

Partner telomeres during anaphase in crane-fly spermatocytes are connected by an elastic tether that exerts a backward force and resists poleward motion

James R. LaFountain, Jr^{1,*}, Richard W. Cole² and Conly L. Rieder^{2,3}

¹Department of Biological Sciences, 657 Cooke Hall, University at Buffalo, Buffalo, NY 14260-1300, USA

²Laboratory for Cell Regulation, Division of Molecular Medicine, Wadsworth Center for Laboratories and Research, New York State Department of Health, PO Box 509, Albany, NY 12201-0509, USA

³Department of Biomedical Sciences, State University of New York, Albany, NY 12222, USA

*Author for correspondence (e-mail: jrl@buffalo.edu)

Accepted 19 January 2002

Journal of Cell Science 115, 1541-1549 (2002) © The Company of Biologists Ltd

Summary

As chromosomes move polewards during anaphase in crane-fly spermatocytes, trailing arms commonly stretch backwards for a brief time, as if tethered to their partners. To test that notion, a laser microbeam was used to sever trailing arms and thereby release telomere-containing arm segments (called acentric fragments because they lack kinetochores) from segregating chromosomes. Analysis of the movement of acentric fragments after their release provided clear evidence that previously conjoined partners were indeed tethered at their telomeres and that tethers exerted backward forces that were sufficient to move the fragment across the equator and into the opposite half-spindle. To address concerns that tethers might be artifacts

of in vitro cell culture, spermatocytes were fixed in situ, and stretched arms within fixed cells provided strong evidence for tethers in vivo. The substantial resistance that tethers impose on the poleward movement of chromosomes must normally be over-ridden by the poleward 'pulling' forces exerted at kinetochores. In spermatocytes, poleward forces are supplied primarily by the 'traction fibers' that are firmly attached to kinetochores through end-on attachments to the plus ends of kinetochore microtubules.

Key words: Meiosis, Anaphase, Spindle, Chromosomes, Telomeres, Kinetochores, Laser, Microbeam

Introduction

The poleward movement of chromosomes during anaphase usually occurs with kinetochores leading and arms trailing behind. Kinetochores lead because pole-directed tensile forces generated by, or transmitted through, them 'pull' them and their attached arms to the pole. The mechanism by which pulling forces are generated is not understood, but there is increasing evidence implicating minus end-directed motors (i.e. dynein) (Savoian et al., 2000; Sharp et al., 2000) and microtubule flux (Desai et al., 1998) as important participants. Poorly defined 'resistance' forces are thought to act on chromosome arms and cause them to be dragged behind the leading kinetochore. As to how resistance is imposed on chromosome arms, any one or combination of the following three possibilities could be involved. Besides the viscosity of the cytoplasm, which surrounds chromosomes and therefore may offer resistance to their poleward movement (Nicklas, 1988; Alexander and Rieder, 1991), two specific mechanisms have been proposed: polar ejection forces (Rieder and Salmon, 1994), possibly based on plus-end-directed motors (i.e. chromokinesin) associated with chromosome arms (Fuller, 1995); and ill-defined 'tethers' (reviewed by Ilagan et al., 1997) that connect segregating partners and thereby resist their movement away from one another. Based on the recent discovery that chromokinesin in *Xenopus* oocyte extracts is actually destroyed at anaphase (Antonio et al., 2000; Funabiki and Murray, 2000), a role for it in imposing resistance to

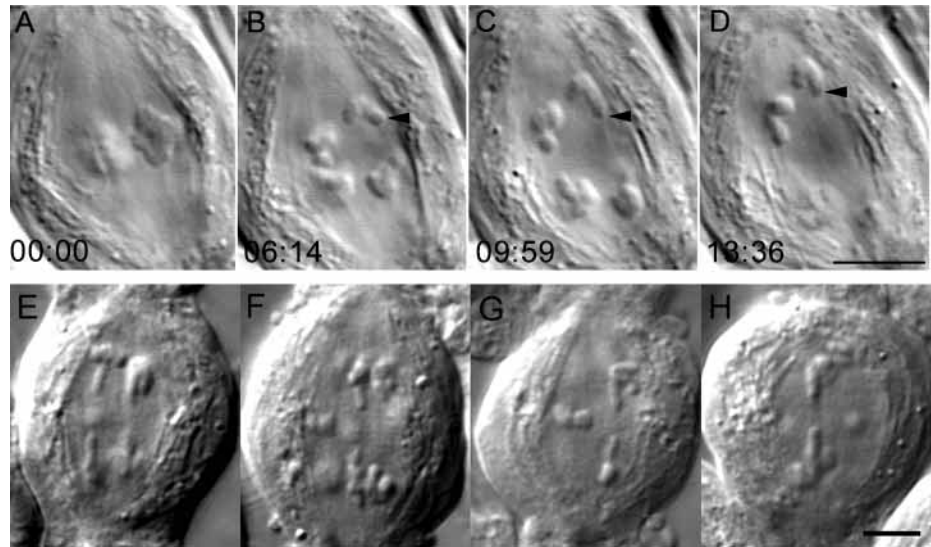
anaphase movement appears unlikely, at least in the *Xenopus* system.

The possibility that segregating partners, although no longer conjoined as at metaphase, remain tethered during anaphase was raised previously (Forer, 1966). Forer found that the movement of a segregating half-bivalent, as well as the movement of its previously conjoined partner, could be interrupted following UV microbeam irradiation of the one half-bivalent's kinetochore fiber. Subsequent micromanipulation studies provided further evidence for linkage between segregating partners (Forer and Koch, 1973), but controversy has revolved around whether such linkages are bona fide structures or 'sticky bridges' artifactually induced by in vitro culture conditions (for reviews, see Begg and Ellis, 1979; Ellis and Begg, 1981).

Our interest in tethers was piqued by recent findings from crane-fly spermatocytes (Ilagan et al., 1997) that showed backward movement of an entire half-bivalent following UV irradiation of its kinetochore domain or its kinetochore fiber. Although the reported result was observed in only 2 out of 23 cells on which kinetochore irradiations were performed, it raised the possibility that tethers might be exerting the forces for backward movement.

This study was undertaken in an attempt to understand both why UV micro-irradiation was so inefficient in eliciting the above results and what the mechanism underlying the results might be. We found that chromosome arm behavior during

Fig. 1. Transient stretching of trailing chromosome arms is common during anaphase in crane-fly spermatocytes. (A-D) Selected frames from a time-lapse DIC recording of a primary spermatocyte in which arms of segregating partner half-bivalents stretched backwards during mid-anaphase. (A) The dichiasmic bivalent at metaphase. (B) The two half-bivalents began to segregate. (C) As anaphase progressed, trailing arms (arrowhead) of partners stretched backwards. (D) Partner arms retract as the half-bivalents continue moving polewards. Times are given in minutes and seconds. Bar, 5 μ m (D). (E-H) Spermatocytes that were fixed in situ with glutaraldehyde. In each, arms of partner chromosomes are stretched backwards as if their telomeres were connected. The sex chromosomes normally lag at the spindle equator during anaphase in these cells and they are especially prominent in G and slightly in focus in E, F and H. Bar, 5 μ m (H).



anaphase gave a strong impression that partner homologues were indeed tethered. When we used a laser microbeam to cut supposedly tethered arms, the backward movements displayed by the resultant arm fragments provided clear evidence that elastic tethers were present and that they were imposing resistance to poleward chromosome movement. Normally during anaphase, such resistance is overcome by forces exerted by the 'traction fibers' that connect chromosomes to the spindle poles. But, as the findings of this study show, that can happen only if chromosomes are firmly attached to their kinetochore microtubules.

