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Research paper

Elastic 'tethers' connect separating anaphase chromosomes in a broad range of animal cells

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ABSTRACT

We describe the general occurrence in animal cells of elastic components ("tethers") that connect individual chromosomes moving to opposite poles during anaphase. Tethers, originally described in crane-fly spermatocytes, exert force on chromosome arms opposite to the direction the anaphase chromosomes move. We show that they exist in a broad range of animal cells. Thus tethers are previously unrecognised components of general mitotic mechanisms that exert force on chromosomes and they need to be accounted for in general models of mitosis in terms of forces on chromosomes and in terms of what their roles might be.

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1. Introduction

This article describes the presence of elastic connections between separating anaphase chromosomes in cells from a broad range of animals, from flatworms to humans, suggesting that such connections are universal. Elastic connections between chromosomes moving to opposite poles represent previously unrecognised forces on anaphase chromosomes, acting in opposite direction to the forces moving chromosomes to the poles. While the composition and function of these connections are unknown, their universal presence has broad implications to how chromosome movements in anaphase are coordinated and controlled.

Elastic physical connections between arms of separating chromosomes were originally demonstrated in anaphase crane-fly spermatocytes by LaFountain et al. (2002), who called them "tethers". Tethers were identified operationally: fragments cut from ends of arms moved 'backwards' to the partner telomeres that were moving to the opposite pole. They moved with speeds considerably greater than the chromosomes while the severed chromosome continued moving to its original pole. Further experiments by LaFountain et al. (2002) indicated that these 'backwards' movements of arm fragments were not due to forces from motors associated with spindle microtubules, and pointed strongly to forces arising from elastic connections between separating telomeres, as follows.

When backward-moving arm fragments were cut into two pieces, the sub-fragment with the telomere kept moving but the other one did not: it stopped. Thus arm-fragment movement is not due to generalised spindle transport forces acting on arms in general but requires the telomere. When the arm-fragment telomere was ablated its movement stopped, and when the fragment itself was not cut but the telomere of the other chromosome (to which the fragment was moving) was ablated, arm-fragment backward movements also stopped. Thus both telomeres need to be intact for backward movement to take place, suggesting that there are elastic connections between separating arms. In meiosis-I, arm fragments from only two of the four arms of each separating half-bivalent pair move backward (LaFountain et al., 2002), which was interpreted to mean that arm fragments connected to their partner move backwards but other arm fragments do not, and that backwards movements of arm fragments require specific connections between partner telomeres. When the backward-moving arm-fragment nears the partner telomere, that intact arm loses length (Sheykhani et al., 2017), it 'shrinks', further indicating that

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the connections, the 'tethers', are elastic. Thus the forces for movement are applied specifically at the telomere, both telomeres need to be intact, and the forces seem to arise from elastic connections ('tethers') that extend between separating telomeres.

Tethers were identified operationally in anaphase meiosis-II cells (LaFountain et al., 2002) as well as meiosis-I cells, so they are not restricted to reduction divisions. The tethers seem to become less elastic as anaphase progresses. Arm fragments produced in early anaphase move fast and reach the telomere of the separating chromosome. Those produced later move across the equator at slower speeds and stop before reaching the partner telomere. Those produced yet later move somewhat toward the partner telomere but not across the equator, or do not move at all. LaFountain et al. (2002) interpreted this behaviour to mean that as anaphase proceeds tethers lose elasticity, as indicated by fragments slowing down, and that eventually tethers are no longer attached to telomeres, as indicated by absence of motion.

In this report we identify and characterise tethers in PtK (marsupial tissue culture) cells, and identify tethers in cells ranging from flatworms to humans, namely from a turbellarian flatworm, two classes of insects, two distinct lineages of arachnids, a marsupial, and humans. Thus, elastic tethers connect separating anaphase chromosomes in animal cells in general, if not universally.

2. Materials and methods

2.1. Cells

Asynchronous or rapidly proliferating PtK2 and U2OS cells were maintained in Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) supplemented with 10% bovine calf serum, L-glutamine, and sodium pyruvate at 37 °C and 5% CO_2.

Adult *Pholcus phalangioides* (Fuesslin) (cellar spider) males were collected from a field site near Bucknell University, Lewisburg PA. Adult *Latrodectus mactans* (Fabricius) (black widow spider) males were obtained from Spider Pharm Inc. (Yarnell, AZ). The abdomens of spiders were cut away from the cephalothorax. Testes were removed from the abdomen and placed in a culture chamber as described in Ault et al., 2017 Testes contents were spread under a layer of Kel-F oil (Ohio Valley Specialty Chemical, Marietta, OH).

Juvenile *Acheta domesticus* (Linnaeus) male crickets were purchased from PetCo (Clairemont, San Diego, California). Testes were removed through a small incision cut directly behind the wing buds on the dorsal side of the cricket abdomen. Testes were placed directly into Kel-F oil and testes contents were spread under a layer of Kel-F oil.

Crane flies, *Nephrotoma suturalis* (Loew), were from a laboratory culture (Forer, 1982). Spermatocytes were put into a fibrin clot and immersed in Ringers solution as described in Forer and Pickett-Heaps (2005).

The turbellarian flatworms, *Mesostoma ehrenbergii*, were from laboratory cultures (Hoang et al., 2013). Spermatocytes were removed from animals, placed in fibrin clots, and immersed in Ringers solution as described in Hoang et al. (2013) and Ferraro-Gideon et al. (2014). The highly unusual chromosome movements in these cells are described in detail in Ferraro-Gideon et al. (2014).

2.2. Experimental methods

The viewing, irradiation, and analysis methods are described detail in Forer et al. (2013). In brief, cells were observed using phase-contrast microscopy (using a Zeiss Plan-Neofluar 63X lens, NA 1.4) in a microscope fitted for laser microbeam surgery (Harsono et al., 2012; Shi et al., 2012). Regions of interest were irradiated using a 200-fs pulsed laser (Mai Tai, Newport Co., Irvine, CA, USA) emitting 740 nm wavelength light. Cells were observed at room temperature, including some of the PtK cells, but for all experiments on U2OS cells and most experiments using PtK cells, the cells were placed on a heated stage maintained at 36.5 °C. Images recorded every 2 or 3 s were cropped, stamped with date and time, and converted into bmp files using *IrfanView* freeware. The bmp files were assembled into avi files using *VirtualDub* freeware. We analysed the results of the experiments by visual observation and by obtaining distance versus time graphs, as described in Forer et al. (2013), using an in-house program, *WinImage* (Wong and Forer, 2003). The graphical data on chromosome arm shortening were only from cuts that caused arms to shorten, except for Figs. 10 and 12, which included all irradiations. Montages were assembled from individual images using Photoshop. The images were adjusted only for image contrast and brightness levels.

Table 1
Comparison of PtK2 cell arm fragment and anaphase chromosome velocities.

Arm fragment velocities	1.8 μm/min ± 1.58 μm/min (SD)	N = 50
Anaphase chromosome velocities	0.4 μm/min ± 0.29 μm/min (SD,	N = 37

3. Results

3.1. Tethers in PtK2 cells

Tethers connect separating anaphase chromosomes in PtK2 cells. When chromosome arms in anaphase cells are cut using a laser microbeam, the resultant arm fragments move away from the arm stub, toward their partner telomeres, either (a) reaching them, or (b) stopping across the equator before reaching them, or (c) stopping before crossing the equator (Fig. 1). The velocities of arm fragments were faster than anaphase chromosomes in the same cells (Table 1), with fragment velocities as high as 6.2 μm/min.

