thomson prism as a polarizer. The condenser optics consists of a Leitz UMK 32 (working NA 0.4) long working-distance objective mounted on a rectifier from an American Optical condenser. The objective lenses were either a Leitz UMK 32 (NA 0.4) or Nikon rectified (18) 20X(NA 0.4). For illumination, light from an Osram HBO-200 W mercury arc lamp was filtered with a 546-nm narrow band pass, high transmission, interference filter in a combination mount with a glass heat-cut filter.

Spindle retardation is measured visually using a Brace-Köhler compensator equipped with electrical readout for rapid measurements (22, 24). The spindle pole-to-pole axis is set at 45° to the polarizer-analyzer axes. The spindle fibers are brought to extinction and both the compensator angle (θ_{sp}) and the background extinction (θ_{BG}) adjacent to the cell are recorded. Measurements of (θ_{sp}) are taken about every 3–4 s from the time of nuclear membrane breakdown to the end of telophase. The retardation of the spindle fibers (r_{sp}) is determined by the equation:

$$r_{(sp)T} = r_{comp} \sin 2 (\theta_{sp} - \theta_{BG}),$$

where r_{comp} is the retardation of the compensator and T is the temperature to which the cell is subjected. This measurement of $r_{(sp)T}$ reflects a per unit volume amount of oriented subunits in the spindle, and should be a direct function of the spindle polymer (tubulin) concentration, on the assumption that spindle microtubules in the region measured are essentially parallel.

Photographic records of the cell under observation are made at about 1-min intervals with a Leitz Ortholux Orthomat system using 35 mm Kodak Plus-X film. Magnification is calibrated by photographing a standard stage micrometer.

The temperature control apparatus used for heating and cooling the cells has been previously described (22, 24). Fogging at low temperatures of the temperature-control slide bearing the living cells is prevented by blowing dry air over the windows of the slide or by coating the windows with a thin layer of Kodak Foto-Flo (Eastman Kodak Co., Rochester, N. Y.) (41).

RESULTS

Normal mitosis occurs in *Tilia* endosperm over a temperature range of 10°–25°C. In *Asterias* eggs this temperature range is 8°–23°C. I shall call these ranges "physiological." Temperatures outside these ranges inhibit development by blocking the ability of the cell to enter anaphase. At temperatures of 10°C or less, anaphase in *Tilia* fails because the chromosomes do not move pole-

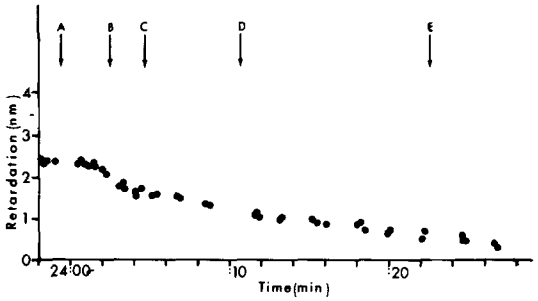

FIGURE 1 *a* An example of spindle retardation changes measured 4–5 μm from a row of chromosomes during the time course of anaphase in *Tilia americana* endosperm (Cell 72 h 1 III: T°C = 20). Time of experiment in hours, minutes; A, late metaphase; B, onset of anaphase; C, early anaphase; D, midanaphase; E, telophase.

ward after the kinetochores separate. The same phenomenon occurs in *Asterias* at temperatures below 8°C. Anaphase inhibition at high temperatures is the result of the inability of the kinetochores to separate. Above 25°C the *Tilia* spindle remains arrested in a metaphase configuration and the half-spindle retardation slowly decreases, becoming undetectable in about 60 min. At temperatures greater than 23°C the *Asterias* spindle can undergo pole-to-pole elongation, but the characteristic anaphase gap at the spindle equator fails to appear, indicating that there is no chromosome separation. Thus, in animal and plant cells, high temperature inhibits kinetochore separation and subsequent kinetochore-to-pole shortening of spindle fibers.

