Precocious cleavage furrows simultaneously move and ingress when kinetochore microtubules are depolymerized in *Mesostoma ehrenbergii* spermatocytes.

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ABSTRACT

A "precocious" cleavage furrow develops and ingresses during early prometaphase in *Mesostoma ehrenbergii* spermatocytes (Forer and Pickett-Heaps, 2010). In response to chromosome movements which regularly occur during prometaphase, and that alter the balance of chromosomes in the two half-spindles, the precocious furrow shifts its position along the cell, moving 2-3 µm towards the half cell with fewer chromosomes (Ferraro-Gideon et al. 2013). This process continues until proper segregation is achieved and the cell enters anaphase with the cleavage furrow again in the middle of the cell. At anaphase the furrow recommences ingression. Spindle MTs are implicated in various furrow positioning models and our experiments studied the responses of the precocious furrows to the absence of spindle microtubules (MTs). We depolymerized spindle MTs during prometaphase using various concentrations of nocodazole (NOC) and colcemid. The expected result is the furrow should regress and chromosomes remain in the midzone of the cell (Cassimeris et al. 1990). Instead, the furrows commenced ingression and all three bivalent chromosomes moved to one pole while the univalent chromosomes, that usually reside at the two poles, either remained at their poles or moved to the opposite pole along with the bivalents, as described elsewhere (Fegaras and Forer, 2018). The microtubules were completely depolymerized by the drugs, as indicated by immunofluorescence staining of treated cells (Fegaras and Forer, 2018), and in the absence of microtubules the furrows often ingressed (in 33/61 cells) at a rate similar to normal anaphase ingression (~1 µm/min), while often simultaneously moving toward one pole. Thus, these results indicate that in the absence of anaphase and of spindle microtubules, cleavage furrows resume ingression.

KEYWORDS: *meiosis, cytokinesis, cleavage furrow, microtubules, nocodazole*

INTRODUCTION

In a typical cell division, anaphase begins before the cleavage furrow forms. In animal cells the cleavage furrow is a contractile ring made of actin and myosin II as well as various other regulatory proteins (Eggert et al. 2006). After anaphase begins the furrow ingresses at the midzone of the cell, eventually cleaving the progenitor cell into two equally sized daughter cells (Barr and Gruneberg 2007). Furrow position is determined before or during anaphase onset, usually during the metaphase-anaphase transition, and is irreversible even if the mitotic apparatus is removed (Rappaport 1981). The three main furrow positioning models are the astral stimulation, astral relaxation and central spindle stimulation models (Barr and Gruneberg 2007). These models all rely on the presence of microtubules to transmit a signal to the cell cortex, whether it be astral microtubules that produce either a positive or a negative furrow stimulus (Rappaport 1961, 1965, 1985), or interzonal (non-kinetochore) microtubules which transmit signaling proteins to the cortex by an unknown mechanism as shown in various cell types through addition of drugs that selectively target astral microtubules while conserving interzonal microtubules (von Dassow 2009) or the creation of asterless mutants (Bonaccorsi et al. 1998). Although there are several other theories that we have not mentioned, the two requirements that all these theories share is the presence of microtubules and the commencement of anaphase.

Certain cell types do not require anaphase commencement to have their cleavage furrows form, however. In various diatom species such as *Hantzschia, Nitzschia, Pinnularia, Cymatopleura* and *Surirella* (Pickett-Heaps et al. 1978, 1979; Pickett-Heaps 1980; Pickett-Heaps 1991), furrows appear at the start of prometaphase. Precocious cleavage furrows also are also common in various green algae, such as *Spirogyra* (McIntosh et al. 1995). In these organisms, the precocious furrows partially ingress and arrest until anaphase when they fully ingress and cleave the cell in two. Likewise, spermatocytes from a species of hermaphroditic flatworm *Mesostoma ehrenberghii* have precocious furrows (Forer and Pickett-Heaps 2010). The precocious furrows in *Mesostoma* spermatocytes form in early prometaphase, arrest and slowly ingress somewhat (Ferraro-Gideon et al. 2013; Forer and Pickett-Heaps 2010), and have the same dynamics as regular cleavage furrows, in that anti-actin or anti-myosin drugs cause the furrows to regress (Forer and Pickett-Heaps 2010).

