Tethers: elastic connections between separating partner chromosomes in anaphase

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Abstract

Recent work has demonstrated the existence of elastic connections, or tethers, between the telomeres of separating partner chromosomes in anaphase. These tethers oppose the poleward spindle forces in anaphase. Functional evidence for tethers has been found in a wide range of animal taxa, suggesting that they might be present in all dividing cells. An examination of the literature on cell division from the 19th century to the present reveals that connections between separating partner chromosomes in anaphase have been described in some of the earliest observations of cell division. Here we review what is currently known about connections between separating partner chromosomes in anaphase, and we speculate on possible functions of tethers, and on what they are made of and how one might determine their composition.

Introduction

Recent experiments have described elastic connections between the telomeres of separating anaphase chromosomes that are present in most if not all animal cells. These "tethers" were defined operationally in two ways: (1) telomere-containing fragments of arms created in anaphase move backwards, across the equator, until they reach the partner telomere moving to the opposite pole (backwards movement shown in Fig 1) (LaFountain et al. 2002), and (2) cutting the tether between separating chromosomes causes the arms to contract (to around 90% of their normal length) (Forer et al. 2017). Cutting the tether also stops the backwards movement (Fig 1). Originally described in crane-fly spermatocytes as extending between two of the four separating arms of anaphase-I chromosomes (LaFountain et al. 2002), these connections have been found in a broad range of meiotic and mitotic cells (Forer et al. 2017). Tethers seem to be a general component of the mitotic apparatus. That there are spindle forces opposing those that move chromosomes to the poles needs

to be considered in dealing with force balances during mitosis, and the structure and composition of the mechanical links need to be understood. Their presence in spindles raises some questions. What is their function? How did they evolve? Before discussing these issues, we explore the long history of descriptions of connections between separating chromosomes, seeking any hint of function that might be revealed.

Interzonal connections

The first studies of chromosomes in cell division were done in fixed, stained preparations and figures were drawn from camera lucida projections. Almost invariably in these preparations, drawings of anaphase cells show some substance connecting the separating chromosomes (Carnoy 1885; Montgomery 1899; Wilson 1905; Stevens 1905; Stevens 1909; Andrews 1915; and many others). Montgomery (1899), when studying the mitotic and meiotic cell divisions of Pentatomid bugs, described the material as, "true connective fibres, derivatives of the chromatin contained in chromosomes," and as chromatin that is drawn out between separating chromosomes forming a hollow tube. Hughes-Schrader (1931) observed a tube connecting separating partner chromosomes in primary spermatocytes in the coccid *Llaveia bouvari*. Schrader (1935) in studying meiosis in both the salamander (*Amphiuma*) and the bug (*Protenor*) showed and discussed "interzonal connections" between separating partner chromosomes. Schrader described these connections as a tube or sheath around separating anaphase chromosomes that appears to stretch and eventually break (Fig 2), though Schrader never addressed a possible function for the connection. Carothers (1936) discovered interzonal connections in primary spermatocytes in grasshoppers. She did not see a tube surrounding the separating chromosomes, but did see fibers connecting the anaphase I chromosomes (Fig 3). Carothers called them parallel fibers of "viscous protoplasm" that eventually separated. Centrifugation of *Tradescantia* stamen hair cells could both

shift the position of the interzonal connections and move the telomeres to which they were connected, suggesting that they were flexible, yet sufficiently sturdy to maintain a connection to their associated chromosome (Fig 4) (Andrews 1915). Interzonal connections were observed in many species of animals (Carnoy 1885; Montgomery 1899; Wilson 1905; and many others), plants (Andrews, 1915;Gentscheff and Gustafsson 1940; Schaede 1930; Ostergren 1949; Bajer and Mole-Bajer 1986;and others), and were also observed in protists (McAllister 1931). Interzonal connections were also observed in holocentric mitosis (Montgomery 1899), and in meiosis in species with holocentric chromosomes, whose chromosomes could be functionally monocentric or holocentric during the meiotic divisions (Montgomery 1899; Wilson 1905; Ostergren 1949; Hughes-Schrader 1931; Schrader 1935). We could find no instance in which the authors discussed their possible function.