The spindle fiber retardation measured 4–5 μm from a row of chromosomes in *Tilia* (Fig. 1 *a*), and 5 μm from the edge of the gap at the spindle equator in *Asterias* (Fig. 2*a*), and thus, retardation clearly exhibits an exponential decay during anaphase. The rate of anaphase spindle-retardation decay is temperature sensitive and increases with increasing temperature over the physiological temperature range of each species. The exponential decay of anaphase retardation when plotted according to the first-, second-, and third-order reaction equations (42) suggests it to be a first-order process. A typical example for *Asterias* anaphase at 17.0°C is shown in Fig. 3. The first-order rate equation for the anaphase depolymerization of microtubules measured as decrease of spindle retardation is:

$$kt = ln(r_o)/(r_t),$$

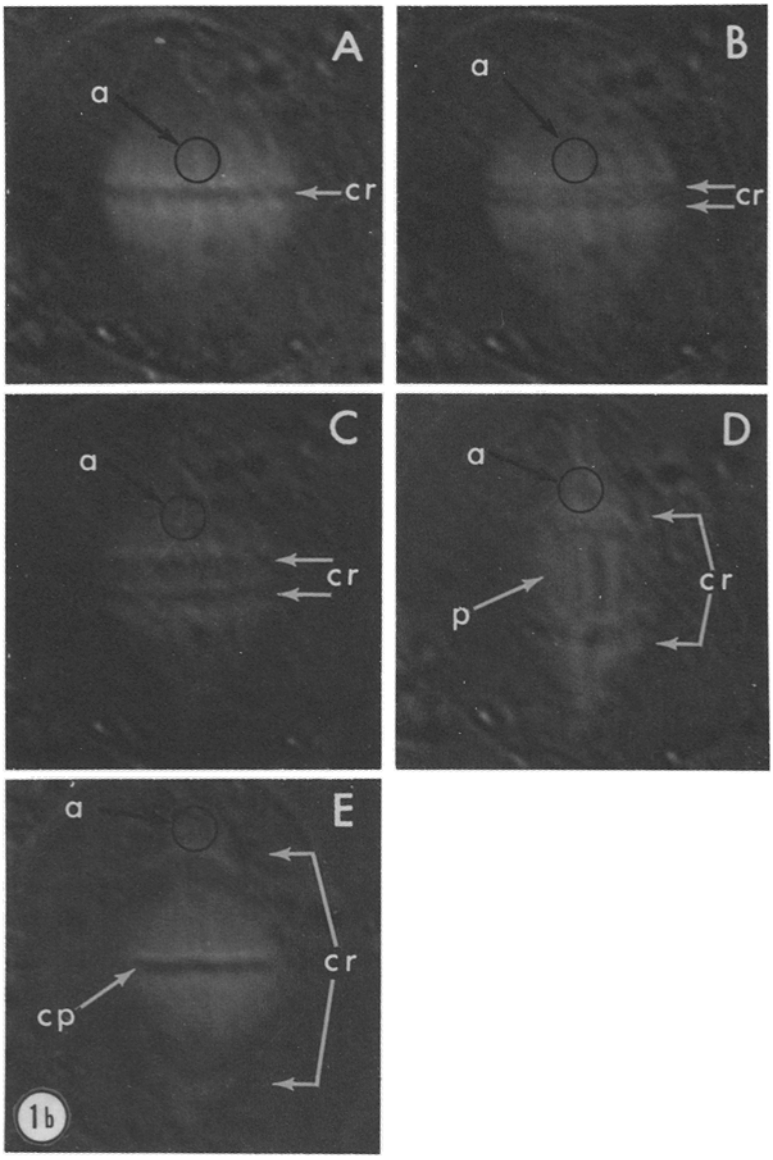

FIGURE 1 *b* Selected examples of *Tilia americana* endosperm mitotic spindle (same cell as in Fig. 1 *a*). These show the area (*a*) in which spindle retardation is measured (see Fig. 1 *a*) and separation of the chromosome rows (*cr*). Scale interval, 10 μm. Capital letters refer to the same stage of mitosis given in Fig. 1 *a*. *p*, phragmoplast fibers; *cp*, cell plate.

where k is the rate constant, (r_t), the observed values of spindle retardation of time t, and (r_o) the maximum value of spindle retardation of the onset of anaphase. A plot of $\log (r_o)/(r_t)$ vs. time for each experimental temperature yields a series of straight lines whose slopes are equal to $k/2.303$ (Fig. 4). The rate constant (k) for anaphase spindle depolymerization increases as an exponential function over the physiological temperature range described for *Tilia* (Fig. 5 A) and *Asterias* (Fig. 5 B).