Materials and Methods

Crane flies (*Nephrotoma suturalis*) were maintained in the laboratory, and fourth instar male larvae were selected for study. In vitro preparations of living spermatocytes were made after testes had been removed into tricine buffer (Begg and Ellis, 1979) by rupturing individual testes under oil (Votalef 10s oil; Uguine Kuhlmann, Paris, France) on a coverslip (LaFountain et al., 2001). Spermatocytes cultured in vitro under oil were used both for live cell imaging (60 \times /1.4 NA planapochromatic objective) and for laser microsurgery as outlined in an earlier report (LaFountain et al., 2001).

For the analysis of chromosome arms in fixed cells, testes were fixed with Pipes-buffered 0.5% glutaraldehyde [in situ fixation is described elsewhere (LaFountain et al., 1999)] as they were removed from the larva prior to rupturing them under oil, as described above. Such oil preparations of fixed spermatocytes were stable for months.

Results

Trailing arms of anaphase half-bivalents were stretched back towards their partners

During anaphase A of meiosis I in crane-fly spermatocytes, the three sets of previously conjoined homologues move polewards during a period that usually lasts about 15-20 minutes. Recently made time-lapse recordings show that one or two chromosome arms per homologue are under tension, as though tethered to their partners. For a brief time (lasting only 3 to 6

minutes) during the course of anaphase, trailing arms appeared stretched backwards (Fig. 1C). Prior to being stretched, those arms were indistinguishable from the other trailing arms of the half-bivalent (Fig. 1B), and subsequently they retracted back to their approximate initial configuration (Fig. 1D). Stretched trailing arms were observed with half-bivalents derived from either monochiasmatic or dichiasmic bivalents. Stretching was independent of the poleward extension of arms (Adames and Forer, 1996; LaFountain et al., 2001). It was possible for a half-bivalent to have a pole-directed arm in addition to a stretched arm, while others had only normally trailing and transiently stretched arms.

Although back-stretched arms were common in freshly made living cell preparations, we extended our analysis to address whether the observed stretching of arms was a consequence of artifacts, such as 'sticky bridges', that arise in cell cultures either spontaneously or under unhealthy conditions (Begg and Ellis, 1979; Ellis and Begg, 1981). For this, we analyzed cells from testes that had been removed from larvae directly into glutaraldehyde. Spermatocytes fixed in that way (in situ fixation) had no opportunity to experience the possible deleterious effects of cell culture. Backward stretching of arms was clearly evident in fixed anaphase cells (Fig. 1E-H). In the 28 larvae included in our analysis, all contained anaphase cells with back-stretched arms. Fixed testes from four larvae were used to determine the percentage of anaphase cells with stretched arms (Fig. 1E-H). Forty percent (136/340) of the anaphase A spermatocytes in those eight testes had at least one pair of obviously back-stretched arms. It is important to emphasize that these numbers represent a lower limit of the extent of stretching that may be possible, because many of those anaphase A spermatocytes were either in early anaphase (prior to the time when arms stretch backwards, for example, Fig. 1B) or in late anaphase (subsequent to the time when stretching would be expected, for example, Fig. 1D). Hence, it is possible that this phenomenon occurs in most, if not all, primary spermatocytes of *Nephrotoma suturalis*. Within each of the four larvae tested, the ratio of the number of

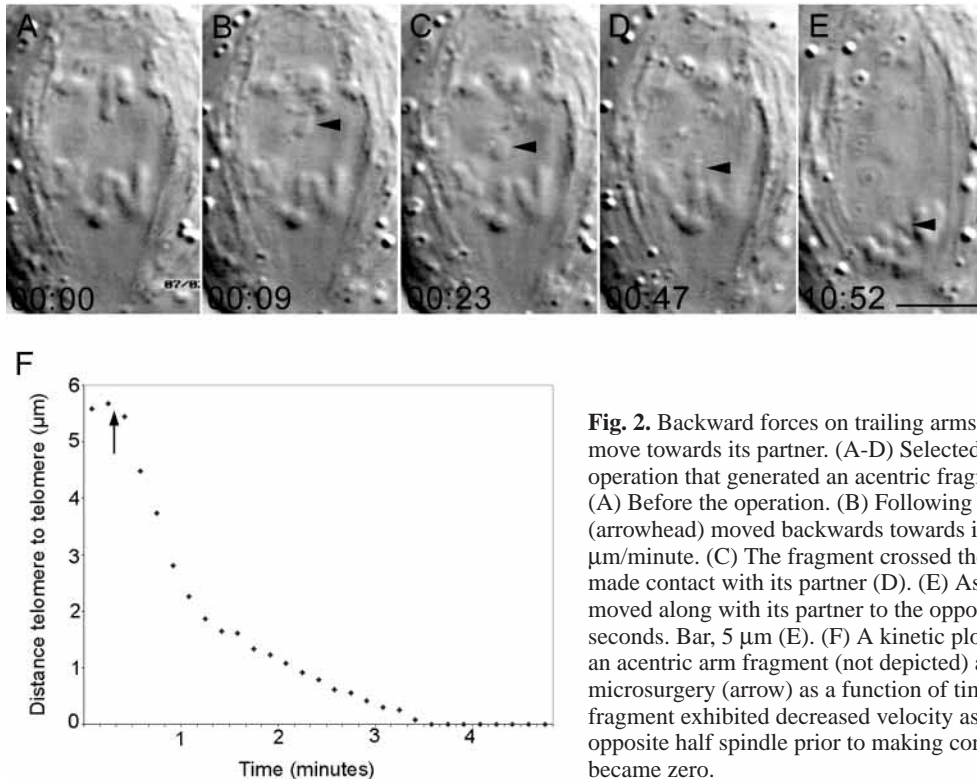


Fig. 2. Backward forces on trailing arms cause a detached acentric arm fragment to move towards its partner. (A–D) Selected frames from a time-lapse series of an operation that generated an acentric fragment from a trailing arm during anaphase. (A) Before the operation. (B) Following the operation, the acentric fragment (arrowhead) moved backwards towards its partner with an initial velocity of $\sim 8 \mu\text{m}/\text{minute}$. (C) The fragment crossed the equator and as its velocity decreased, it made contact with its partner (D). (E) As anaphase progressed, the fragment moved along with its partner to the opposite pole. Times are given in minutes and seconds. Bar, $5 \mu\text{m}$ (E). (F) A kinetic plot of the distance between the telomere of an acentric arm fragment (not depicted) and its partner telomere following laser microsurgery (arrow) as a function of time. The backward motion of the tethered fragment exhibited decreased velocity as the fragment approached its partner in the opposite half spindle prior to making contact, when the distance between telomeres became zero.

spermatocytes with stretched arms to the total number of anaphase A spermatocytes was 20/47, 47/121, 26/57, and 43/115. Based on these findings, we conclude that backward stretching of chromosome arms is indeed a common feature of anaphase in crane-fly spermatocytes and not simply an artifact, such as ‘sticky bridges’, resulting from *in vitro* culture.