Arm fragment movements are due to elastic connections between telomeres, because cutting the region between the two telomeres causes the arm fragment to stop moving toward the opposite chromosome (Fig. 2). [This was our operational definition of cutting tethers.] When tethers were cut, some arm fragments reversed direction and moved back towards the arm stub (Fig. 3), as reported for arm fragments in crane-fly spermatocytes (Sheykhani et al., 2017), presumably because the initial arm-severing cut was incomplete and elastic chromosome components remained in the cut region.

Tension from tethers stretches anaphase crane-fly spermatocyte chromosome arms backward, toward the separating partner telomeres (LaFountain et al., 2002). Stretched arms shorten when their tethers are cut (Sheykhani et al., 2017). Thus we cut PtK-cell tethers themselves, to test whether their chromosomes were stretched. After cutting their tethers, arms shortened (Fig. 4). In those cells in which we were able to see both kinetochore and telomere sufficiently clearly to measure arm lengths, the arms shortened to about 89% of their initial value (0.89 ± 0.025 sd, N = 31), over a period of up to a minute, independent of the initial length of the chromosome arm (Fig. 5). The stretch appears to be uniform along the entire length of the chromosome arm, because, independent of the initial length of the stub, the arm stubs shortened to about the same extent (0.85 ± 0.06 sd, N = 10) as entire arms (Fig. 6).

Not all arms shortened when we attempted to cut their tethers. This may be because we did not actually cut them; we have no visible marker of tethers, so it is possible that sometimes they were not cut.

Not all arm fragments moved backwards toward the partner telomere. Lack of movement might be because not all arms are connected to their partners with tethers. For example, in some cells only one or two arm fragments moved backwards toward the other

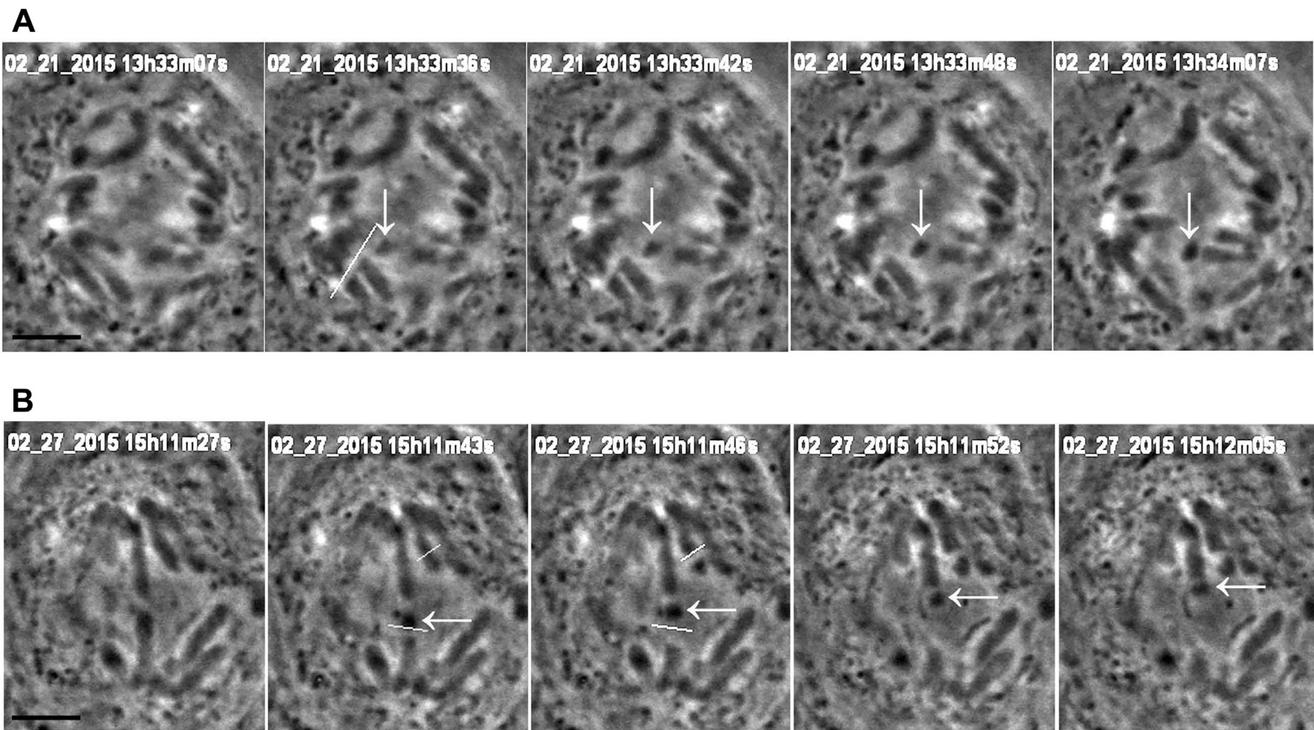

Fig. 1. Arm fragments move across the equator to their partner telomeres in a PtK2 cell. A: the white line in the second image is the laser position immediately after the arms were cut. The arm fragment indicated by the white arrow moved to the partner telomere. The other arm fragments did not. The black line in the first image represents 5 μm. B: The white lines in the second and third images represent the laser positions just as and just after the arms were cut. The arm fragment indicated by the white arrow moved to the partner telomere and when it did the partner chromosome shortened, and the arm stub shortened. The other arm fragment did not move. The black line in the first image represents 5 μm.

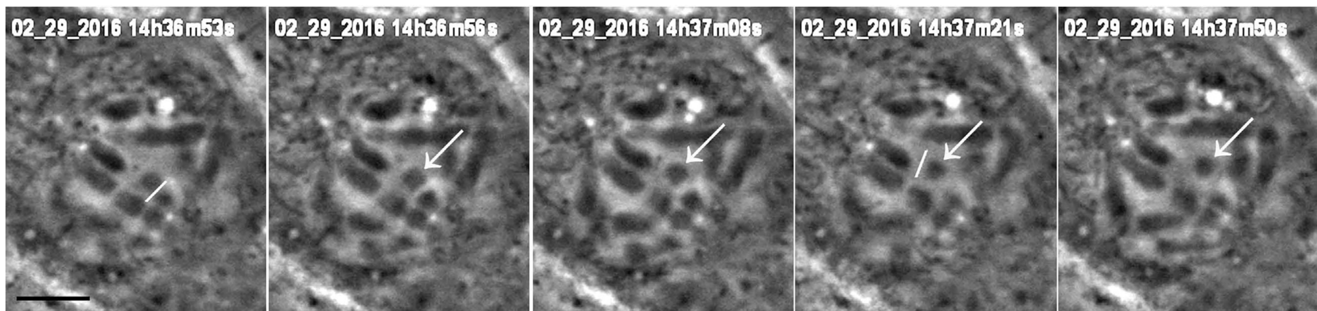

Fig. 2. Cutting the tether stops movement of the arm fragment in a PtK2 cell. The white arrow indicates the arm fragment. The arm fragment moved toward the partner telomere after the arm was cut (white line in the first image) but once the tether was cut (white line in the fourth image) the arm fragment stopped moving and drifted away. The black line in the first image represents 5 μm.