The chromosomes of *Tilia* are clearly visible (Fig. 1 *b*) at all stages of anaphase, and their poleward displacement at different temperatures is

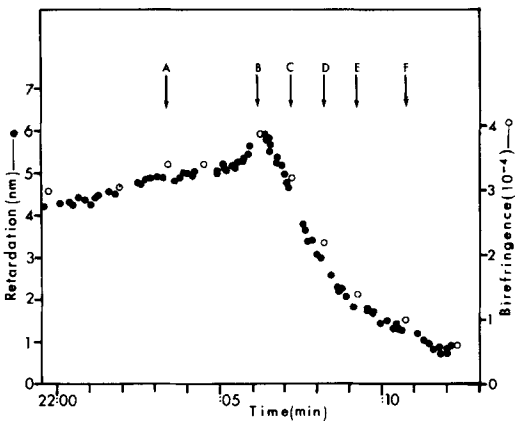

FIGURE 2 *a* Example of retardation and birefringence changes in the half-spindle of *Asterias forbesi* eggs in first mitosis (Cell 72 f 21 I: T°C = 17.0). The retardation and birefringence (retardation per unit thickness of the spindle) curves are parallel, indicating that the observed decay in anaphase in the result of the loss of numbers of microtubules. A, metaphase; B, onset of anaphase; C, early anaphase; D, midanaphase; E, late anaphase; F, telophase.

easily measured (Fig. 6). Poleward velocity of the chromosomes is taken as one-half the rate of increase in the interkinetochore separation (displacement of the chromosome rows) along the spindle axis. Between 10°C and 25°C the poleward velocity increases temperature (Fig. 7 A). Determination of the positions of anaphase chromosomes in *Asterias* cells is difficult because the chromosomes are small and lack sufficient contrast to be readily visible in polarized light. In many cases, however, the chromosomes can be faintly seen and no problems are encountered. The poleward displacement (Fig. 8) of *Asterias* chromosomes during anaphase is determined by subtracting the combined length of the two daughter half-spindles from the spindle pole-to-pole length. The resulting value corresponds to the "gap" between daughter half-spindles (Fig. 2 *b*, G). The actual velocities of chromosomes as a function of temperature for *Tilia* and *Asterias* are in close agreement with each other and with other species over their respective temperature ranges (Fig. 9).

For both *Tilia* and *Asterias* the rate constant for anaphase spindle birefringence decay and chromosome velocity are similar exponential functions of temperature (Figs. 5 and 8). These anaphase parameters when plotted against each other yield a linear-positive relationship (Fig. 10 for *Tilia* and Fig. 11 for *Asterias*). This is the first description of the existence of a correlation between anaphase chromosome movement and spindle fiber retardation changes. The linear-positive correlation of these rates shows that the more rapidly the anaphase spindle microtubules depolymerize, the faster the chromosomes are moved poleward and vice versa.

In contrast to the rate of retardation decay, when equilibrium retardation and chromosome velocities are plotted on the same axis against temperature, the two curves are totally dissimilar. The chromosome velocity continues to increase with increasing temperature even after the spindle fibers have attained their maximum value of retardation (Figs. 12 and 13 for *Tilia* and *Asterias*, respectively). At temperatures above 23°C for *Asterias* and 25°C for *Tilia*, chromosomes stop moving (Fig. 8, 9) and the equilibrium spindle retardation begins to decrease (Figs. 12 and 13). This is suggestive of a single temperature-sensitive process whose thermal inactivation blocks the cells from entering into anaphase.

Thus, within the physiological temperature range, chromosome velocity is correlated directly with the rate of spindle-fiber depolymerization. Chromosome velocity is not correlated with the absolute amount of spindle retardation in either anastral plant or astral animal spindles.

DISCUSSION

Early investigations (4, 33) established that chromosomes in anaphase move faster at higher temperatures. Before the present study, however, only an approximate relation of chromosome movement to temperature was known. It is a striking fact that chromosome velocity for the two species reported in this paper and those reported by Barber (4) and Ris (33) all follow the same exponential function of temperatures (Fig. 9). This suggests a universal control mechanism for anaphase chromosome velocities. Analysis of chromosome velocity vs. temperature by the Arrhenius (1) equation results in a "critical thermal increment" (U) (8) or liminal activation energy (32) of 18.1 ± 1.3 kcal/mol for *Tilia* and 16.2 ± 0.3 kcal/mol for *Asterias* (14). The quantity U is independent of temperature and the actual velocity of the process. It is also more sensitive and theoretically a more significant index than the Q_{10} ratio for the characterization of a process (8, 9).