Detachment of a stretched arm revealed backward forces acting on it

To assess the basis of arm stretching, we performed laser microsurgery on stretched arms during early and mid-anaphase to cut off a portion of a stretched arm (Fig. 2A–B). We then tracked the movement of the resultant acentric arm fragment following the operation (Fig. 2C–E). Stretched arms were ideal targets for laser microsurgery. Since they extended backwards and had minimal lateral contact with the other unstretched arms, it was a simple operation to sever them from the half-bivalent.

The outcome of operations on stretched arms varied (see below), but the majority involved the immediate and rapid backward movement of the arm fragment across the equator and into the opposite half-spindle (Fig. 2C). In the case of the fragment depicted in Fig. 2, that movement led to contact with the fragment’s homologous partner (Fig. 2D); then the fragment moved with the partner to the other pole as anaphase was completed (Fig. 2E). The remainder of the cut half-bivalent, including its kinetochores and uncut arms, showed no apparent effect from the loss of the fragment. The cut half-bivalent proceeded through anaphase, arriving at the pole on schedule with the other two uncut autosomes. That is in accord with our earlier findings (LaFountain et al., 2001), which demonstrated that chromosome cutting operations performed

during metaphase had no detectable effect on either cell viability or the completion of anaphase. A total of 20 such operations were performed on back-stretched arms, and in all cases, except one, backward movement of the fragment was observed (see below).

Among the fragments that displayed the behavior described above (i.e. backward movement to make contact with its partner), the initial backward velocities ranged between 2.5 and $20 \mu\text{m}/\text{minute}$ (average $\sim 7 \mu\text{m}/\text{minute}$; $n=5$), clearly many times greater than the average velocities ($\sim 0.5 \mu\text{m}/\text{minute}$) of half-bivalents during anaphase (LaFountain et al., 2001). In all of these cases, fragment velocities progressively decreased as they moved across the equator and into the opposite half-spindle. In the example given (Fig. 2F), backward velocity decreased to zero when the fragment made contact with its partner.

The extent of backward movement of a fragment correlated with the time course of anaphase

We next performed operations on trailing arms at different times during the progression of anaphase A. We especially wanted to investigate whether trailing arms at late anaphase, after retractions had occurred (Fig. 1D), would display backward motion to as great an extent as arms that were clearly stretched at the time of the operation. This was based on the finding that the one fragment (above) that did not display any backward movement had been cut from a half-bivalent that was well into anaphase. Since its telomere at the time of operation was separated from its partner by a distance greater than that of the other fragments that displayed backward movement (Fig. 2), it was possible that the arm was in the process of reacting when it was cut. Thus, for these additional operations, we cut

Table 1. The extent of backward movement of acentric fragments generated from trailing arms during anaphase correlates with the distance separating partner telomeres

Stage of anaphase	Telomere-to-telomere distance at time of cut	Type 1* fragment (contact with partner)	Type 2* fragment (opposite half-spindle)	Type 3* fragment (not to equator)
Early anaphase	Less than 4 μm	8/14 (57%)	5/14 (36%)	1/14 (7%)
Mid anaphase	4–8 μm	3/20 (15%)	6/20 (30%)	11/20 (55%)
Late anaphase	Greater than 8 μm	0/13	2/13 (15%)	11/13 (85%)

*For diagrams of these types of fragments, refer to Fig. 3.

some unstretched arms during late anaphase, as well as some during early anaphase before they had become maximally stretched (Fig. 1B).

We pooled the data from these additional 27 operations with the data obtained above from stretched arms (Table 1). Partner half-bivalents were categorized as being in early, mid or late anaphase at the time of the operation when their telomere-to-telomere distances were less than 4 μm , 4–8 μm or greater than 8 μm , respectively. Since, in crane-fly spermatocytes, anaphase A proceeds with little if any spindle elongation (Ilagan et al., 1998), the progressive increase in inter-telomere distance is a fairly accurate indicator of the proportionate decrease in kinetochore-to-pole distance as anaphase A progresses. Also, categorization based on the objective criterion of telomere-to-telomere distance was chosen over chromosome stretching, a subjective criterion (e.g. slightly stretched, highly stretched, somewhat retracted) that could not be quantified.

The fates of fragments generated during anaphase conformed to one of the three sets of diagrams in Fig. 3A–C. Type 1 fragments (Fig. 3A) moved backwards across the equator into the opposite half spindle to make contact with their partners before the partners reached their poles. The fragment depicted in Fig. 2 is a type 1 fragment. Type 2 fragments (Fig. 3B) moved backwards across the equator into the opposite half-spindle and if they did make contact with partners, it was not until after the partners had reached the pole (type 2 fragment not depicted). Type 3 fragments (Fig. 3C) either moved backwards a short distance without crossing the equator or, as was found with 10 of the 23 fragments of this type, they did not move backwards at all (type 3 fragment not depicted). Subsequent movement of type 3 fragments was then towards its ‘proper’ pole (Fig. 3C, step 4; see Discussion).

The data in Table 1 reveal two important findings. First, backward movement of type 1 was displayed only by fragments from arms cut during early and mid-anaphase. Second, backward movement of type 3 was the most prominent type among arms generated at late anaphase.

These findings correlate well with the appearance of arms when the fragments were generated. Among the 23 type 3 fragments, 11 were generated from arms that appeared to have already retracted (Fig. 1D). One of those arms was monitored during anaphase as it stretched backwards and then retracted polewards. When it was cut, the distance between it and its partner was $\sim 8 \mu\text{m}$, and it displayed no backward movement. Five type 1 fragments were generated from arms at early anaphase before they appeared to be stretched backwards (Fig. 1B), thereby demonstrating that back-stretching is not a necessary requirement for backward motion of fragments generated at early anaphase.