Interzonal connections were described in a period when cytologists were studying fixed/stained cells to try to piece together spindle structures and their dynamics. For many, there was skepticism about whether spindle fibres themselves were artefacts produced by fixation (reviewed in McIntosh and Hays 2016). Once polarisation microscopy observations showed that spindle fibres indeed existed in living cells (Hughes and Swann 1948; Swann 1951; Inoue and Dan 1951;Inoue 1952;Swann and Mitchison 1953) and scientists were able to study living cells using phase-contrast microscopy, most of the subsequent work was directed at measuring how chromosomes moved and what the forces might be. There were many hypotheses for how chromosomes moved, including electrostatic forces and forces from the interzone pushing chromosomes poleward (reviewed in Schrader 1944). Most of these hypotheses were eliminated, except for the hypothesis that forces arose from kinetochore spindle fibres (e.g., Schrader 1934; Cornman 1944; reviewed in McIntosh and Hays 2016). Because interzonal connections between

separating chromosomes were not seen in living cells, and because most of the attention was directed at forces from kinetochore fibres, any possible role of putative connections between separating partner chromosomes (i.e. sister chromatids or homologues) were just not considered, except for evidences we now describe.

Functional evidence for tethers

The first evidence that some form of "interzonal connection" could have a functional role in tethering separating partner chromosomes came from Carlson's micromanipulation experiments. Carlson, using a Chambers micromanipulator, swept a small needle across the spindle midzone of anaphase grasshopper neuroblasts. When the needle swept through the region between the separating sister-chromatid arms (which had no visible connections between them), the two separating arms moved following movements of the needle (Carlson 1952). This suggested the presence of a persistent connection between the separating sister chromatids. Later experiments confirmed this: micromanipulation of early anaphase I grasshopper spermatocytes revealed that moving one chromosome arm with a micromanipulation needle would cause movement in the partner chromosome from which it was separating (Paliulis and Nicklas 2004). UV microbeam experiments in silkworm spermatocytes suggested a function for persistent connections. Irradiation of one spindle pole in telophase I silkworm spermatocytes causes the entire chromosome group associated with the irradiated pole to move to the opposite spindle pole (Nakanishi, 1965). This result suggested that there was something applying force to separating chromosomes that opposed the anaphase poleward force.

Further evidence that separating anaphase chromosomes are not independent came from ultraviolet microbeam irradiations of individual kinetochore fibres in anaphase crane-fly spermatocytes. These irradiations stopped the movements of the associated chromosomes when

the ultraviolet light was of sufficient power and proper wavelength (Forer 1966; Sillers and Forer 1981). Other chromosomes in the cell were not affected by the irradiation except for the partner chromosome moving to the opposite pole: the half-bivalent connected to the irradiated kinetochore fibre and its partner moving to the opposite pole both stopped moving. This surprising result does not require or imply mechanical connections between the partners, though such connections might help explain them, nor was the possibility of such connections discussed, but the result emphasised that the movements of partner chromosomes to opposite poles were coordinated. Subsequent experiments on crane-fly spermatocytes also pointed to movements of separating anaphase chromosome partners being coordinated (Sillers and Forer 1981); here too there was no attempted tie-in of the results to mechanical connections between partner chromosomes.

Mechanical linkages between partners later were identified in micromanipulation experiments on crane-fly spermatocytes: when individual anaphase chromosomes (half-bivalents) were pushed sideways (i.e., perpendicular to the spindle axis), the partner chromosomes also moved sideways (Forer and Koch 1973). Thus the conclusion that separating chromosomes *are* connected mechanically. Begg and Ellis (1979) disputed this conclusion, however: when they moved anaphase crane-fly spermatocyte chromosomes sideways, the partner chromosomes did not move. Therefore their conclusion was that the separating chromosomes are *not* connected mechanically. They said that only when culture conditions 'deteriorated' did they see linkages between separating chromosomes, in that both pushed and partner chromosomes moved sideways. They thus suggested that the results of Forer and Koch (1973) were because their cells were not healthy. However, evidence from other taxa supported the existence of linkages tethering separating chromosomes and applying forces that oppose the anaphase poleward force. Ultraviolet microbeam irradiation that severed spindle fibres associated with separating anaphase

chromosomes in newt fibroblasts caused the associated chromosome to suddenly and briefly move away from the spindle pole toward which it had previously been moving (Spurck et al. 1997), again suggesting that there are forces opposing the anaphase poleward chromosome movements. Further suggestion of these opposing forces comes from laser experiments. In *Fusarium* (fungus), anaphase chromosomes moved at a mean speed of 7.6 μm/min. When the midzone of an anaphase spindle was irradiated using a laser, the rate of chromosome movements increased to 22.4 μm/min. One interpretation of this result is that cutting the tethering linkages removed the opposing force and sped the rate of chromosome separation (Aist and Berns 1981).