Although the formation of a precocious furrow is not unique to *Mesostoma*, what is unique is that the furrow changes position in the cell in response to chromosome imbalances in the cell (Ferraro-Gideon et al. 2013, 2014). Throughout prometaphase, which can last up to 2 hours, univalents often move between the two poles, temporarily resulting in unequal numbers of univalents at the two poles (Oakley 1984). By the start of anaphase there is one of each kind of univalent at each pole (Oakley 1984). When there are unequal numbers of univalent at the two poles, the furrow shifts 2-3 μm towards the pole with fewer univalents (Ferraro-Gideon et al. 2013, 2014; Forer and Pickett-Heaps 2010). This creates an asymmetrically positioned furrow, with one half cell being larger than the half cell with fewer chromosomes. It is important to note that the asymmetric furrow in *Mesostoma* is not the same as asymmetrically dividing cells such as *C. elegans* or *Drosophila* embryos in which the spindle is positioned asymmetrically in the cell (Cytrynbaum et al. 2005; Grill et al. 2001; Gonczy 2008). In *Mesostoma* the spindle

remains symmetric throughout division even when the cleavage furrow is not; by anaphase there are equal numbers of univalent at the two poles, and a symmetrically placed furrow that ingresses to produce two equally-sized daughter cells (Forer and Pickett-Heaps 2010; Ferraro-Gideon et al. 2013).

In our experiments we wanted to test whether microtubules are required for positioning of the cleavage furrow, as required by many models. We chose to target microtubules by adding nocodazole (NOC) to *Mesostoma ehrenbergii* spermatocytes. NOC has been used to lock the cell cycle in the G2 to M phase as a means to synchronize cell cultures (Poxleitner et al. 2008), and has been used in cancer cell studies (Choi et al. 2011; Mollinedo and Gajate 2003; Jordan and Wilson 2004). NOC targets the dynamic nature of microtubules which in cells constantly switches between phases of growth or shrinking. When microtubules are treated with concentrations of NOC above 1μM, they enter a third, paused state with arrested growth, as observed in newt lung epithelial cells (Vasquez 1997). In a cell undergoing division, this equates to the kinetochore fiber losing its formerly tight connection to the kinetochore. High concentrations of NOC also cause microtubules to detach from the microtubule organizing centre (MTOC), generating microtubule fragments (Yang et al. 2010). Microtubules are a key in furrow positioning models, and they are also considered the primary force producers of chromosome movements. When microtubules are depolymerized during prometaphase or metaphase it is expected that chromosomes stay in the middle of the cell and arrest at the equator (Cassimeris et al. 1990) and that the cleavage furrow will regress (Alsop and Zhang 2003; Barr and Gruneberg 2007; Glotzer 2004; Murthy and Wadsworth 2008). However when Mesostoma spermatocytes are treated with NOC, in each cell the bivalents (and sometimes the univalents) detach from one pole and move towards the opposite pole (Fegaras and Forer 2018), and the cleavage furrow usually ingresses, as described in the present article.

Our results indicate that after the chromosomes move as a consequence of NOC treatment, the precocious furrow remains ingressed or ingresses further and simultaneously moves towards the half-cell with fewer chromosomes. This movement results either in the furrow reaching the far end of the half-cell and disappearing or the furrow partially cleaving the cell into two grossly unequally sized daughter cells which together look like shmoos (Capp 1948). Thus, furrows can ingress and change position in the apparent absence of spindle microtubules. The mechanism by which it does so remains unknown.

MATERIALS AND METHODS

Living cell preparation:

Mesostoma are reared in the lab as described by Hoang et al. (2013). We dissected animals that contain resting eggs. The animals are chosen based on the opaque white colour and large size of their testes. We extracted the entire testes using pulled 5, 10 or 15 μL micropipettes (Fisher) and then laid them onto a cover slip into a small drop of 0.2 mg/mL bovine fibrinogen (Calbiochem) diluted in *Mesostoma* Ringers solution (61 mM

NaCl, 2.3 mM KCL, 0.5 mM $CaCl_2$ and 2.3 mM phosphate buffer), prepared fresh daily. We used the pulled needle tip to pierce the testes and break the lobes, after which cells lie flat on the cover slip. Thrombin is added, which causes the fibrinogen to clot and the clot holds the cells in place, as previously described (Forer and Pickett-Heaps 2005; Ferraro-Gideon et al. 2014). We then mounted the coverslip preparations on a perfusion chamber containing *Mesostoma* Ringers solution to submerse the cells, and sealed the edges with wax. Live primary spermatocytes were viewed with phase-contrast microscopy using a Nikon 100x oil immersion lens (NA 1.3) and recorded in real-time on a DVD recorder. Cells were periodically streamed with 0.5-1 mL Ringers to exchange their immersion medium before the addition of drug. The cells were then perfused with 2-5 mL of either 5µM, 10µM or 20µM NOC (Sigma) or 50 µM or 100 µM colcemid (Sigma) in *Mesostoma* Ringers streamed through the perfusion chamber. Both drugs were in a 1000x concentrated stock solution diluted with Dimethyl sulfoxide (DMSO); the 0.1% concentration of DMSO has no effect on *Mesostoma* spermatocytes (Forer and Pickett-Heaps 2010). The drugs were usually washed out with 5-10 mL of Ringer's solution 10-15 minutes after initial immersion in the drugs. The cells were followed by video microscopy for 45 to 120 minutes depending on the experiment.