Initial velocities of backward movement were highest

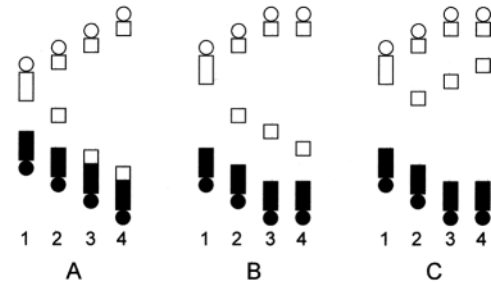


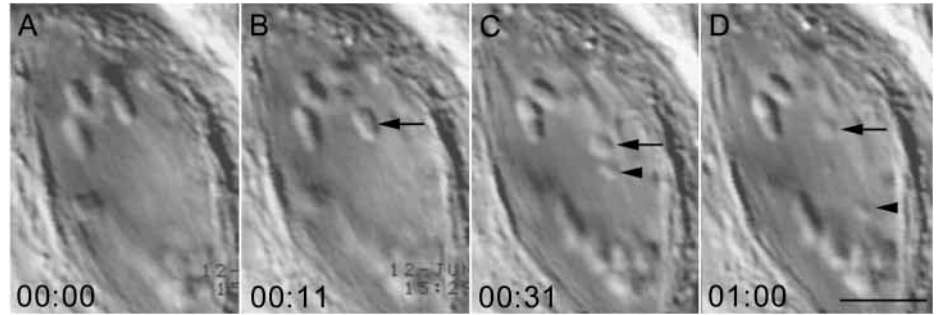
Fig. 3. The movement of an acentric fragment generated by cutting a trailing arm from a segregating half-bivalent conformed to one of three types. (A–C) The single trailing arm that was to be severed is represented by an open rectangle and the acentric fragment generated by cutting is represented by an open square. The kinetochore-containing fragment is represented by an open square connected to a circle, the latter representing the centromere/kinetochore domain of the half-bivalent. For simplicity, only the to-be-cut arm and its partner are included. (A) Type 1 fragment: the fragment moved rapidly backwards across the equator into the opposite half spindle (A2) to make contact with its partner (A3) and then moved along with its partner to the lower pole (A4). The tethered arm of the partner half-bivalent is shown with a solid rectangle and solid circle. (B) Type 2 fragment: the fragment moved backwards across the spindle into the opposite half spindle (B2), but it did not make contact with its partner (B3) as in (A). Further movement of the fragment to the opposite pole occurs after the partner (solid) reaches the pole (B4). (C) Type 3 fragment: this fragment moved backwards a short distance (or did not move backwards at all) without crossing the equator (C2) then shifted from backward movement to movement to the correct pole (C3) and at late anaphase it was located near its original pole (C4).

among type 1 fragments, averaging $\sim 7 \mu\text{m}/\text{minute}$ (range=1–22 $\mu\text{m}/\text{minute}$; $n=11$). Among the 13 type 2 fragments, initial velocities averaged $\sim 4 \mu\text{m}/\text{minute}$ (range=1–8 $\mu\text{m}/\text{minute}$), and among the 13 type 3 fragments that displayed backward movement, initial velocities averaged $\sim 1 \mu\text{m}/\text{minute}$ (range=1–4 $\mu\text{m}/\text{minute}$).

The backward movement of an acentric fragment was mediated by its telomere

To determine the site(s) on a trailing arm fragment where the force for its movement was exerted, we performed two different two-step operations. The first involved making two laser cuts on a trailing arm (Fig. 4A–D). The initial cut severed the arm from its half-bivalent (Fig. 4B) and then, during the backward movement of the detached fragment, it was cut again (Fig. 4C). The second cut generated two fragments (Fig. 4C): (1) a telomere-containing fragment and (2) an interstitial fragment. In the four operations of this type that were

Fig. 4. Backward movement of an acentric fragment was mediated by its telomere. (A-D) Selected frames from a time-lapse recording of a double-cut operation. (A) Before the first cut. (B) After the first cut, the fragment (arrow) moved backwards. (C) After the second cut, the telomere-containing fragment (arrowhead) continued moving backwards, but the interstitial fragment (arrow) halted at the location of the second cut. Times are given in minutes and seconds. Bar, 5 μ m (D).



performed, the telomere-containing fragment continued moving backwards, but the interstitial fragment halted at the cut site and did not move further backwards (Fig. 4D). In the example presented (Fig. 4), the telomere-containing fragment actually made contact with its partner and then moved with its partner to the opposite pole (not depicted). These results not only implicated the telomeric ends of arms in the mechanism of backward movement, but they also ruled out any possibility of backward movement based on lateral interactions between the sides of chromosome arms and linear elements of the spindle.

As was expected, because of the progressive decrease in velocity during backward motion, as described above (Fig. 2F), the initial velocities of the telomere-containing fragments generated by the second cut (V_2) were on average ~ 2 -3 μ m/minute lower than the velocities after the initial cut (V_1). For example, V_1 of the fragment generated in Fig. 4B was ~ 9 μ m/minute, but V_2 after the second cut (Fig. 4C) was only ~ 7 μ m/minute. (range $V_1 = \sim 5$ -13 μ m/minute; range $V_2 = \sim 4$ -8 μ m/minute; $n=4$).

For the second type of operation, an initial cut severed a stretched arm from its half-bivalent and that was followed by a second operation to ablate either the fragment's telomere or the telomere of its partner (Fig. 5A-D). The goal here was to initiate backward movement of a fragment and then to stop it by destroying the connection between the two stretched arms. Backward motion of fragments stopped immediately upon irradiation of either telomere (Fig. 5C). Stopped fragments, however, did not remain motionless indefinitely at the equator, as is shown in the sequence included in Fig. 5. The action of the transport properties (LaFountain et al., 2001; LaFountain et al., 2002) of the half-spindle that contained such a fragment

eventually took over (Fig. 5D) and transported it towards one or the other pole (see Discussion).

We performed these telomere ablation operations on eight cells: four in which the telomere of the fragment was irradiated and four in which the telomere of the partner was ablated. A different strategy was used on three additional cells in which telomere irradiation was performed prior to fragment detachment. In those cases, no backward movement of the fragment was observed.

Taken together, the results of the above experiments suggested that the mechanism underlying the backward motion of a detached arm fragment involved its telomere.

Detachment of kinetochores from segregating half-bivalents generated some fragments that moved backwards and others that did not

We performed another set of experiments to determine which of the four arms of a segregating half-bivalent were capable of backward motion. To do that, we used the laser to cut segregating half-bivalents at early anaphase along a plane just below their kinetochores to sever them from the four trailing arms. For those operations, we selected half-bivalents that had disjoined from dichiasmatic bivalents, because their kinetochores are especially protuberant and therefore readily sliced off with the laser. That operation resulted in the generation of a small kinetochore-containing fragment (K-fragment) that continued moving polewards (Fig. 6A-D), and the four arms of the half-bivalent were detached. In eight of the kinetochore detachment operations that we performed, arms were released as distinct fragments, which indicated that the objective of removing the centromere/kinetochore domains

Fig. 5. Backward movement of a fragment halted upon irradiation of the telomere of its partner. (A-B) Selected frames from a recording of a telomere ablation operation. (A) Before the operation. (B) The acentric fragment (arrowhead) moved backwards rapidly and was probably a type 1 fragment (Fig. 3A). (C) After the telomere (arrow) of the fragment's partner was irradiated, the backward movement of the fragment stopped (D). Times are given in minutes and seconds. (E) As anaphase progressed, both partners continued polewards, but the transport properties of the spindle (see text) began to act on the fragment (arrowhead) and moved it away from the equator and into the half-spindle. Bar, 5 μ m (E).