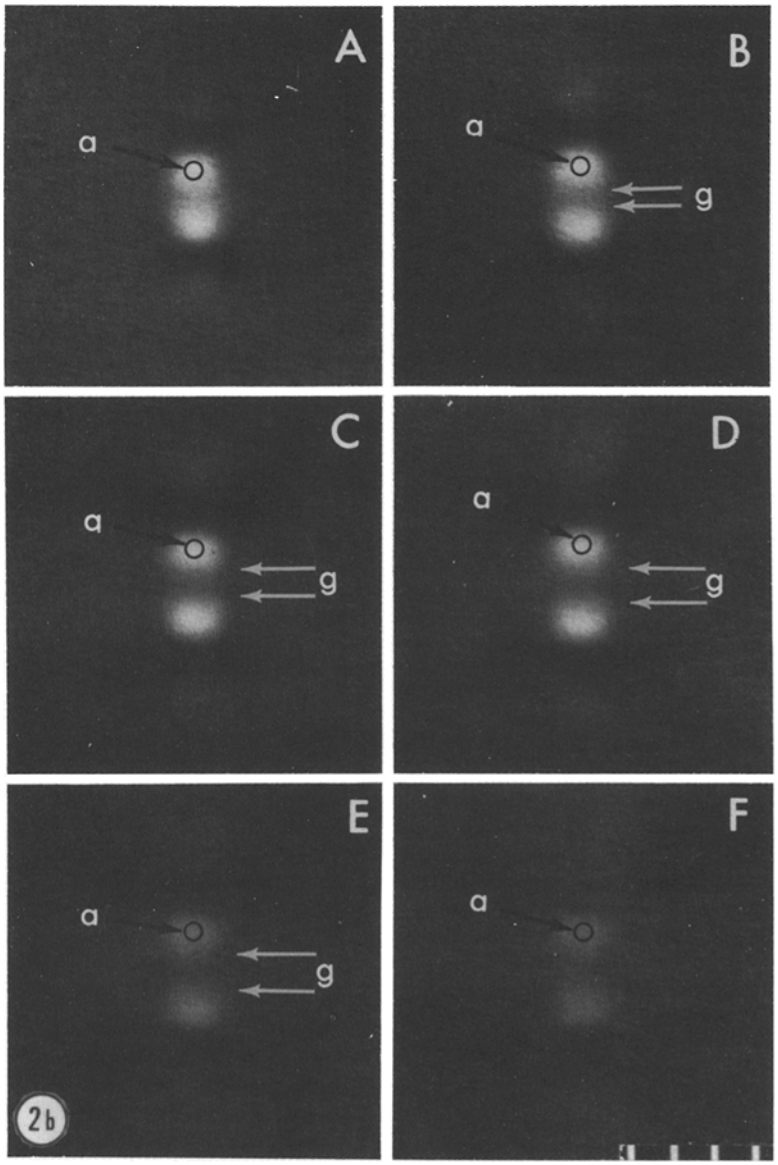

FIGURE 2 *b* Selected examples of *Asterias* first mitosis spindle (same cell as in Fig. 2 *a*). These photographs illustrate where the spindle retardation (see Fig. 2 *a*) is measured (*a*) and the increase in separation of the daughter half-spindles resulting in the anaphase "gap." Scale interval = 10 μm. Capital letters refer to the same mitotic stages given in Fig. 2 *a*.

For any given temperature, spindle retardation decays exponentially with time (Figs. 1 *a* and 2 *b*; reference 16). The anaphase retardation-rate constants plot on a logarithmic function of temperature (Fig. 5). The U value for spindle retardation decay in *Tilia* is 11.8 ± 1.4 kcal/mole and 13.9 ± 0.3 kcal/mol for *Asterias* (14).

Chromosome velocity and the rate of anaphase spindle-fiber decay are similar functions of temperature and energetics in active anaphase. This

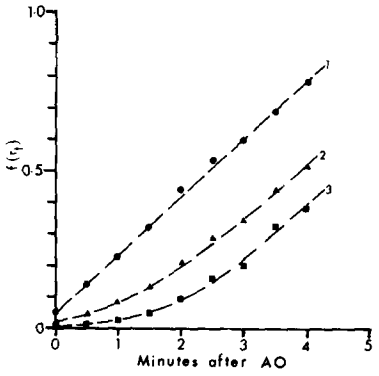

FIGURE 3 Determination of the reaction order for the decay of anaphase spindle fiber retardation. The observed data for Asterias egg (72 f 21:I) is plotted according to the first-order rate equation, circles, $f(r_t) = \log (r_o)/(r_t)$, second-order rate equation, triangles, $f(r_t) = 1/(r_t) - 1/(r_o)$, and third-order rate equation, squares, $f(r_t = 1/(r_t)^2 - 1/(r_o)^2$. The first-order equation yields the best straight line fit to the observed data, indicating the process of anaphase birefringence decay to be first order.