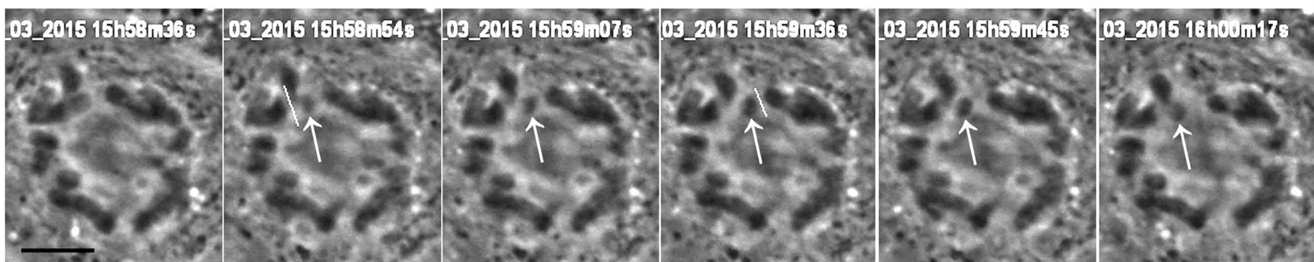

Fig. 3. An arm fragment moved back to the arm stub when its tether was cut in a PtK2 cell. The arm fragment (indicated by a white arrow) moved toward the other telomere after the arm was cut (white line in the second image) and when its tether was cut (white line in the fourth image) the arm fragment moved back to its original arm. The black line in the first image represents 5 μm.

telomeres while others cut at the same time and same tether length (as measured by inter-telomere distance) did not (Fig. 7, and Supplemental Video S1). It is unclear which arms are connected by tethers and which not, though it may be that, as in crane-fly spermatocytes, there are tethers between only one of the two arms of each separating chromosome. Another possibility why some arm

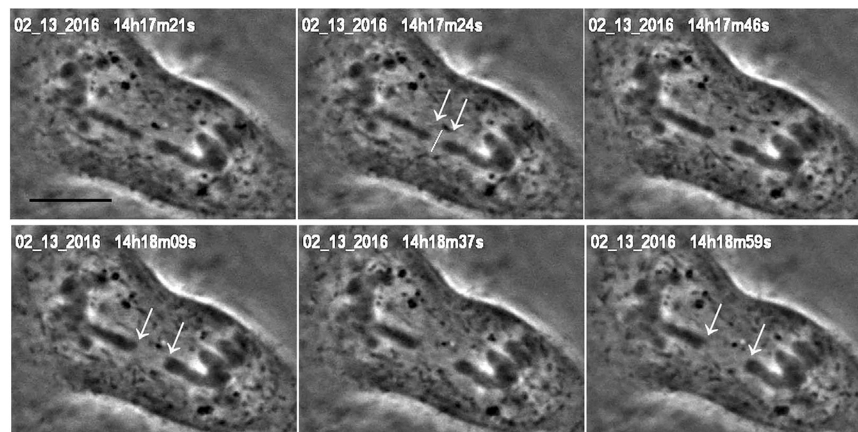

Fig. 4. Arms shrink when their tethers are cut in a PtK2 cell. After their tether was cut (white line in the second image) both chromosome arms shortened. The white arrows indicate the two telomeres. The black line in the first image represents 10 μm.

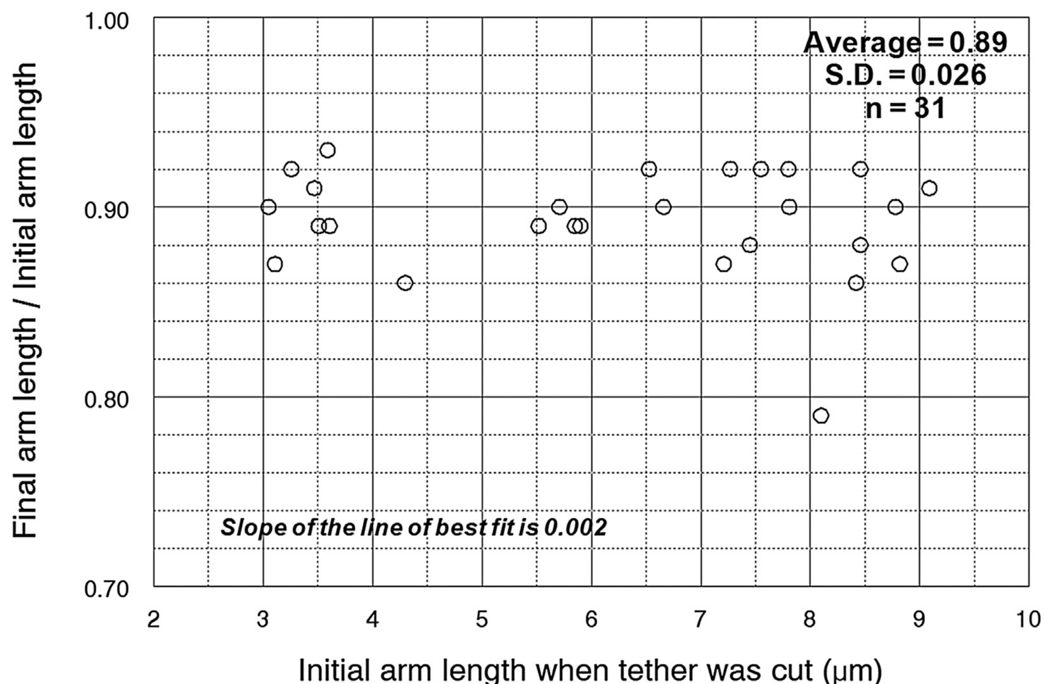

Fig. 5. The percentage shortening of chromosome arms when tethers are cut in PtK2 cells is independent of initial arm length. The ordinate is the final arm length divided by the initial arm length, and the abscissa is the initial arm length when the tether was cut (in μm).

fragments did not move might be that tethers in PtK cells become less elastic as anaphase progresses. To test whether longer tethers are less elastic than shorter ones we compared arm fragment velocities with arm shortening after cutting tethers.

Arm fragment movement varied with tether length. The longer the tethers, (a) the slower were the velocities of arm fragments (Fig. 8), (b) the less likely the arm fragments were to reach the partner telomere or to cross the equator (Fig. 9), and (c) the less likely that arm fragments would move at all (Fig. 10). On the other hand, the *amounts the arms shortened* after cutting tethers did not change with tether length: at all tether lengths the arms shortened on average to 89% of their initial length (Fig. 11). Thus while arm fragment velocities were slower as anaphase progressed, the tension that the arms were under did not change. This suggests that tethers are present between separating telomeres at all tether lengths, at least to the maximum we studied (9.6 μm), and that their elasticity is reduced as they become longer. As further indication that this is the case, arms shortened after cutting tethers at tether lengths at which fragments did not move: at tether lengths longer than 7 μm, none of 17 arms fragments moved (Fig. 10) yet at those same lengths (>7 μm) arms shortened after 6 out of 8 cuts of tethers (Fig. 12). Thus tethers are present and produce a constant tension on the arms throughout anaphase, but the elasticity of the tethers decreases as the tethers elongate.

We did not detect any change in poleward movement of anaphase chromosomes after their tethers were cut (Fig. 13), so the force from the tethers that stretches chromosome arms is much smaller than the force moving the chromosome to the pole.