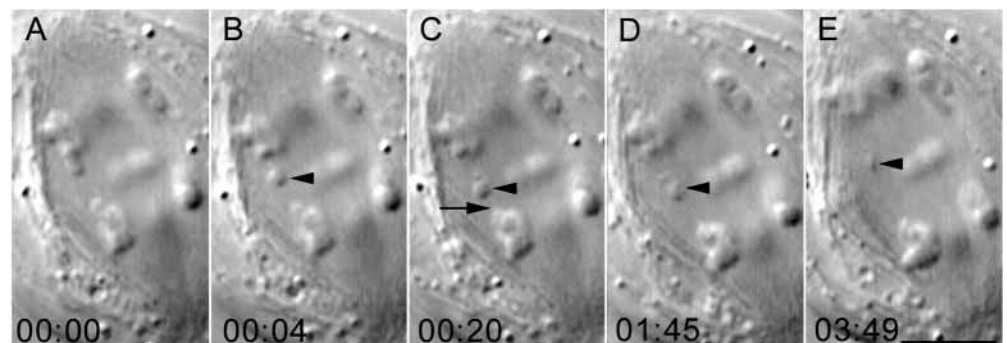
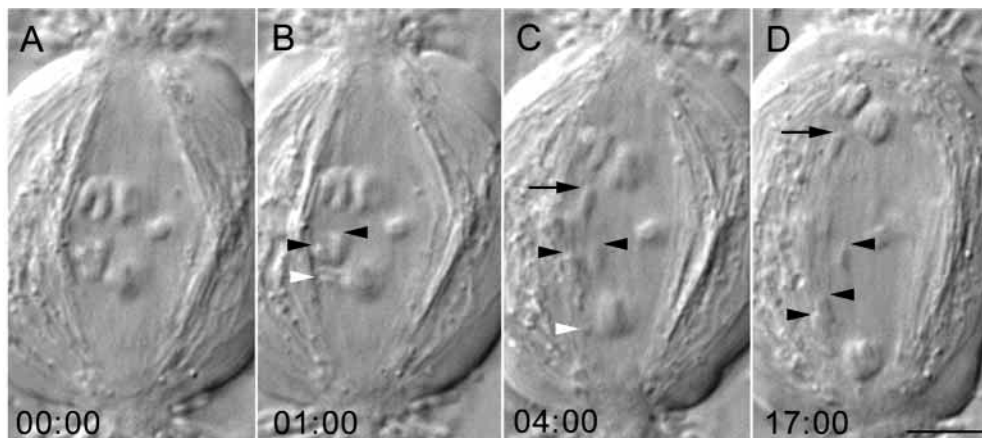


Fig. 6. Kinetochore detachment operations generated both tethered and untethered fragments.

(A-E) Selected frames from a time-lapse recording of a kinetochore detachment operation. (A) Before the operation. (B) The K-fragment (white arrowhead) was detached and continued moving polewards; four acentric fragments were generated and the telomeres of two of them are located with black arrowheads.

(C) The K-fragment (white arrowhead) approaches the spindle pole; the tethered, type 2 fragment (arrow points to its telomere) exhibited rapid backward motion;

among the other three fragments were a weakly tethered fragment (left-pointing arrowhead) and two untethered fragments (the telomere of one is located with a right-pointing arrowhead). (D) The positions of all four fragments generated by laser microsurgery are evident: the arrow locates the telomere of the type 2 fragment, one left-pointing arrowhead locates the telomere of the weakly tethered fragment near the equator and the other two arrowheads locate the telomeres of the untethered fragments, which are in the process of being transported to the lower pole. Times are given in minutes and seconds. Bar, 5 μ m (D).



from the half-bivalent had been achieved. Out of the total of 32 fragments generated by those operations, 12 exhibited backward movements as described above, but 19 of the remaining 20 did not exhibit backward movement. The fate of one fragment could not be determined.

Among those 12 backward moving fragments, only 8 (one per half-bivalent) were capable of the most rapid backward motion that resulted in it making contact with its partner (type 1 fragment; Fig. 3A). Four half-bivalents released another fragment that moved across the equator and into the opposite half-spindle, but its backward movement was clearly less rapid than the one that preceded it, and it did not make contact with its partner (Type 2 fragment; Fig. 3B). Thus, detachment of the kinetochores from a half-bivalent generated at most two arms capable of backward motion. None of the half-bivalents released three backward-moving arms.

All eight half-bivalents released at least two fragments that displayed no backward motion. Those fragments paused for

a brief period (1-6 minutes), as their kinetochores moved polewards. Then, as anaphase progressed, they were transported polewards at velocities ranging between 0.2 and 0.5 μ m/minute (normal anaphase velocity \sim 0.5 μ m/minute) in a manner similar to that seen previously during anaphase with acentric fragments generated prior to anaphase (LaFountain et al., 2002). The final destination of such fragments was the vicinity of the uncut half-bivalents and kinetochores from which they were detached.

With regard to the post-op behavior of K-fragments, their poleward velocities were the same as those of the other segregating half-bivalents, as well as to their own velocities before the operation, averaging \sim 0.5 μ m/minute (range: 0.4-0.5 μ m/minute, $n=5$). K-fragments commonly became less visible as they moved polewards. It is not clear whether that was simply due to diminished refractility that is normally observed during anaphase (LaFountain et al., 1998) or whether it involved additional laser-inflicted effects (LaFountain et al., 2001; LaFountain et al., 2002).

Fig. 7. Backward forces were sufficiently strong to achieve the backward movement of an entire acentric half-bivalent.

(A-E) Selected frames from a time-lapse recording of an operation in which the kinetochores were removed from a segregating half-bivalent. Here the acentric arms remained 'stuck' together following the operation and moved backward as a unit, in this case with an initial velocity of \sim 2 μ m/minute.

(A) Before the operation. (B) After the operation, the removal of kinetochores is evident from the truncated appearance of the half-bivalent; the operation also generated a ribbon of denatured nucleoprotein, called a sniglet (arrowhead).

(C) The sniglet (arrowhead) was transported polewards, but the truncated half-bivalent moved backwards towards its partner.

(D) The acentric arms continued moving towards the partner's pole, as the sniglet (arrowhead) was transported further polewards. (E) At late anaphase, the truncated half-bivalent was located in the opposite half-spindle and the sniglet (arrowhead) was located near the spindle pole. Times are given in minutes and seconds. Bar, 5 μ m (E).