In further work with crane-fly spermatocytes, it was noted that some individual chromosome arms that ordinarily trailed their kinetochores to the pole instead moved ahead of their kinetochores. This occurred in crane-fly spermatocytes after treatments that slowed anaphase speeds, for example after ultraviolet microbeam irradiations (Adames and Forer 1996) or after treatments with anti-actin drugs (Forer and Pickett-Heaps 1998). Because only one or two of the four arms moved ahead of the kinetochore, and never more than two, Adames and Forer (1996) suggested that this is because the other two arms are mechanically linked. That is, they suggested that two arms are mechanically linked to partners and therefore cannot swing ahead of the kinetochores during poleward movement, whereas the other two are not linked and thus are able to move ahead of their kinetochores. This suggestion explained their observations, but there was no solid evidence that separating chromosomes are mechanically linked.

Yin and Forer (1996) implicated something in the interzone (the region between the separating anaphase chromosomes) as being essential for the interdependence of partner anaphase chromosomes in crane-fly spermatocytes. They deduced this because they uncoupled the movements by irradiating the interzone with an ultraviolet microbeam. Ultraviolet microbeam

irradiation of kinetochore fibres blocked poleward movements of both the chromosome attached to the irradiated fibre and its partner moving to the opposite pole, but the movements were uncoupled when the interzone was irradiated either prior to or after irradiation of the kinetochore fibre: only the chromosome attached to the irradiated kinetochore fibre stopped moving. They suggested as a likely explanation that mechanical linkage between separating chromosomes might explain their results. But they, too, had no real evidence for such linkage. Further evidence for mechanical linking of separating chromosomes arose from ultraviolet microbeam irradiations of anaphase kinetochores.

Ultraviolet microbeam irradiations of single kinetochores in anaphase crane-fly spermatocytes cause the motion of all anaphase chromosomes to temporarily stop (Ilagan and Forer 1997). However, in 2/23 cells irradiated at a kinetochore the anaphase chromosome with irradiated kinetochore moved backwards, to the partner moving to the other pole, at speeds much faster than anaphase chromosome movements (Ilagan et al. 1997). Similar backward movements were seen in rare cells in which kinetochore fibres were irradiated just in front of the kinetochore (Ilagan et al. 1997). Because the chromosomes moved backward with an extended rear-facing arm moving toward the extended arm of the partner chromosome, Ilagan et al. (1997) suggested that the movements were caused by elastic mechanical connections between the arms of separating anaphase chromosomes. While these experiments provide indirect evidence for such connection, the backward movement was not regularly seen, so one could argue that they are not a regular occurrence.

LaFountain et al. (2002) presented definitive experimental proof that arms of separating chromosomes in crane-fly spermatocytes are connected by elastic links, that they called ‘tethers’. When they used a laser to cut off the ends of arms from anaphase chromosomes, the fragments

they produced moved across the equator to the telomeres of the partner chromosomes moving to the opposite poles, at speeds many times faster than anaphase chromosome movement. This occurred for no more than 2 of the 4 arms of each separating chromosome pair, so only two of the 4 arms are connected with tethers. They ruled out that this occurred only when the cells had 'deteriorated' by noting that tethers identified in this way were consistently associated with arms that appeared pulled backwards, and that these arms are seen in fresh cells fixed immediately upon dissection. Thus they are not due to 'deteriorating' conditions. In further experiments they ruled out that movements of the arm fragments were due to the action of interzonal spindle microtubules. Arm fragments stopped moving when the laser ablated either the telomere of the arm fragment or the telomere of the partner. This indicates that arm fragment movement is not caused by microtubules propelling the arm fragment because if it was, motion would not stop after ablation of the telomere toward which the fragment is moving. Similarly, when the moving arm fragment was cut in half, only the telomere-containing arm sub-fragment moved. Because arm fragment movement requires that both telomeres be undamaged, arm-fragment movement seems to be due to an elastic connection between separating telomeres.