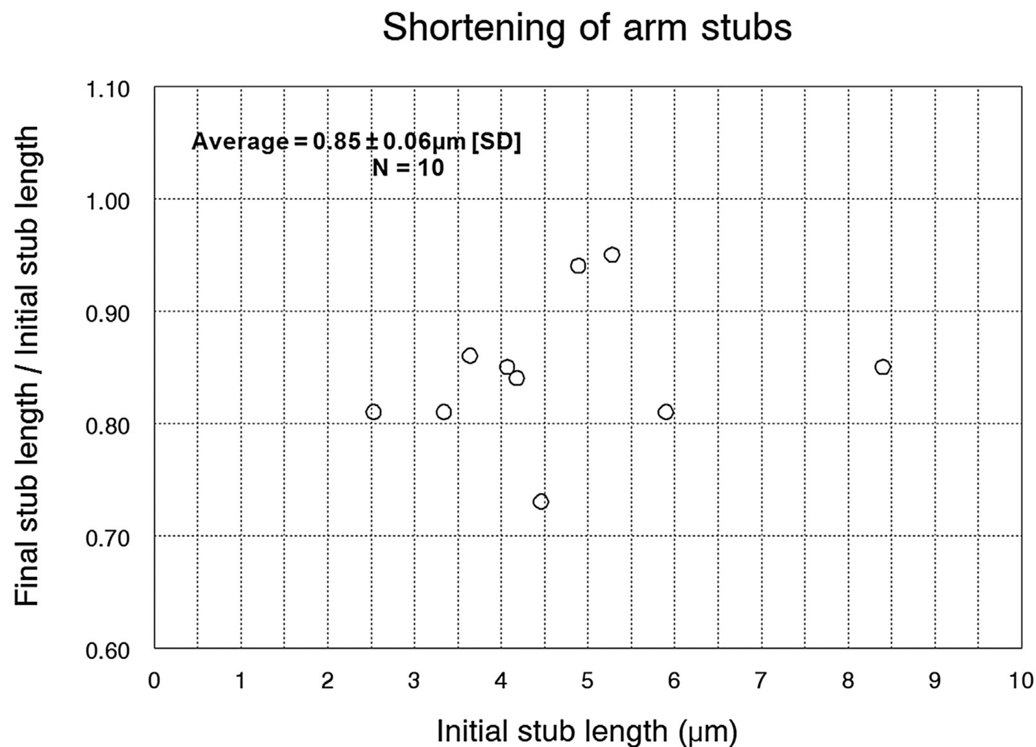

Fig. 6. The percentage shortening of chromosome arm stubs when tethers are cut in PtK2 cells is independent of initial stub length. The ordinate is the final stub length divided by the initial stub length, and the abscissa is the initial stub length (in μm).

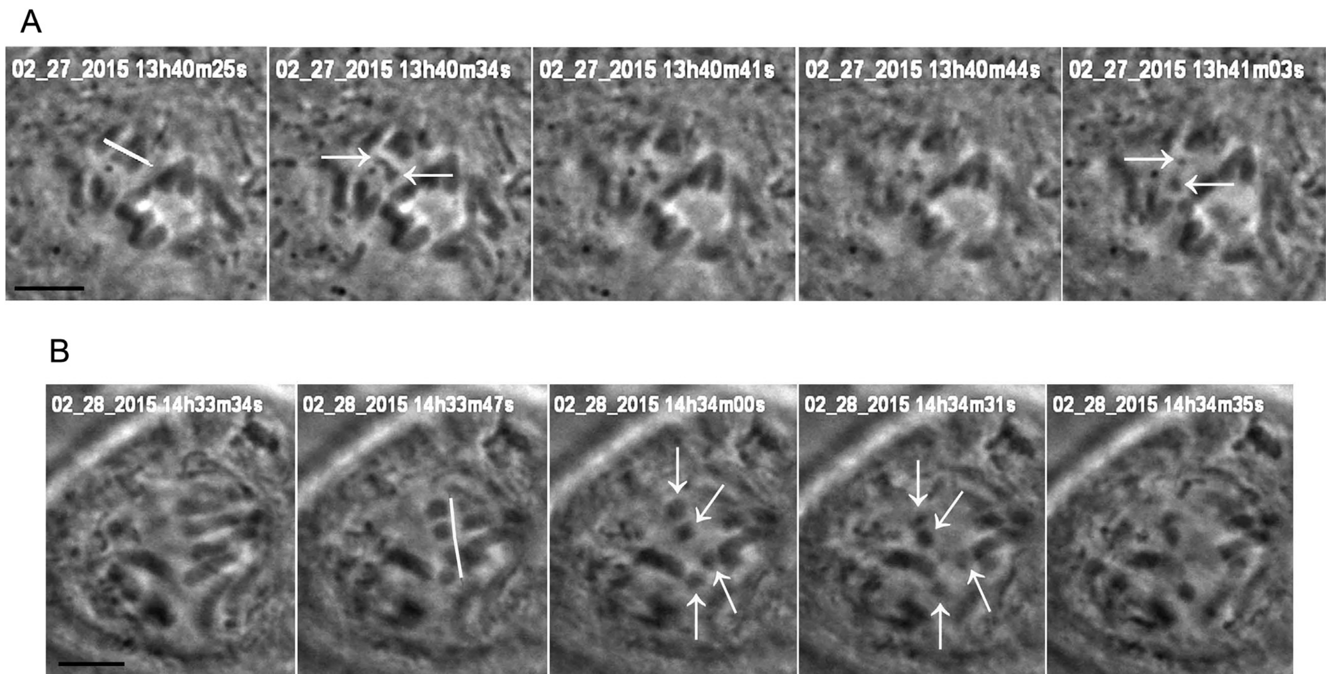

Fig. 7. Not all arm fragments move in PtK2 cells even at the same tether length. A: only one of the two arm fragments (indicated by white arrows) formed when the two arms were cut (white line in the first image) moved toward the opposite telomere. The black line in the first image represents 5 μm. Time-lapsed images of these cuts (and others) in this cell are presented in the Supplemental Video S1. B: one of the four arm fragments (white arrows) formed when the arms were cut (white line in the second image) does not move toward the partner telomere. None of the three that moved reached the other telomeres. The black line in the first image represents 5 μm.

3.2. Tethers in crane-fly spermatocytes

Tether properties described in previous work on crane-fly spermatocytes (LaFountain et al., 2002; Sheykhani et al., 2017) are the same as those we found in PtK cells. Some issues had not been determined for crane-fly spermatocytes, however, namely, whether the tethers lose elasticity as they get longer and how much the tethers stretch the chromosome arms to which they are attached. We studied crane-fly spermatocytes to look at these particular issues.

In crane-fly spermatocytes, chromosome arms shortened on average to 89% of their initial length, similar to that in PtK cells (Table 2). Arms shortened in late anaphase when their tethers were

Fig. 8. Arm fragments in PtK2 cells tend to move more slowly when their tethers are longer. The ordinate is the velocities of the arm fragments (in μm/min.), and the abscissa is the tether lengths (distance between telomeres) in μm when the arms were cut.