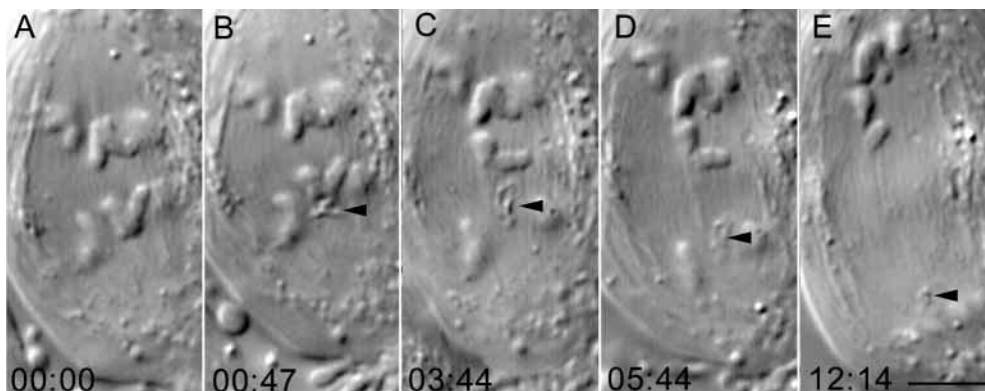
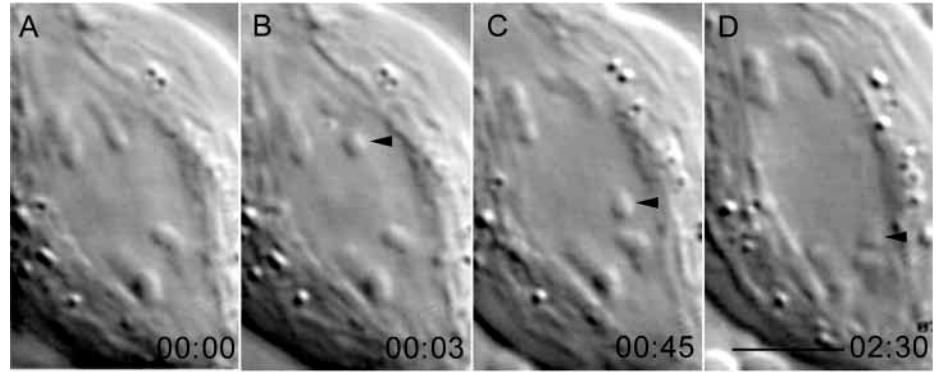


Fig. 8. Severing of trailing arms during meiosis II also resulted in backward movement of acentric fragments. (A–D) Selected frames from a time-lapse recording of a secondary spermatocyte undergoing anaphase II. (A) Sister chromatids segregating to opposite poles before the operation. (B) A trailing arm (arrowhead) of one of the sex chromosomes was cut. (C) The acentric fragment (arrowhead) moved backwards to make contact with its sister. (D) The fragment moved along with its sister as anaphase was completed. Times are given in minutes and seconds. Bar, 5 μ m (D).



The products of our kinetochore removal operations also included acentric arms that remained stuck together following the operation and thus appeared as a truncated half-bivalent. This was the outcome of four of our operations. It is not clear why arms did not separate from one another in those cases. Following the operation, the K-fragment continued moving polewards, as above. The detached arms moved backwards as an interconnected unit across the equator, and they then moved together with their partner half-bivalent as it completed anaphase.

Similar backward movements were also observed following five operations in which the kinetochores of a segregating half-bivalent were destroyed with the laser (Fig. 7). In these cases, no K-fragment was generated, but the absence of kinetochores was clearly evident by the obvious truncated appearance of the half-bivalent's centromere/kinetochore domain (Fig. 7B).

Acentric fragments generated during meiosis II also displayed backward movement

Trailing arms of segregating chromatids in secondary spermatocytes were cut with the laser to test whether backward movements were possible during meiosis II. The results were similar to those obtained during meiosis I. Acentric arm fragments moved backwards rapidly to make contact with their sisters (Fig. 8). The extent of backward movement diminished as anaphase progressed. Moreover, this behavior was evident with fragments derived from either autosomes or sex chromosomes, which segregate along with the autosomes during the second division, instead of lagging behind them as they normally do during meiosis I. Further analysis involving double cut experiments and telomere ablations was not performed on meiosis II cells.

Discussion

We sought to determine whether disjoined, segregating chromosomes are tethered during anaphase. New data suggested that stretched chromosome arms were under tension during a brief portion of anaphase, before which and after which such tension was not manifested. When stretched arms were cut off with a laser, their rapid backward movement and the velocity profiles of that movement suggested that they were connected to their partners by an elastic tether. Our interpretation is that arms appear stretched backwards because of tension built up both within them and within the elastic tether connecting them. Upon severing one of the stretched

arms, that tension was relieved, and the tethered fragment moved backwards as the stretched tether recoiled.

Backward stretching of trailing arms was observed extensively in living spermatocytes, as well as in spermatocytes that had been fixed *in situ*, thus allaying all concerns that the phenomena observed were a result of 'sticky bridges' or some other artifact stemming from *in vitro* culture of spermatocytes.

We have also shown that the backward movement of arm fragments was mediated by their telomeres. Thus, we conclude that telomeres of partner half-bivalents during meiosis in crane-fly spermatocytes are tethered *in vivo*, as originally proposed (Forer, 1966).

Arm cutting operations revealed that the extent of backward motion of a fragment correlated with the time course of anaphase. The majority of fragments severed during early anaphase were type 1 fragments that moved across the equator to make contact with their partners and then appeared to be dragged behind them as anaphase was completed. By contrast, the majority of fragments severed from segregating half-bivalents during late anaphase were type 3 fragments that either did not display any backward motion or moved backwards to a limited extent, stopping before reaching the spindle equator. We view these findings, plus our results on arm stretching and arm retraction during anaphase, as a strong indication that the elasticity of tethers drops as anaphase progresses and that eventually during late anaphase connections between partners are completely lost. These losses provide a ready explanation for why trailing arms appear stretched for only a brief portion of anaphase and then retract polewards at mid-to-late anaphase (Fig. 1).

We propose that as anaphase progresses, the tether connecting partner telomeres is stretched beyond its elastic limit and therefore is unable to recoil and facilitate the backward movement of an attached fragment. Such reduced elasticity was evident in the behavior of type 2 fragments (Fig. 3B) whose tethers must have been less elastic than those of type 1 fragments (Fig. 3A). Tethers of type 2 fragments were capable of facilitating limited backward motion but incapable of achieving contact between the fragment and its partner. Thus, we propose that further movement of a type 2 fragment, after its tether either lost its elasticity or lost its attachments to telomeres, is not tether-based. Rather, the transport properties of the spindle, which we have proposed act on acentric fragments located near the spindle equator during anaphase through a microtubule flux-based mechanism (LaFountain et al., 2001; LaFountain et al., 2002), must move type 2 fragments into closer proximity with their partners, after the latter have reached the spindle pole (Fig. 3B, step 4).

Loss of elasticity within the tether also explains why type 3 fragments moved backwards only a short distance prior to being transported towards the proper pole. The tethers connecting such fragments must have lacked sufficient elasticity to effect extensive backward movement. Along with losing their elasticity, connections between telomeres must not exist during late anaphase, allowing the transport properties of the spindle to act on type 3 fragments to move them to the 'proper' pole (Fig. 3C, steps 3,4). Since type 3 fragments did not move backwards into the opposite half-spindle, the flux-mediated transport properties of their 'original' half-spindle would be expected to move them towards the pole to which they normally would have segregated had they remained attached to their half-bivalents. The action of spindle transport properties on fragments is further evident in fragment behavior after either its own or its partner's telomere was ablated. Such fragments were transported polewards within either half-spindle after their tethers had been detached microsurgically. It is not yet known how connections between partner telomeres are normally lost during late anaphase, that is, whether they break upon reaching their elastic limit, or detach from telomeres, or just disintegrate.