Cleavage Furrow and Chromosome Movement Analysis:

We used the free computer program virtual dub (www.virtualdub.org) to time lapse the image sequences into videos at 60 times the original speed. Our in-house program Winimage (Wong and Forer 2004) was used to plot furrow width (the 'diameter' of the cell at the position of the furrow) and furrow position along the length of the cell as shown in the diagram in Figure 7a; the measured furrow width is the distance between black arrowheads in Figure 7a. Measuring the distance between the two black arrowheads at different times gave data on the rate of furrow ingression or regression, and measuring the distance between one of the black arrowheads and the red "X" (Figure 7a) gave data on the movement of the furrow along the length of the cell. Furrow movement and ingression graphs were generated in the program SlideWrite 7.0. Adobe Photoshop was used to overlay cell outlines as seen in Figures 2 and 4, and to create image montages from single video frames. Chromosome velocity as shown in Table 2 was collected in a similar fashion to furrow position, as described in detail in Fegaras and Forer (2018). The position of each bivalent's and/or univalent's kinetochore was plotted in comparison to a fixed point at the cell's edge, near the pole (e.g., the X in Figure 7a). Distances calculated in WinImage were converted to graphs of distance versus time using the commercial program SlideWrite. Student's t-test was performed on all data to compare the different times and velocities for furrow and chromosome movement at the different concentrations of drug.

RESULTS

Control cells

Of the several unusual features in *Mesostoma* spermatocytes (Ferraro-Gideon et al. 2013), we concentrate here on the precocious, pre-anaphase cleavage furrow which

forms during prometaphase, up to two hours before anaphase commencement (Forer and Pickett-Heaps 2010). Spermatocytes have 3 bivalent (b) and 4 univalent (u) chromosomes (Fig. 1). Throughout prometaphase the three bivalents persistently oscillate towards and away from either pole, at speeds roughly 5-6 μm/min, as first described by Fuge (1987, 1989). The univalents are at the poles throughout prometaphase. By the start of anaphase, they have assorted themselves into one of each kind of univalent at each pole (Fig. 1), which they achieve by moving to opposite poles. When a univalent changes poles, this causes an imbalance in the number of chromosomes at the two poles (Oakley 1984), after which the furrow shifts 2-3 μM towards the pole with fewer chromosomes (Forer and Pickett-Heaps 2010). Throughout prometaphase the furrow can either be symmetrically (Figure 2a and a') or asymmetrically oriented (Figure 2b and b') depending on the distributions of univalent chromosomes. By the start of anaphase, the univalents have achieved proper distribution and the furrow always is in a symmetrical position. After anaphase the furrow ingresses, eventually cleaving the cell into two equally sized daughter cells.

Drug-treated cells

We treated cells with NOC or colcemid to depolymerize the spindle microtubules. The effects on chromosome movement were the same for both anti-microtubule drugs, as were effects on the furrow, but we studied in detail only the effects of NOC on the cleavage furrow.

Chromosome movement

To help understand the effects on the cleavage furrow of removal of microtubules we first need to describe the effects on the chromosomes. The effects of NOC and colcemid on chromosome movements are described in detail elsewhere (Fegaras and Forer 2018). Briefly, immediately after addition of NOC at all concentrations (5μM, 10μM, 20μM), the bivalent chromosomes stretch out towards both poles (Figure 3a-c). They pause there for a short time period, and then detach from only one pole (Figure 3d). The detached kinetochores quickly move towards the opposite pole and eventually end their movement (Figure 3e and f). Sometimes only bivalents detach (Figure 4i) but sometimes both the bivalents and univalents detach, both detaching from the same pole (Figure 4ii). The effects of 50μM and 100μM colcemid are very similar to NOC, verifying that the chromosome response is due to depolymerizing the microtubules. Immunofluorescence observations indicated that the spindle microtubules are almost completely gone when chromosome detachment and movement occurs (Fegaras and Forer 2018), and remain absent afterwards as the cleavage furrow ingresses and or moves, which we now describe.