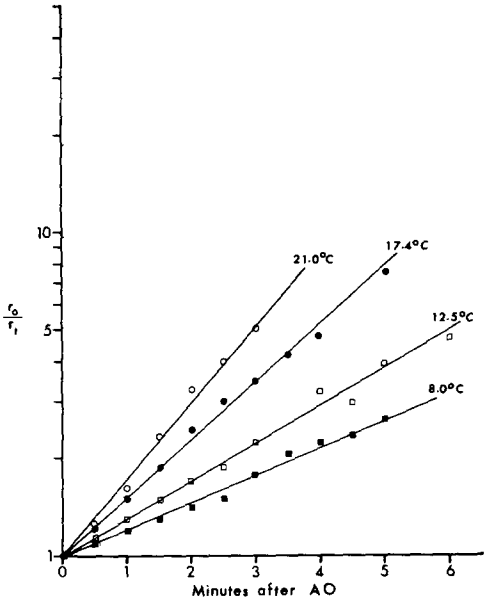

FIGURE 4 The rate constant (k) for the decrease in spindle retardation for *Tilia* or *Asterias* at different temperatures is obtained from the slope of a plot of log r_o/r_t vs. time. This example shows *Asterias* anaphase at four different temperatures. r_o is the spindle retardation at anaphase onset, and r_t is the spindle retardation at time in minutes after anaphase onset.

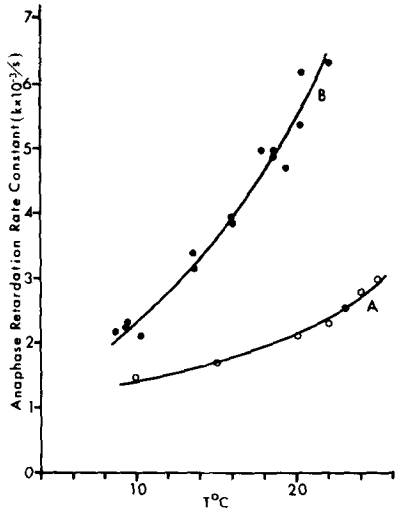

FIGURE 5 The rate of anaphase spindle fiber retardation decay for *Tilia* endosperm cells (curve A) and *Asterias* eggs (curve B), given as a first-order rate constant (k) defined by Eq. (1), increases exponentially with increasing temperatures over the range at which the two species can complete normal mitosis. The solid lines are each fitted by a least squares exponential function (30, 6).

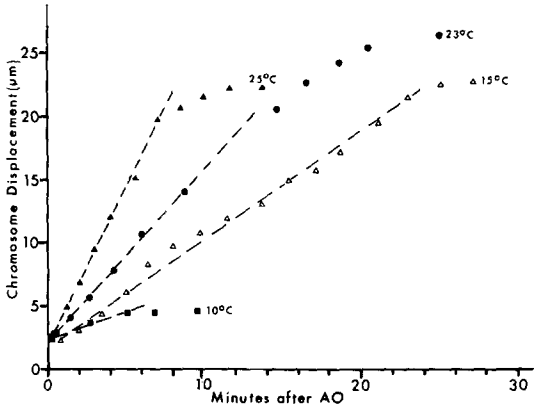

FIGURE 6 The mean poleward displacement of chromosomes on the spindle axis during *Tilia* anaphase maintains a basic sigmoid response regardless of temperature. One-half the slope of the dashed line is used for chromosome velocity. Time zero is defined as anaphase onset as seen in Fig. 1 *a*.

suggests that the same rate-limiting reactions controlling anaphase are common to both anastral plant and astral animal spindles.