The tethers identified in crane-fly spermatocytes seem to lose elasticity as they increase in length, because as they became longer the movements of severed arms toward the opposite telomere were slower and ended before the arm fragments reached the other telomere, or (later) ended before the equator, or (later) did not take place. Thus LaFountain et al. (2002) suggested that the elongated tethers either became completely non-elastic or were detached from telomeres.

Subsequent experiments suggested that tethers were involved in coupling the movements of separating (partner) chromosomes. When Sheykhani et al. (2017) cut tethers prior to cutting kinetochore spindle fibres they uncoupled the dependent movements of the partner chromosomes.

They suggested that the tethers are involved in coordinating their movements, but this was based solely on a correlation. While the correlation was clear, it is not absolutely certain that the movement coupling arises from cutting tethers: it could arise from cutting another not-identified component in the interzone.

The idea that between separating anaphase chromosomes there might be connections that can exert force was substantially expanded through experiments on cells other than crane-fly spermatocytes.

Tethers were identified and characterised in PtK cells (Forer et al. 2017), in two ways. One was to cut arms (with a laser) and seeing that the arm fragments moved backwards to the partner telomere. The other was to cut tethers directly (with a laser), after which the associated arms contracted (to about 90% of their initial length). Since arms contracted even at tether lengths at which arm fragments did not move, they concluded that tethers remain present until late anaphase but they lose elasticity as they elongate. They identified tethers in various other cells as well, using motion of arm fragments as criterion. In all, tethers were identified in: meiosis-I spermatocytes of a turbellarian flatworm; meiosis-I and meiosis-II crane-fly spermatocytes; meiosis-I and meiosis-II cricket spermatocytes; meiosis-I black widow and cellar spider spermatocytes, from two distinct lineages of spiders; mitotic marsupial (PtK cells) cells; and mitotic human (U2OS) osteosarcoma cells. In these cells the tethers were not found on all of the arms. Thus tethers are found in mitotic spindles of a broad range of cells and are associated with most if not all chromosomes, though not with each arm of the chromosomes. Together with the identification of interzonal connections between anaphase chromosomes, cited above, in animals, plants, protists, and fungi, the overall conclusion is that tethers may be universal in mitotic and meiotic cells.

The function(s) of tethers

What is the function of tethers? Do they facilitate communication between separating partner chromosomes? Do they transmit forces between separating partner chromosomes? Do they have multiple roles? We do not know what tethers are made from but to get a clear understanding of the function of tethers, we will need to identify what composes the tether. Montgomery (1899) wrote that the tethers are made of "linin," a hypothesized chromatin matrix component. We do not know what "linin" is composed of and we also do not know what might be tethering chromosomes. Recent work shows evidence of persistent connections between some separating sister chromatids in anaphase in mammalian tissue culture cells (Wang et al. 2008; Nakajima et al. 2007), and in yeast (Harrison et al. 2009; Renshaw et al. 2010). Strands of DNA are visible in the interzonal space, and components of the cohesin complex could be present in that region as well (Wang et al. 2008; Nakajima et al. 2007; Harrison et al. 2009; Renshaw et al. 2010). Are the persistent, visible connections the tether? Is DNA present in the tether? Are other chromatin components like the cohesin complex part of the tether? Previous papers speculated that tethers could be composed of titin, because titin is present in the spindle midzone in anaphase crane fly spermatocytes seeming to connect chromosome arms (Fabian et al. 2007); but the presence of titin or any other substance does not necessarily prove that titin or the other substance is the tether.

From present evidence for presence of tethers, using the 'functional assay' of whether arm fragments move toward the opposite telomere, it is strange that not all arms from any given chromosome seem to have tethers. That is certainly true for crane-fly spermatocytes, which has the best data in this regard (LaFountain et al., 2002; Sheykhani et al., 2017). Not all arms have tethers in other cells studied (Forer et al., 2017), but the situation is less clear in these less well-studied cells.