Not all of the arms of segregating half-bivalents were tethered to their partners. We found only one, and in some cases two tethers, per half-bivalent. Only one per bivalent was sufficiently 'strong' to move an attached acentric arm fragment backwards to make contact with its partner. The force exerted by tether recoiling, however, was obviously sufficient to drag an entire (truncated) half-bivalent across the equator and into its partner's half-spindle, as shown already (Ilagan et al., 1997). Moreover, our data on the behavior of the four arms of a segregating half-bivalent are consistent with earlier findings (Adames and Forer, 1996) that suggested that only two arms of a half-bivalent may be tethered and the other two are not.

Data from other systems, including spermatocytes from grasshoppers (Izutsu and Sato, 1992) and silkworms (Nakanishi and Kato, 1965), point to a more widespread occurrence of tethering between segregating partner chromosomes. In the case of silkworms, it is not clear why the backward motion was still possible at telophase. Perhaps tethers in these cells are more long-lived than in crane flies. To date, there is no evidence for elastic tethers in mitotic cells, but we did find them connecting telomeres of segregating sister chromatids during meiosis II (discussed below).

With regard to why the UV micro-irradiations performed by Ilagan et al. were so inefficient in causing the backward movement of bivalents, it is likely that either kinetochores were incompletely destroyed by UV or tethers were unable to recoil (Ilagan et al., 1997). The effect of UV irradiation of a kinetochore is 'paling', an outcome that may vary in intensity as well as in location. Thus, although Ilagan et al. reported that kinetochore domains paled, that may not have caused sufficient destruction to detach them from kinetochore fibers and permit tether recoiling. In addition, if the elastic limit of the tether had already been exceeded, then that tether could not have promoted backward movement.

A goal for future work will be to resolve the structure and composition of tethers. The origins and functions of tethers also remain unresolved. Ilagan et al. have proposed that tethers may serve as conduits through which signals may be transmitted from one half-bivalent to another. It is also possible that the tension imposed by tethers on segregating

chromosomes could be playing a role in stabilizing kinetochore/kinetochore fiber linkages during anaphase. It is well known that the stability of kinetochore attachments of bivalents during meta- and prometaphase depends on tension (Nicklas, 1997). Perhaps there is a need to maintain that tension for at least part of anaphase.

Alternatively, tethers may not perform any required function during anaphase. The molecules comprising tethers may simply be remnants of important structures (e.g. the molecular complexes that function during prophase to attach telomeres to the nuclear envelope or possibly elements of chiasmata) that existed and performed an essential function at an earlier stage of division, yet have no specific role to play in anaphase. Whichever, if any, of the above is correct, the data demonstrate that tethering is a feature of both meiotic divisions, and thus, any proposed function must take that into account.

The relationship of the telomere-telomere associations reported here to telomere interactions reported elsewhere (reviewed by Cenci et al., 1997) remains to be determined. Likewise, although there appears to be no relationship between tethers in crane-fly spermatocytes and aberrant telomere-telomere linkages in *Drosophila UbcD1* (Cenci et al., 1997) and *polo* (Donaldson et al., 2001) mutants, the possible roles of the proteins encoded by those genes during anaphase in crane-fly spermatocytes deserve investigation.

Our earlier studies (LaFountain et al., 2001) on acentric arm fragments that were generated prior to the onset of anaphase demonstrated that they are transported polewards at velocities similar to those of anaphase chromosomes. Such transport is viewed to be a result of lateral associations along the length of a fragment with the sides of spindle microtubules undergoing poleward flux. Based on that view, it is entirely possible that such lateral associations between chromosome arms and fluxing spindle microtubules could also achieve the poleward movement of an entire chromosome. In fact, the dispensability of kinetochores in the mechanism of chromosome movement has been entertained for a number of years (Fuge, 1989; Fuge, 1990). Our earlier work (LaFountain et al., 2001; LaFountain et al., 2002) ruled out a related anaphase mechanism that invoked the interaction between spindle microtubules and chromosome-associated motors. In the present study, the results indicate that, even in a spindle composed of fluxing microtubules, kinetochores are absolutely required for the anaphase movement of chromosomes.

The necessity of kinetochores in the anaphase mechanism was revealed by the findings that only untethered fragments were transported polewards during anaphase, while tethered fragments invariably displayed backward movement into the opposite half-spindle. In the latter case, flux-mediated transport was unable to prevent the backward force of the recoiling tether. The force exerted by the tether must have been greater than the forces mediating the lateral associations between the fragment and fluxing microtubules. The same must be true for truncated half-bivalents, that contained all four arms stuck together and moved backwards into the opposite half-spindle. In these cases, the binding affinity between all of the arms of the half-bivalent and fluxing microtubules must have been weaker than the backward force exerted by the tether. In essence, the flux-mediated transport properties of the spindle were unable to 'grip' tethered fragments and tethered truncated half-bivalents. As a consequence of this, the backward

movement of tethered fragments put them into the opposite half-spindle and out of their normal 'flux field'.

It is still possible that during normal anaphase, tethered chromosomes are transported polewards primarily by a flux-mediated mechanism, provided they are firmly attached to the microtubules undergoing flux. Normally during anaphase, that firm attachment is mediated by the kinetochores of chromosomes. Kinetochores are required because the end-on connections they make with kinetochore microtubules are strong (Begg and Ellis, 1979) versus the weak lateral associations between chromosome arms and microtubules. Thus, flux-mediated forces normally generated by 'traction fibers' firmly attached to the kinetochores of chromosomes are capable of over-riding backward forces exerted by tethers.

The traction fiber may not need to generate more force to 'break through' the resistance of a tether but, if need be, it has the capability to do so (Nicklas, 1983) without effecting any change in velocity (Nicklas, 1965). It is possible that kinetochore motors might be playing a role in over-riding the resistance of a tether but, as our findings (LaFountain et al., 2001) and those of Wilson et al. (Wilson et al., 1994) suggest, their contribution to the anaphase mechanism cannot amount to more than ~20%.

With this characterization of tether-based forces, the list of forces that act on chromosomes in crane-fly spermatocytes is increased to a total of five. The others are polar ejection forces, kinetochore-based pulling forces (traction fibers), the flux-mediated transport properties of the central spindle domains, and transverse equilibrium forces. All of these forces apparently are deployed within the framework of a dynamic spindle in which microtubules are in a constant state of flux. The shortcoming of our studies to date is that we have not yet visualized flux directly, as others have done in vertebrate cells (Mitchison and Salmon, 1992; Zhai et al., 1995) and in *Xenopus* oocyte extracts (Desai et al., 1998). That is on our agenda for future studies, which we hope will lead to a better understanding of how these various forces participate in the meiotic process.