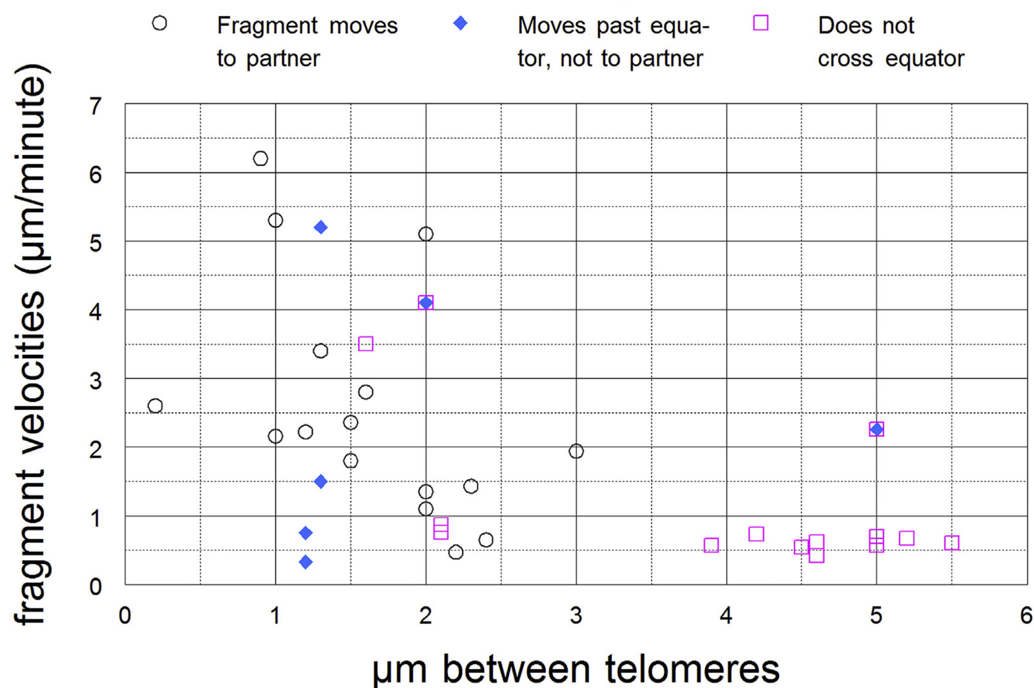

Fig. 9. In PtK2 cells, arm fragments with longer tethers are less likely to move to the opposite telomere, or to move across the equator. The ordinate is the velocities of the arm fragments (in μm/min.), the abscissa is the tether lengths (distance between telomeres) in μm when the arms were cut, and the different symbols indicate whether the arm fragment moved to the partner [O], past the equator but not to the partner [♦], or did not cross the equator [□].

cut, up to tether lengths of at least 15 μm (the longest we have studied); at these tether lengths arm fragments do not move, or move very slightly (LaFountain et al., 2002). Thus our data suggest that, similar to PtK cells, tethers in crane-fly spermatocytes are present throughout anaphase but lose their elasticity as anaphase progresses.

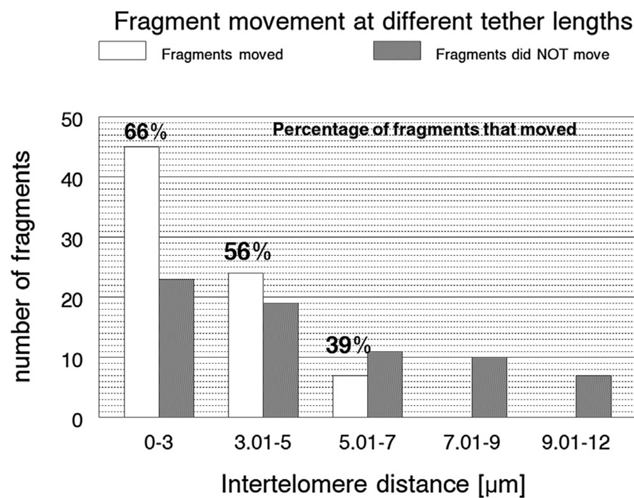

Fig. 10. In PtK2 cells, the longer the tether the less likely that the arm fragment will move across the equator or to the equator. The ordinate is the number of arm fragments and the abscissa is the tether length (inter-telomere distance) in μm. The open bars indicate the arm fragments that moved, the shaded bars the arm fragments that did not move, and the percentages above the open bars indicates the percentages of arm fragments that moved out of the total that were formed in that range of tether lengths.

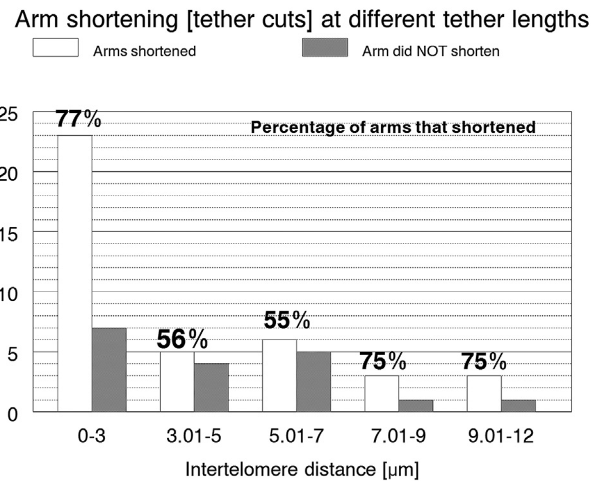

Fig. 12. In PtK2 cells, at all tether lengths chromosome arms shorten when tethers are cut. The ordinate is the number of arms, and the abscissa is the tether length (inter-telomere distance) in μm at the time the tethers were cut. The open bars indicate the arms that shortened, the shaded bars the arms that did not shorten, and the percentages above the open bars indicates the percentages of arms that shortened out of the total that were cut in that range of tether lengths.

Table 2
Shortening of chromosome arms after cutting their tethers.

Final length/initial length		
PtK2 cell chromosome arms	0.89 ± 0.025 (SD)	N = 31
PtK2 cell chromosome stubs	0.85 ± 0.06	N = 10
Crane-fly spermatocyte chromosome arms	0.90 ± 0.047	N = 24

3.3. Tethers in other cells

We have identified tethers in other cell types as well, from flatworms to humans. Arm fragments produced in early anaphase moved across the equator to the partner telomeres in spermatocytes from the turbellarian flatworm *Mesostoma*; in spermatocytes from an orthopteran insect, the house cricket *Acheta domesticus*, in both meiosis-I and meiosis-II; in spermatocytes from two arachnids from distinct lineages, cellar spiders (*Pholcus*) and black widow spiders (*Latrodectus*); and in human osteosarcoma cancer cells, U2OS (Fig. 14). We have seen at least 3 examples of rapid arm fragment

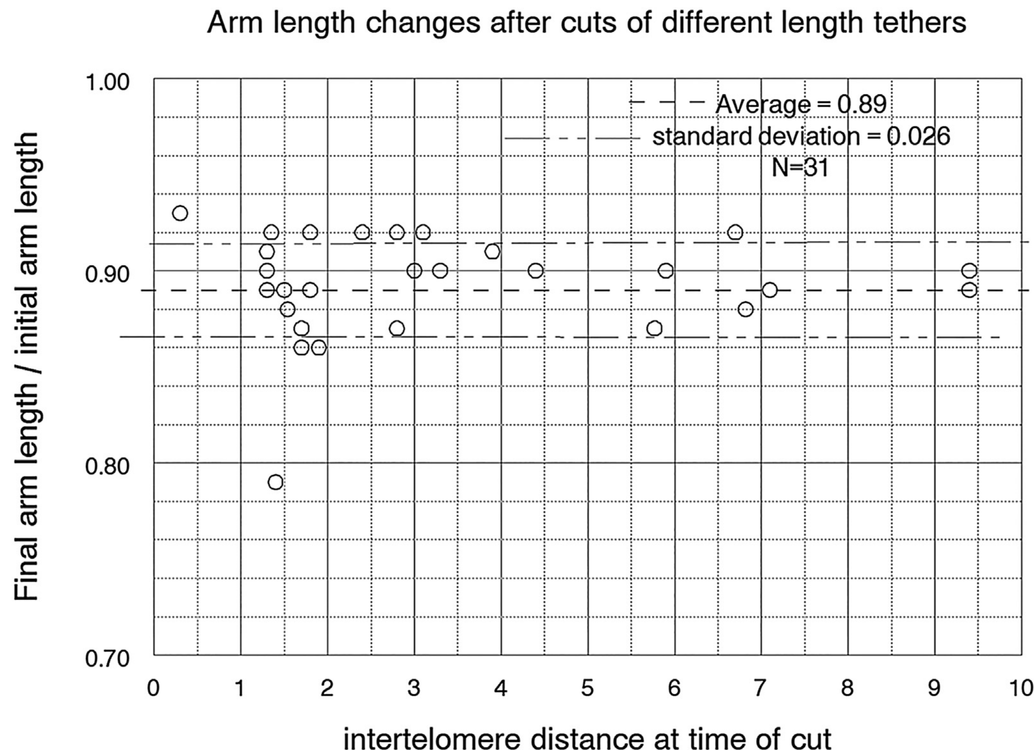

Fig. 11. In PtK2 cells, chromosome arms shorten the by same fractional amount at all tether lengths after their tethers are cut. The ordinate is the final arm length divided by the initial arm length and the abscissa is tether length (inter-telomere distance) in μm at the time the tether was cut.