In agreement with the critical thermal incre-

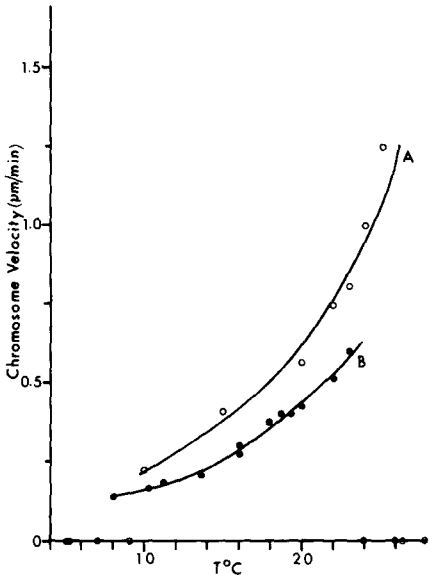

FIGURE 7 Example of the poleward displacement of *Asterias forbesi* chromosomes taken as the increase in the gap between anaphase daughter half-spindles at three different temperatures. Time zero is defined as anaphase onset as seen in Fig. 2 *a*. One-half the slope of the dashed lines indicated the chromosome velocity toward the spindle pole at different temperatures.

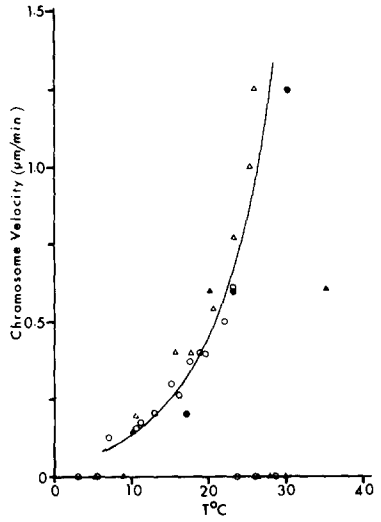

FIGURE 9 Chromosome velocity measured as the rate of separating daughter chromosomes in *Asterias* eggs (open circles), grasshopper spermatocytes (solid circles) (33), plant cells, *Tilia* endosperm (open triangles), and *Tradescantia* stamen hair cells (solid triangles) (4) increases exponentially with increasing temperature. Barber's data (4) contains an odd point at 35°C, but the velocities obtained at the two lower temperatures agree with the present data. The solid line is fitted to all the observed points by a least-squares exponential function. The odd point at 35°C was omitted from the analysis.

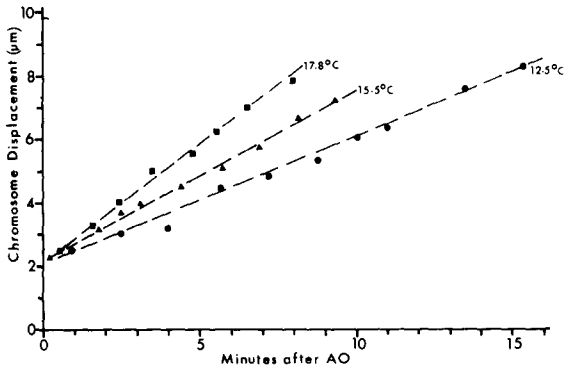

FIGURE 8 The poleward velocities of chromosomes in *Tilia* endosperm (curve A) and *Asterias* eggs (curve B) are similar functions of temperature (Fig. 5 vs. Fig. 8). Chromosome velocity for *Asterias* anaphase is expressed as one-half the rate of separation of the daughter half-spindles. No chromosome movement is observed at temperatures greater than 26°C for *Tilia*, or above 23°C and below 8°C for *Asterias*. Chromosome velocity outside of three respective temperature ranges is zero. Within these temperature ranges the velocity of chromosomes increases exponentially. The solid lines are fitted by the method of least squares for exponential functions.

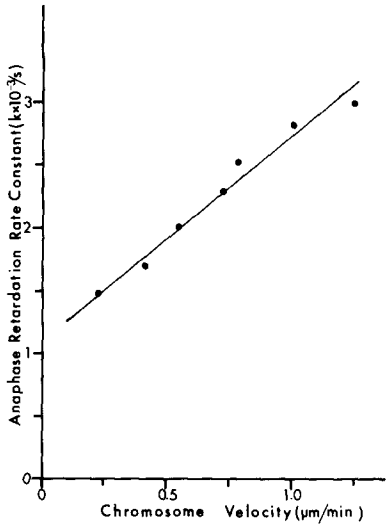

FIGURE 10 The rate of anaphase spindle fiber retardation decay rate constant (k), as defined by Eq. 1, when plotted against chromosome velocity in *Tilia* endosperm mitosis yields a linear equation. The straight line was fitted to the experimental points by the method of least squares.

ments, the rate of anaphase spindle retardation decay and chromosome velocity correlate linearly with each other (Figs. 10 and 11). This relationship states that within the physiological temperature range of a species in which anaphase chromosome movement can occur, the faster the spindle fibers depolymerize, the faster the chromosomes move poleward. This correlation is consistent with the conditions stated for chromosome movement by the dynamic equilibrium model, and strongly suggests that depolymerization of a single component, the spindle microtubules, into nonbirefringent subunits is responsible for anaphase chromosome movement.