Whatever the tether is composed of, tethers are elastic when shorter and become inelastic as they lengthen during anaphase. We might speculate that phosphorylation is involved with maintaining the elasticity, and that dephosphorylation causes the tethers to become inelastic, as follows.

Calyculin A inhibits protein phosphatases PP1A and PP2A, so that phosphorylated proteins such as myosin do not become dephosphorylated and thereby maintain their active state (described in Fabian et al. 2007). When Calyculin A is added to early anaphase crane-fly spermatocytes, the chromosomes continue moving to the pole and after they reach the pole they move backwards towards their partners at the opposite pole, arms first (Fabian et al. 2007). It seems reasonable to suspect that this backward movement is due to elastic tethers. If so, the Calyculin A has prevented the tethers from becoming inelastic since tethers in crane-fly spermatocytes are inelastic at late stages of anaphase: arm fragments formed in late anaphase do not move toward the equator or partner. If blocking protein phosphatase 1 indeed prevents tethers from losing elasticity this may be a tool to determine the composition of the tethers.

We suggest that the identity of the tether might be discovered in a second look at various mutant screens. If the tether plays a key signaling or force-transmitting role in anaphase, it is likely that the absence of a tether would lead to a subtly higher rate of chromosome loss or gain after cell division. Mutants that have a slight increase in aneuploidy, or small-molecule inhibitors whose use leads to slight increases in aneuploidy (with no obvious reason) could be tether-mutants or tether-inhibitors, and a screen of such mutants/inhibitors could be worthwhile in discovering the identity of the tether.

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FIGURES

Figure 1. Images of a crane-fly spermatocyte, illustrates movement of an arm fragment, and that the movement was stopped by cutting its tether. The two sex chromosome univalents are indicated by vertical open arrows in the first image. The two separating half bivalents are indicated by the two angled arrows in the second image. The horizontal line in the third frame indicates the position of the laser line immediately after cutting the chromosome arm. The horizontal arrows (from the right) in the fourth through sixth images point to the arm fragment formed after cutting the arm. The horizontal line in the middle image bottom row indicates the position of the laser line just after the laser cut the tether. The arm fragment moves toward its partner (third, fourth and images) but stops moving after its tether is cut (fifth image). Times are given at the top of each frame as hour:minute:second.

Figure 2. Diagram showing interzonal connections between separating anaphase I chromosomes. The connections are shown as a tube connecting the separating chromosomes (From Schrader 1935, Figs. 14 and 15).

Figure 3. Interzonal connections between separating anaphase I chromosomes of the grasshopper *Trimerotropis fallax* (From Carothers 1936, Fig 21).

Figure 4. Interzonal connections and the chromosomes to which they are connected bend in response to centrifugation in *Tradescantia* stamen hair cells (From Andrews 1915, Figs. 16-21).