Table 3
Velocities of arm fragments in different cells.

CELL	Range of velocities	number measured
PtK2 cells	0.5–6.2 μm/min	50
crane-fly (*Nephrotoma*) primary spermatocytes[a]	1–22 μm/min	37
crane-fly (*Nephrotoma)* primary spermatocytes[b]	1–18 μm/min	13
Cricket *(Acheta)* primary spermatocytes	1–11 μm/min	5
Cellar spider (*Pholcus*) primary spermatocytes	1–7 μm/min	4
Black widow spider (*Latrodectus*) primary spermatocytes	5–7 μm/min	2
Flatworm (*Mesostoma*) primary spermatocytes	7–13 μm/min	2
U2OS (human) cells	2–13 μm/min	6

[a] Data from LaFountain et al. (2002).
[b] Data from Sheykhani et al. (2017).

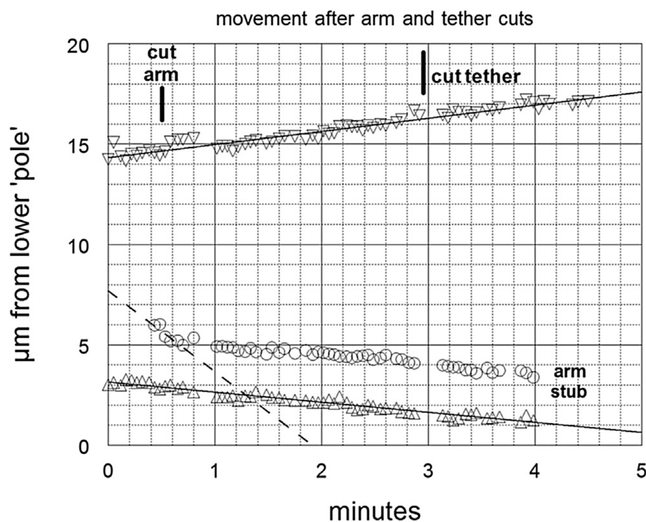

Fig. 13. In PtK2 cells anaphase chromosome movements do not change when arms and/or tethers are cut. The ordinate is the distance (in μm) from a fixed point near the lower 'pole' and the abscissa is time (minutes). The triangles (△) and inverted triangles (▽) indicate the positions of the two kinetochores moving to opposite poles. An arm of the chromosome moving to the lower pole was cut at about 0.5 min, and the open circles (O) represent the position of the end of the arm stub; the stub shortens immediately after the cut, and then moves poleward at the same speed as its kinetochore. The tether was cut at about 3 min. Neither kinetochore increased speed when arm or tether were cut.

movements in each of the cells studied. Those whose speeds we have been able to measure are presented in Table 3. In addition, we have verified several other characteristic of tethers in these cells: in particular, in all of the cells not all arm fragments move toward their partner; in flatworm spermatocytes, cricket spermatocytes and human cells tethers stretch arms; and cutting tethers in meiosis-I cricket spermatocytes stops the movements of arm fragments.

4. Discussion

A main conclusion from our experiments is that there are mechanical connections (tethers) between separating anaphase chromosomes in a broad range of cells: flatworm spermatocytes, crane-fly spermatocytes (meiosis-I and –II), cricket spermatocytes (meiosis-I and -II), cellar spider spermatocytes (meiosis-I), black widow spider spermatocytes (meiosis-I), marsupial (PtK2) cells, and human U2OS (osteosarcoma) cells. Detailed characterisation of tethers in PtK2 cells and crane-fly spermatocytes indicates that tethers are elastic, but that they lose elasticity as they become longer: the arm fragments move slower and slower toward the opposite telomere, and eventually do not cross the equator or do not move. Severing tethers releases the connections between separating arms which causes the arms to shorten. Arms shorten at all tether lengths, whereas arm fragments do not move at longer tether lengths. Thus separating chromosomes are connected by tethers throughout anaphase but the longer the tether the less elastic it is. The force exerted by tethers on separating chromosomes stretches the chromosome arms by around 12% (100/89) regardless of tether length; the stretch seems to be throughout the length of the chromosome arms since the chromosome stubs (that remain after the arm is severed) also contract to the same extent as the unsevered arm, at least in PtK2 cells, in which the arm stubs are long enough to measure length changes. The force exerted by tethers is enough to stretch chromosome arms but is considerably smaller than that exerted on the kinetochore toward the poles since chromosomes do not speed up when tethers are cut (Fig. 13; also LaFountain et al., 2002; Sheykhani et al., 2017).

Because tethers are found in cells from widely separate organisms, from flatworms to humans, it seems likely that they are present in most cell types, and perhaps are even present universally in cells, at least those with localised kinetochores. It seems likely that tethers between partner chromosomes are responsible for the backwards movements observed after cutting individual anaphase spindle fibres in PtK2 cells (Fig. 1C of Elting et al., 2014,), which have tethers (this paper), and, by extension, in grasshopper spermatocytes (Chen and Zhang, 2004) and newt fibroblasts (Spurck et al., 1997), in neither of which have tethers been demonstrated. We might also speculate that backwards movements seen after cutting across entire half-spindles in anaphase *Haemanthus* cells (Bajer, 1972) are due to tethers, and backwards movements after spindle poles are irradiated in silkworm meiosis-I (Nakanishi and Kato, 1965) are due to tethers.

Our purpose in the experiments presented herein was to test whether tethers were present in a variety of cells, to generalise their presence. We envision that subsequent experiments will deal with the question, what are tethers composed of? We did not pursue this question, and we have no data regarding their composition. But perhaps brief discussion is worthwhile.

It is difficult to get a handle on tethers if one cannot identify them so we would like to be able to see them in living cells, or at least in stained cells. Since cutting between partner telomeres in PtK2 cells caused chromosome arms to shorten 75% of the time (Fig. 12), we expect tethers to extend between separating telomeres in at least 75% of the separating chromosomes, and most likely 100%. In crane-fly spermatocytes tethers were identified operationally for each pair of separating chromosomes, but only for half the arms (La Fountain, 2002b). In trying to identify tethers morphologically we therefore expect to see them between all separating chromosomes, though not necessarily between all separating arms. Connections between separating telomeres in anaphase are not visualised by several usual staining methods (discussed in Sheykhani et al., 2017) and no connections between telomeres were seen in PtK2 cells using standard electron microscopy sectioning methods (Roos, 1973). However, connections between separating telomeres were seen electron microscopically in crane-fly spermatocytes by Fuge