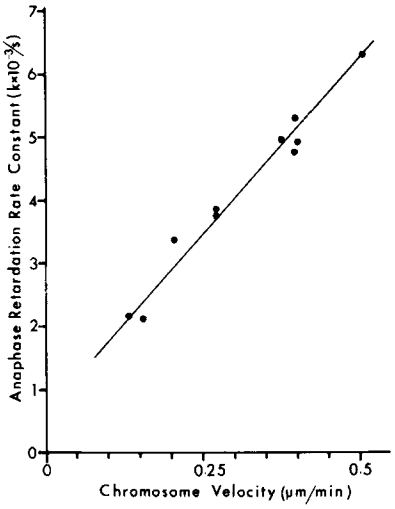

FIGURE 11 The rate of anaphase retardation decay given as the rate constant (k) defined as Eq. 1, plotted against chromosome velocity in *Asterias* mitosis results in a linear response. The straight line was fitted to the experimental points by the method of least squares.

In normal anaphase and cases of induced chromosome movement (24) the rate of spindle microtubule depolymerization must be controlled so that the rate constant (k) of depolymerization is maintained between 1.5 and 6.5 $\times$ 10^{-3} s^{-1}. Rates of spindle-fiber microtubule depolymerization which exceed 6.5 $\times$ 10^{-3} s^{-1} produce a rapid random collapse of the structure. The spindle fibers do not shorten, but fall apart uniformly in place. Motive forces necessary for chromosome movement are not produced and/or transmitted by the rapidly collapsing kinetochore fibers. Rates below 1.5 $\times$ 10^{-3} s^{-1} indicate that kinetochore fibers can be depolymerized too slowly for the production of forces needed to move the chromosomes. No poleward movement of chromosomes occurs when the anaphase spindle microtubules fall apart too rapidly (14) or too slowly (Figs. 10 and 11), or are not present. The rate of spindle microtubule depolymerization must be in a range which allows for the necessary rearrangements in the microtubule structure to maintain a quasista-

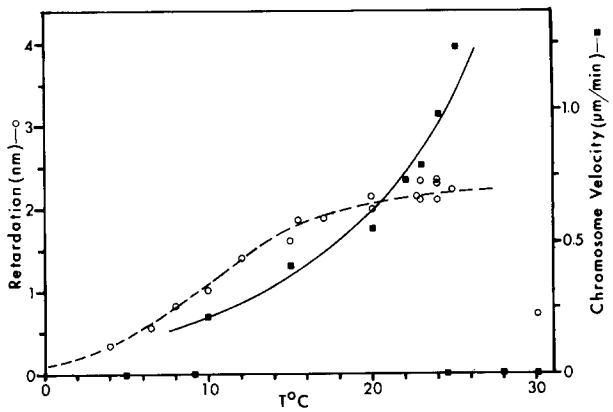

FIGURE 12 The velocity of the chromosomes in *Tilia* continues to increase with increasing temperature even after the spindle has attained its maximum retardation. This would indicate that the velocity of the chromosomes during anaphase does not depend on the absolute amount of microtubules (tubulin) in the spindle. Equilibrium retardation of spindle (open circles); chromosome velocity (solid squares). The solid line fitted to the chromosome velocity values is a least-squares exponential function. The dashed line fitted to the equilibrium retardation data was calculated from the dynamic equilibrium model and the van't Hoff equation (14, 24).