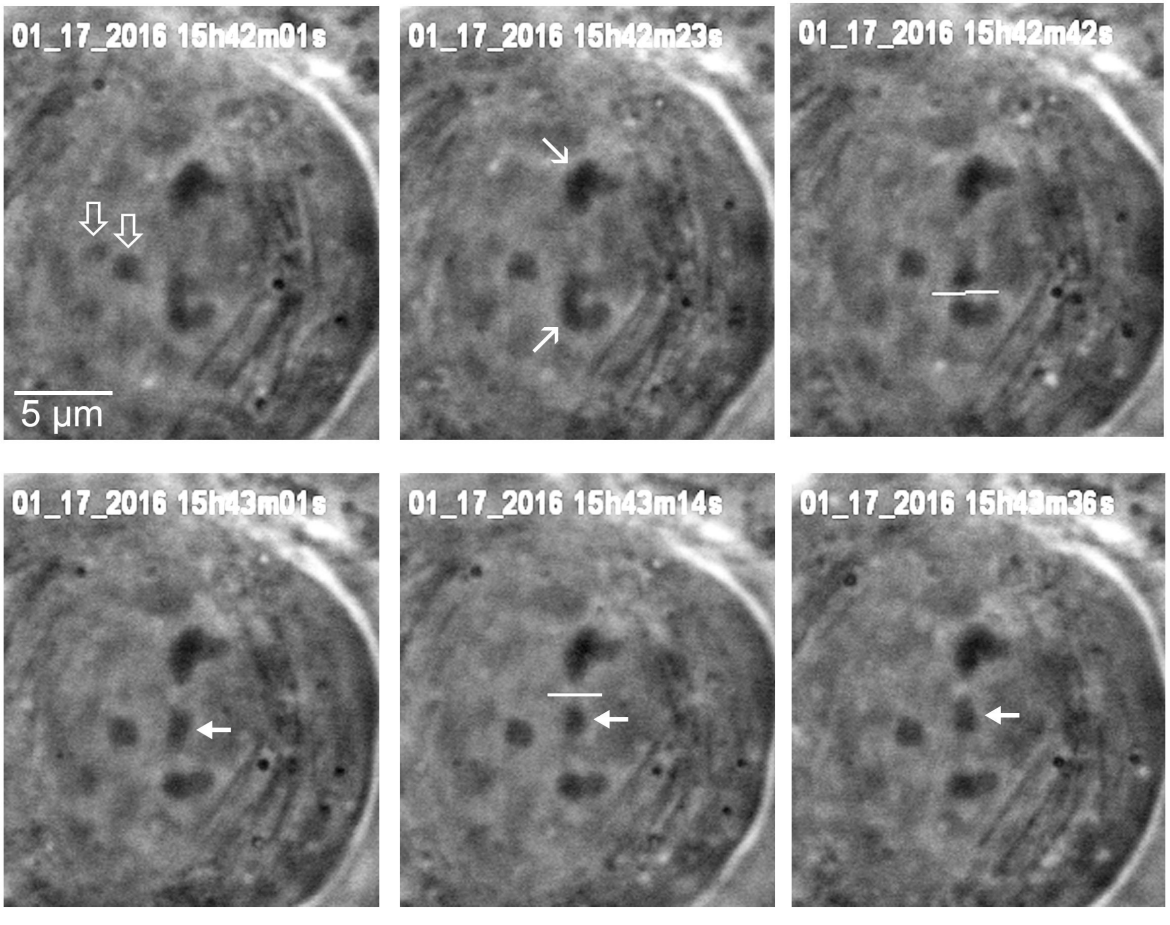
01_17_2016 15h42m01s
5 µm
01_17_2016 15h42m23s
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FIGURE 1