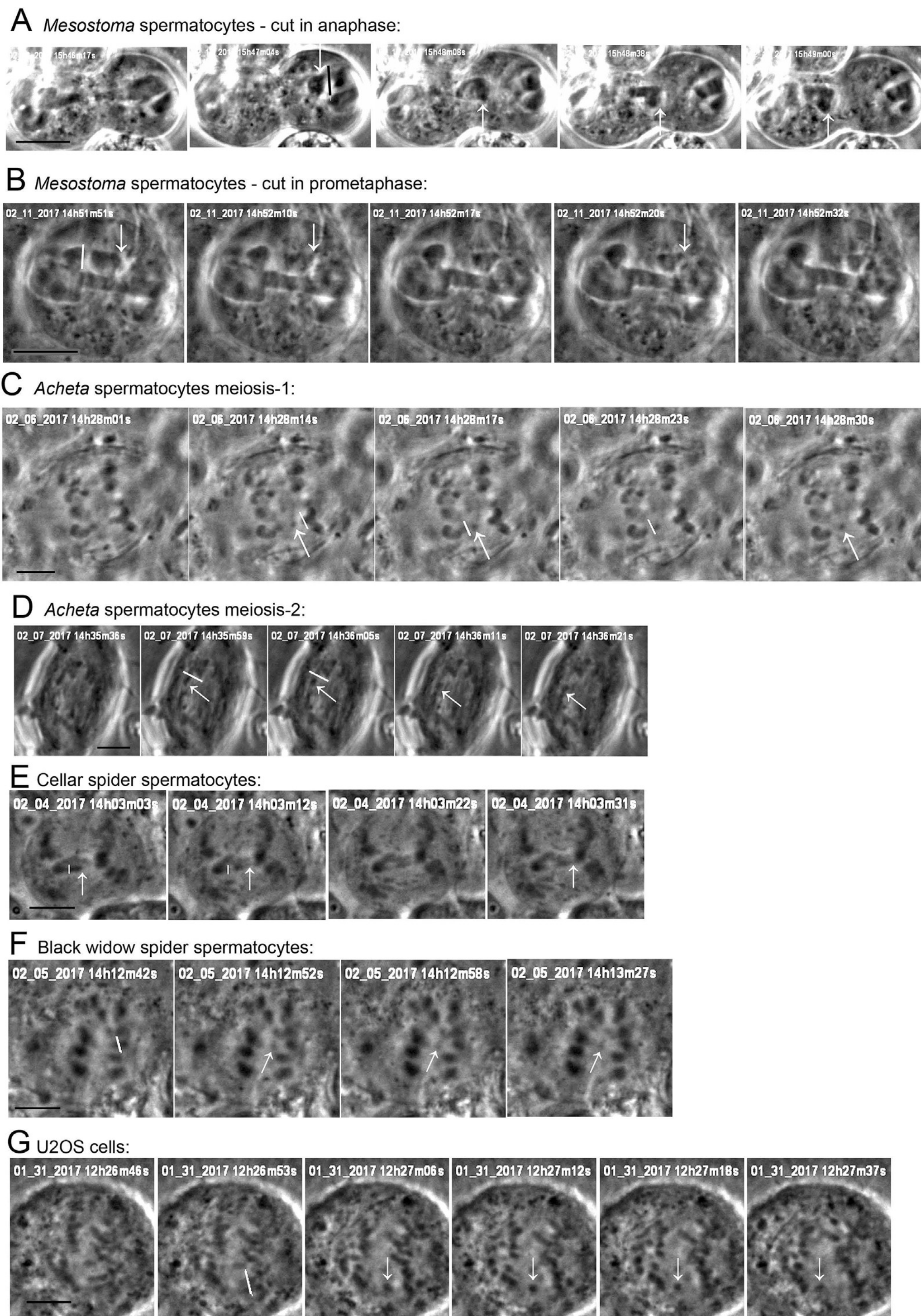

Fig. 14. Arm fragments in different cell types move toward partner telomeres. A: After arms in an anaphase flatworm *(Mesostoma)* spermatocyte were cut (black line in the second image) the arm fragment (indicated by a white arrow) moved across the equator to its partner telomere. B: after an arm was cut in a prometaphase *Mesostoma* spermatocyte, indicated by the white line in the first image, the arm fragment (indicated by a white arrow) moved across the equator to the partner telomere. (Ferraro-Gideon et al., 2014, describe prometaphase in this unusual cell division.) C: after an arm in an anaphase cricket meiosis-I spermatocyte was cut (white line, second image) the resultant arm fragment (indicated by a white arrow) moved toward its partner telomere. When its tether was cut (white line in the third and fourth images) the arm fragment stopped moving and the arm of the partner shortened in length. D: an arm fragment was formed after an arm in an anaphase cricket meiosis-II spermatocyte was cut (white line, second and third images, the second image before the cut and the third after). The arm fragment (indicated by a white arrow) moved backward to the partner telomere. E: after an arm in an anaphase cellar spider spermatocyte was cut (white line, first and second images) the arm fragment (indicated by a white arrow) moved to the partner telomere. F: after an arm in an anaphase black widow spider spermatocyte was cut (white line, first image), the arm fragment (white arrow) moved backwards toward the partner telomere. G: after an arm in an anaphase U2OS cell was cut (white line, second image), the resultant arm fragment (indicated by a white arrow) moved backwards toward its partner telomere. The black lines in the first images in A and B represent 10 μm; those in C, D, E, F and G represent 5 μm.

(1978), who reported that in fortuitous sections of two crane-fly spermatocytes he identified connections between telomeres in three of the 6 bivalent pairs; in one of the pairs there were connections between two of the 4 pairs of arms, similar to the results from cutting arms in living cells (LaFountain et al., 2002; Sheykhani et al., 2017). Connections between separating chromosomes also were seen electron microscopically in meiosis-I of cockroaches (Krishan and Buck, 1965). Given the difficulty of spotting such elements using the electron microscope (Fuge, 1978), it is tempting to think that these components correspond to tethers, but one does not know how regularly this occurs – i.e, whether all chromosomes are connected this way. Without that correlation one does not know, without further experimentation, whether these images are of tethers. Some bridges also have been seen using light microscopy: Feulgen-positive chromatin bridges that are composed of chromatin and are visible using standard light microscopy after staining for DNA, and ultra-fine DNA bridges that do not stain for DNA but are identified by staining for associated proteins BLM, topoisomerase-IIIα, or PICH (Chan et al., 2007). Neither of these DNA bridges seem to fit the criteria for tethers, however. Chromatin bridges (sensu Schrader, 1953, pp 45–46) are present only rarely in healthy normal cells, and when present generally retard or stop movements. Ultra-fine (DNA) bridges, studied primarily in human tissue culture cells, also do not fit the criteria if indeed all the bridges are identified by the present staining procedures: in different reports only 20–80% of normal anaphases have 1 or more ultra-fine bridges (Chan et al., 2007; Barefield and Karlseder, 2012; Gemble et al., 2015), yet tethers are present in all cells. Of those anaphase cells that do have ultra-fine bridges, in different reports there are less than 8 bridges per cell (Chan et al., 2007), or 2 or more bridges in only 50% of the cells (Barefield and Karlseder, 2012), or more than 3 bridges per cell in only 10–15% of the cells (Gemble et al., 2015), considerably fewer than the 46 pairs of chromosomes, yet tethers are associated with at least 75% of the chromosomes, if not all. Further, of the bridges studied, most terminate in centromeric DNA or 'fragile sites', not telomeres (Chan et al., 2007, 2009; Barefield and Karlseder, 2012; Nielsen et al., 2015). Those extending from telomeres at one or both ends of the bridge were only 15% of the identified ultra-fine bridges (Barefield et al., 2012). Thus, if all the ultrafine DNA bridges are identified by these staining methods, they cannot be components of tethers since not all cells have them and there are far too few per cell that extend between telomeres.

We are aware of only one other description of components extending between separating anaphase chromosomes, those containing the protein titin, described as extending between separating anaphase telomeres in crane-fly spermatocytes (Fabian et al., 2007). Though titin has elastic properties consistent with it being a tether component, we do not know from the published report whether all chromosomes have them, or how often they are seen, so without further description and experimentation one cannot be certain whether they might be components of tethers.