This research was supported by grants from the National Science Foundation (MCB-9808290 to J.L.) and the National Institutes of Health (GM 400198 to C.R.). Much of the work was completed in the Video LM Core Facility of the Wadsworth Center. We thank A. Siegel and J. Stamos for help with the figures.

References

- Adames, K. A. and Forer, A. (1996). Evidence for polewards forces on chromosome arms during anaphase. *Cell Motil. Cytoskeleton* **34**, 13-25.
- Alexander, S. P. and Rieder, C. L. (1991). Chromosome motion during attachment to the vertebrate spindle: initial saltatory-like behavior of chromosomes and quantitative analysis of force production by nascent kinetochore fibers. *J. Cell Biol.* **113**, 805-815.
- Antonio, C., Ferby, I., Wilhelm, H., Jones, M., Karsenti, E., Nebreda, A. R., and Vernos, I. (2000). Xkid, a chromokinesin required for chromosome alignment on the metaphase plate. *Cell* **102**, 425-435.
- Begg, D. A. and Ellis, G. W. (1979). Micromanipulation studies of chromosome movement. I. Chromosome-spindle attachment and the mechanical properties of chromosomal spindle fibers. *J. Cell Biol.* **82**, 528-541.
- Cenci, G., Rawson, R. B., Belloni, G., Castrillon, D. H., Tudor, M., Petrucci, R., Goldberg, M. L., Wasserman, S. A. and Gatti, M. (1997). UbcD1, a *Drosophila* ubiquitin-conjugating enzyme required for proper telomere behavior. *Genes Dev.* **11**, 863-875.
- Desai, A., Maddox, P. S., Mitchison, T. J. and Salmon, E. D. (1998). Anaphase A chromosome movement and poleward spindle microtubule flux occur at similar rates in *Xenopus* extract spindles. *J. Cell Biol.* **114**, 703-713.
- Donaldson, M. M., Tavares, A. A. M., Ohkura, H., Deak, P. and Glover, D. M. (2001). Metaphase arrest with centromere separation in *polo* mutants of *Drosophila*. *J. Cell Biol.* **153**, 663-675.
- Ellis, G. W. and Begg, D. A. (1981). Chromosome micromanipulation studies. In *Mitosis/Cytokinesis* (ed. A. M. Zimmerman and A. Forer), pp. 155-179. New York: Academic Press.
- Forer, A. (1966). Characterization of the mitotic traction system and evidence that birefringent spindle fibers neither produce nor transmit force for chromosome movement. *Chromosoma* **19**, 44-98.
- Forer, A. and Koch, C. (1973). Influence of autosome movements and of sex-chromosome movements on sex-chromosome segregation in crane-fly spermatocytes. *Chromosoma* **40**, 417-442.
- Fuge, H. (1989). Traction fibres in chromosome movement: the pros and cons. *Biol. Cell.* **66**, 209-213.
- Fuge, H. (1990). Non-kinetochore transport phenomena, microtubule-chromosome associations and force transmission in nuclear division. *Protoplasma* **158**, 1-9.
- Fuller, M. T. (1995). Riding the polar winds: chromosomes motor down east. *Cell* **81**, 5-8.
- Funabiki, H. and Murray, A. W. (2000). The *Xenopus* chromokinesin Xkid is essential for metaphase chromosome alignment and must be degraded to allow anaphase chromosome movement. *Cell* **102**, 411-424.
- Ilagan, A. B., Forer, A. and Spurck, T. (1997). Backward chromosome movement in anaphase, after irradiation of kinetochores and kinetochore fibers. *Protoplasma* **198**, 20-26.
- Izutsu, K. and Sato, H. (1992). Rapid backward movement of anaphase chromosomes whose kinetochore fibers were cut by ultraviolet microbeam irradiation. *Biol. Cell* **76**, 339-350.
- LaFountain, J. R., Jr, Hard, R. and Siegel, A. J. (1998). Visualization of kinetochores and analysis of their refractility in crane-fly spermatocytes after aldehyde fixation. *Cell Motil. Cytoskeleton* **40**, 147-159.
- LaFountain, J. R., Jr, Siegel, A. J. and Rickards, G. K. (1999). Chromosome movement during meiotic prophase in crane-fly spermatocytes. IV. Actin and the effects of cytochalasin D. *Cell Motil. Cytoskeleton* **43**, 199-212.
- LaFountain, J. R., Jr, Oldenbourg, R., Cole, R. W. and Rieder, C. L. (2001). Microtubule flux mediates the poleward motion of acentric chromosome fragments during meiosis in insect spermatocytes. *Mol. Biol. Cell* **12**, 4054-4065.
- LaFountain, J. R., Jr, Cole, R. W. and Rieder, C. L. (2002). Polar ejection forces are operative in crane-fly spermatocytes, but their action is limited to the spindle periphery. *Cell Motil. Cytoskeleton* **131**, 721-734.
- Mitchison, T. J. and Salmon, E. D. (1992). Poleward kinetochore fiber movement occurs during both metaphase and anaphase A in newt lung cells. *J. Cell Biol.* **119**, 569-582.
- Nakanishi, Y. H. and Kato, H. (1965). Unusual movement of the daughter chromosome group in telophase cells following the exposure to ultraviolet microbeam irradiation. *Cytologia* **30**, 213-221.
- Nicklas, R. B. (1965). Chromosome velocity during mitosis as a function of chromosome size and position. *J. Cell Biol.* **25**, 119-135.
- Nicklas, R. B. (1983). Measurements of the force produced by the mitotic spindle in anaphase. *J. Cell Biol.* **97**, 542-548.
- Nicklas, R. B. (1988). The forces that move chromosomes in mitosis. *Annu. Rev. Biophys. Chem.* **17**, 431-449.
- Nicklas, R. B. (1997). How cells get the right chromosomes. *Science* **275**, 632-637.
- Rieder, C. L. and Salmon, E. D. (1994). Motile kinetochores and polar ejection forces dictate chromosome position on the vertebrate mitotic spindle. *J. Cell Biol.* **124**, 223-233.
- Savoian, M. S., Goldberg, M. L. and Rieder, C. L. (2000). The rate of poleward chromosome motion is attenuated in *Drosophila* *zw10* and *rod* mutants. *Nat. Cell Biol.* **2**, 948-952.
- Sharp, D. J., Rogers, G. C. and Scholey, J. M. (2000). Cytoplasmic dynein is required for poleward chromosome movement in *Drosophila* embryos. *Nat. Cell Biol.* **2**, 922-930.
- Wilson, P. J., Forer, A. and Leggiadro, C. (1994). Evidence that kinetochore microtubules in crane-fly spermatocytes disassemble during anaphase primarily at the poleward end. *J. Cell Sci.* **107**, 3015-3027.
- Zhai, Y., Kronebusch, P. J. and Borisy, G. G. (1995). Kinetochore microtubule dynamics and the metaphase-anaphase transition. *J. Cell Biol.* **131**, 721-734.