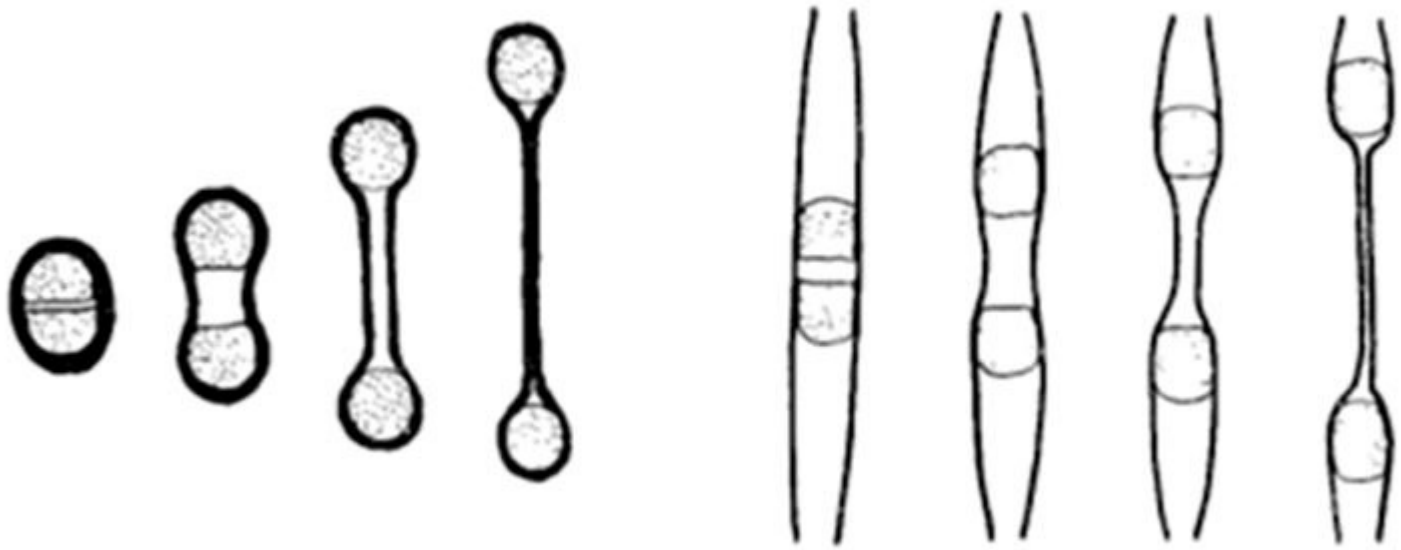

FIGURE 2

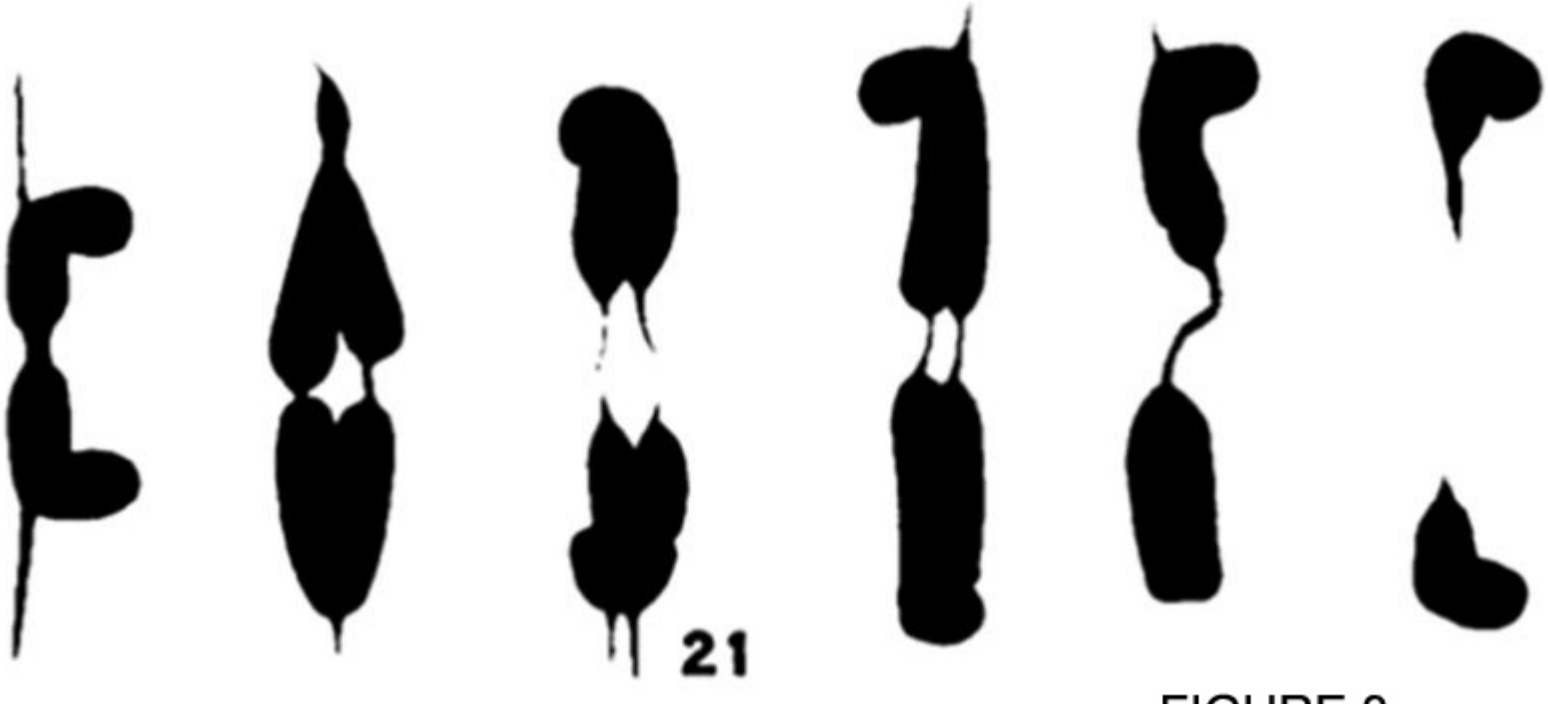

FIGURE 3

FIGURE 4