Why do tethers extend between separating anaphase chromosomes? What is their function? One can only speculate at this time, but experiments on crane-fly spermatocytes (Sheykhani et al., 2017) indicate that tethers in crane-fly spermatocytes function to coordinate movements of separating chromosomes; this conclusion derived from studying movements that were linked when tethers were present but that became unlinked when tethers were severed. Thus one function in crane-fly spermatocytes seems to be to modulate movements between partner chromosomes, possibly through the tension that they generate on chromosome arms. Perhaps their evolutionary function is to coordinate chromosome movements in general, to help ensure that chromosomes are directed to their proper poles.

How much force does a tether exert? The force is enough to stretch chromosomes by 12%, but is smaller than the poleward force that acts on the kinetochore. Nonetheless, small as it may be, the forces from tethers oppose the poleward forces at kinetochores, and should be taken into account when considering the balance of forces acting on chromosomes during anaphase. We plan to measure the force that drives arm fragment motion by using an optical trap to stop the movement of arm fragments shortly after they are formed.

In summary, we have shown that tethers extend between the telomeres of separating chromosomes in a broad range of anaphase cells, both meiotic and mitotic. They exert a force that opposes the forces propelling the chromosome to the pole, enough to stretch the chromosome arm by 12%. Tethers are elastic at the start of anaphase, but they lose elasticity as they elongate. Tethers have been found in the several cell types we have studied, the only ones where they have been looked for, as far as we know. From the range of species in which tethers are present, tethers probably are present in most cells, if not universally present during cell division.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ejcb.2017.07.001.

References

Ault, J.G., Felt, K.D., Nedo, A.O., Doan, R.N., Andreychik, C.M., Paliulis, L.V., 2017. Co-segregation of sex chromosomes in the male black widow spider *Latrodectus mactans* (Araneae, Theridiidae). Chromosoma, http://dx.doi.org/10.1007/s00412-017-0628-7.

Bajer, A., 1972. Influence of UV microbeam on spindle fine structure and anaphase chromosome movements. Chromosomes Today 3, 63–69.

Barefield, C., Karlseder, J., 2012. The BLM helicase contributes to telomere maintenance through processing of late-replicating intermediate structures. Nucleic Acid Res. 40, 7358–7367.

Chan, K.-L., North, P.S., Hickson, I.D., 2007. BLM is required for faithful chromosome segregation and its localization defines a class of ultrafine anaphase bridges. EMBO J. 26, 3397–3409.

Chan, K.L., Palmai-Pallag, T., Ying, S., Hickson, I.D., 2009. Replication stress induces sister-chromatid bridging at fragile site loci in mitosis. Nat. Cell Biol. 11, 753–761.

Chen, W., Zhang, D., 2004. Kinetochore fibre dynamics outside the context of the spindle during anaphase. Nat. Cell Biol. 6, 227–231.

Elting, M.W., Hueschen, C.L., Udy, D.B., Dumont, S., 2014. Force on spindle microtubule minus ends moves chromosomes. J. Cell Biol. 206, 245–256.

Fabian, L., Xia, X., Venkitaramani, D.V., Johansen, K.M., Johansen, J., Andrew, D.J., Forer, A., 2007. Titin in insect spermatocyte spindle fibers associates with microtubules, actin, myosin and the matrix proteins skeletor, megator and chromator. J. Cell Sci. 120, 2190–2204.

Ferraro-Gideon, J., Hoang, C., Forer, A., 2014. Meiosis-I in *Mesostoma ehrenbergii* spermatocytes includes distance segregation and inter-polar movements of univalents, and vigorous oscillations of bivalents. Protoplasma 251, 127–143.

Forer, A., Pickett-Heaps, J., 2005. Fibrin clots keep non-adhering living cells in place on glass for perfusion or fixation. Cell Biol. Int. 29, 721–730.

Forer, A., Ferraro-Gideon, J., Berns, M.W., 2013. Distance segregation of sex chromosomes in crane-fly spermatocytes studied using laser microbeam irradiations. Protoplasma 250, 1045–1055.

Forer, A., 1982. Crane fly spermatocytes and spermatids: a system for studying cytoskeletal components. In: Wilson, L. (Ed.), Methods in Cell Biol, vol. 25. Academic Press, New York, pp. 227–252.

Fuge, H., 1978. Fine structure of anaphase bridges in meiotic chromosomes of the crane fly *Pales*. Chromosoma 65, 241–246.

Gemble, S., Ahuja, A., Buhagiar-Labarchède, G., Onclercq-Delic, R., Dairou, J., Biard, D.S., Lambert, S., Lopes, M., Amor-Guéret, M., 2015. PLoS Genet., http://dx.doi.org/10.1371/journal.pgen.1005384.

Harsono, M.S., Zhu, Q., Shi, L.Z., Duquette, M., Berns, M.W., 2012. Development of a dual joystick-controlled laser trapping and cutting system for optical micromanipulation of chromosomes inside living cells. J. Biophotonics, http://dx.doi.org/10.1002/jbio.201200019.

Hoang, C., Ferraro-Gideon, J., Gauthier, K., Forer, A., 2013. Methods for rearing *Mesostoma ehrenbergii* in the laboratory for cell biology experiments, including identification of factors that influence production of different egg types. Cell Biol. Int. 37, 1089–1105.

Krishan, A., Buck, R.C., 1965. Ultrastructure of cell division in insect spermatogenesis. J. Ultrastruct. Res. 13, 444–458.

LaFountain, J.R., Cole, R.W., Reider, C.L., 2002. Partner telomeres during anaphase in crane fly spermatocytes are connected by an elastic tether that exerts a backward force and resists poleward movement. J. Cell Sci. 115, 1541–1549.

Nakanishi, Y.H., Kato, H., 1965. Unusual movement of the daughter chromosome group in telophase cells following the exposure to ultraviolet microbeam irradiation. Cytologia 30, 213–221.

Nielsen, C.F., Huttner, D., Bizard, A.H., Hirano, S., Li, T.-N., Palmai-Pallag, T., Bjerregaard, V.A., Liu, Y., Nigg, E.A., Wang, L.H.-C., Hickson, I.D., 2015. PICH promotes sister chromatid disjunction and co-operates with topoisomerase II in mitosis. Nat. Commun. 6, 8962|, http://dx.doi.org/10.1038/ncomms9962.

Roos, P., 1973. Light and electron microscopy of rat kangaroo cells in mitosis. I. Formation and breakdown of the mitotic apparatus. Chromosoma 40, 43–82.

Schrader, F., 1953. Mitosis. The Movements of Chromosomes in Cell Division, 2nd ed. Columbia University Press, New York.

Sheykhani, R., Berns, M., Forer, A., 2017. Elastic tethers between separating anaphase chromosomes in crane-fly spermatocytes coordinate chromosome movements to the two poles. Cytoskeleton 74, 91–103.

Shi, L.Z., Zhu, Q., Wu, T., Duquette, M., Gomez, V., Chandsawangbhuwana, C., Harsono, M.S., Hyun, N., Baker, N., Nascimento, J., You, Z., Botvinick, E.B., Berns, M.W., 2012. Integrated optical systems for laser nanosurgery and optical trapping to study cell structure and function. In: Mendez-Vilas, A. (Ed.), Current Microscopy Contributions to Advances in Science and Technology, Formatex, Badajoz, Spain. Microscopy Book Series Number 5, pp. 685–695.

Spurck, T., Forer, A., Pickett-Heaps, J., 1997. Ultraviolet microbeam irradiations of epithelial and spermatocyte spindles suggest that forces act on the kinetochore fibre and are not generated by its disassembly. Cell Motil. Cytoskel. 36, 136–148.

Wong, R., Forer, A., 2003. 'Signalling' between chromosomes in crane-fly spermatocytes studied using ultraviolet microbeam irradiation. Chromosome Res. 11, 771–786.