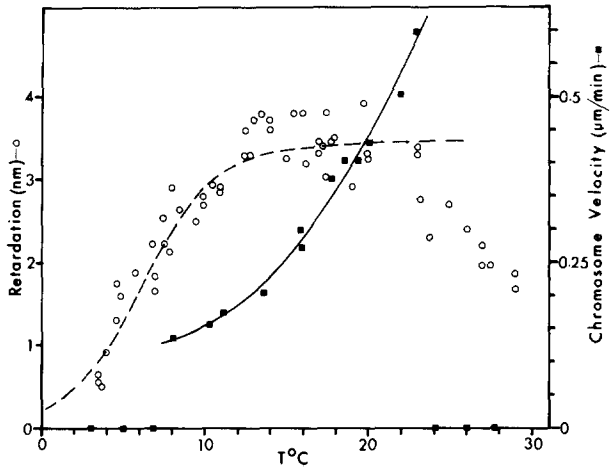

FIGURE 13 Over the temperature range 14°-23°C, where the retardation values (open circles) reach a constant plateau value, the chromosome velocity (solid squares) continues to increase. At temperatures above 23°C the spindle retardation begins to suffer thermal decay and chromosome velocity falls to zero, as the kinetochores appear unable to separate. Chromosome velocity is independent of the absolute amount of spindle fiber retardation present. The solid line fitted to the chromosome velocity values is a least squares exponential function. The dashed line fitted to the equilibrium retardation values was calculated from the dynamic equilibrium model and the van't Hoff equation (14, 24).

ble state which can produce and/or transmit the motive forces required for chromosome movement. Inoué (25) has theoretically shown that slow depolymerization of microtubules can generate sufficient force to move chromosomes through cytoplasm.

The total spindle retardation, which also increases with higher temperatures, is described by a function completely unrelated to the rate of anaphase retardation decay or chromosome velocity. This indicates that anaphase chromosome velocity is independent of the number of microtubules or the concentration of tubulin polymer in the spindle. Instead, it is directly correlated with the rate at which the spindle retardation decays, which measures the rate of microtubule depolymerization. The anaphase microtubule depolymerization rate appears to be governed by a first-order reaction which reduces the pool of free tubulin available for the spindle equilibrium (15).

A mechanism of chromosome movement based on these and earlier observations (14, 15) can be thought of as a series of reaction steps. At anaphase onset, the kinetochores separate and concurrently the pool of free spindle subunits begins being inactivated at a rate defined by the temperature of the cell. As the free subunit concentration decreases, more subunits are drawn to the pool from the microtubules to maintain equilibrium conditions. The loss of subunits creates empty sites in the microtubules, raising the free energy of the system. To minimize again the free energy of the system, the microtubules rearrange their surface lattice (25) to fill the empty sites. Since there are fewer subunits available to maintain the original spindle-fiber length, the more energetically favorable spindle fibers will be shorter. During the time course of anaphase, as the pool of free subunits is continually diminished, the spindle fibers become progressively shorter. This model assumes that the poleward ends of the spindle-fiber microtubules are anchored at or near the centrioles or their equivalent, and that there is a net poleward transport of microtubular material between the microtubule anchor points at the kinetochore and centriolar structures. These assumptions are substantiated by the demonstration that kinetochore fibers in spindles are attached firmly near the spindle pole and to the kinetochore in both grasshopper spermatocytes (5, 29) and *Lilium* pollen mother cells (38), and also by the observation of the poleward transport of birefringent and nonbirefringent material in the spindle (3, 10, 11, 20).

These observations on *Tilia* and *Asterias* add substantial confidence to the dynamic equilibrium model of chromosome movement. Although not eliminating the other popular models, these observations do place serious constraint on and pose questions about the alternative models of chromosome movement.

SUMMARY

The salient points of this study can be summarized as follows. (*a*) Chromosome velocity and rate of anaphase spindle retardation decay correlate linearly. The faster the spindle microtubules fall apart, the faster the chromosomes move, within the physiological temperature range of a given species. (*b*) Chromosome velocity is independent of the total number of microtubules present in the spindle. (*c*) The rate of anaphase spindle retardation decay must be maintained with a rate constant between 1.5 and 6.5 $\times$ 10^{-3} s^{-1} for chromosome movement to occur. Rates out of this range affect the spindle fibers adversely so that sufficient integrity is lacking to produce or transmit the required motive forces for chromosome movement. (*d*) Anaphase chromosome movement and spindle fiber decay appear to be controlled by a first-order reaction which effectively reduces the free pool of microtubule subunits.

These conclusions are consistent with and support the dynamic equilibrium model of chromosome movement proposed by Inoué (20, 26). When combined with the observations of Inoué and co-workers (20, 24–26), they result in constraints which must be considered for any alternate model of chromosome movement to remain meaningful.

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