Precocious furrow moves and changes its width

After the kinetochores move to the opposite pole, the cleavage furrow moves and/or changes its width (its 'diameter', the distance between the arrowheads in Figure a). The furrow always moves toward the pole that has fewer chromosomes, but the distances it moves and whether it changes width vary: furrows respond differently in

different cells, as illustrated in Figure 5 and as shown in two supplemental video sequences (S1 and S2). We categorize 3 different furrow *movements*: (1) partial movement (Figure 5a), where the furrow moves between 0 - 5μm, which therefore includes furrows that did not move; (2) halfway movement (Figure 5c), where the furrow moves between 5 - 10 μm; and (3) full movement (Figure 5b), where the furrow moves > 15 μm, off the end of the cell; that is, the furrow contracts to a smaller and smaller diameter as it moves down the cell, and eventually disappears, as in the second image in Figure 5b. We categorize 3 different furrow *width* ('diameter') *changes*: (1) partial change, where the furrow width increases or decreases slightly if at all, i.e., by less than 2 μm; (2) ingression (Figures 5b and 5c), where the width decreases >2 μm; and (3) regression (e.g., Figure 5a), where the width enlarges > 2 μm. The numbers of cells in each of the combinations that were encountered in this study are shown in Table 1. In 33/61 cells studied the furrow ingressed in the absence of spindle microtubules, the ingression starting from 0 – 6 minutes after the chromosomes detached. Full movement occurred in all cells in which both univalent and bivalents moved to the opposite pole (such as in Figure 4ii); in these cells the furrow moved and ingressed until it reached the end of the cell and apparently dissipated since it was no longer seen. Halfway movement of the furrows occurred in cells in which the univalents remained at the otherwise empty poles (such as in Figure 4i).

In addition to furrow movements and widths, we looked at the timing of width changes and furrow movements - which came first? The most frequent response was that they started at the same time - furrows moved while ingressing. This occurred in 29/61 cells. There were no significant differences between different concentrations of NOC in effects on movement, change in width, or the order of movement vs. width change (Fig. 6), or on the length of time after bivalent detachment that the furrow moved or changed width (Table 2). Thus most of the findings were consistent amongst all three drug concentrations.

The rate of ingression was also consistent amongst all drug concentrations, and is very similar to ingression during regular anaphase in cells with intact spindles. As seen in Table 1 and Figure 6, slightly more than half of cells (33/61) treated with NOC ingressed after chromosome movement to one pole. The rate of ingression for both drug treated and anaphase cells is approximately 1 μm/min, range 0.66-1.89 μm/min. (Figure 7 A, B).

While the cells look quite abnormal after treatment with NOC, they are not dead. When the NOC is washed out shortly after the chromosomes detach and move to the one pole, the cells in time look like non-treated cells that progress to meiosis-II: they reconstitute nuclei, the chromatin becomes arranged around the rim of the nuclei, the nuclei rotate, and then the chromosomes begin to oscillate as if entering second division (Fegaras and Forer 2018). As seen in Figure 3 (f-k) the cleavage furrow remained constricted to form unequal sized portions of the cell (a shmoo), and 45 minutes after the drug was added nuclei reformed (Figure 3 l). In some cells after drug washout the cleavage furrow can even fully ingress and cleave the cell into two unequal portions. In these cells, cleavage occurred between 15-45 minutes after NOC washout (Figure 8). This was observed in only 5 cells.

DISCUSSION

The main results of our experiments are as follows: NOC caused all bivalent kinetochores to detach from one pole and the kinetochores moved to the other pole, sometimes accompanied by the univalents. After chromosome movement the cleavage furrow moved along the length of the cell toward the half-spindle with fewer chromosomes. In all cells in which both the bivalents and the univalents moved to the other pole and there were no chromosomes in the one half-spindle, the cleavage furrow always moved the entire way to that end of the cell and off the end, as exemplified in Figure 5b. When univalents remained in the half-spindle, the furrow was not able to travel the entire way but rather they usually moved "halfway", creating cells that looked like shmoos (Figure 5c) (Capp 1948). As the furrow moved it either maintained its width or ingressed further (53/61 cells, Table 1), at rates similar to those that occur in control cells at anaphase (Figure 7), suggesting that the same acto-myosin mechanisms apply in both circumstances. The continued ingression and movement of the furrow takes place in the absence of microtubules so spindle microtubules determine neither the furrow position nor the continued ingression (or not) of the pre-existing furrow. We do not yet know why the furrow forms in prometaphase, nor why after it appears it does not ingress to cleave the cells until after anaphase, nor why in non-treated cells it moves toward the half-spindle with fewer chromosomes, nor why the furrow ingresses after spindle microtubules are depolymerised by NOC, nor why after NOC treatment it moves toward the depleted half-spindle even to the end of the cell.

These furrow behaviors are not explained by prevailing models since the generally accepted ideas are that cleavage furrows form after anaphase, and that their positions are determined somehow by direct or indirect interactions with spindle asters or spindle microtubules of some kind (discussed in Forer and Pickett-Heaps 2010). The standard models consider furrows set up by signals arriving at the cortex in late metaphase or early anaphase, and therefore cannot accommodate cleavage furrows forming before then, and none incorporate movement of the furrow along the spindle to accommodate unequal distributions of chromosomes in the spindle. The only data we know of in which furrows moved along the length of the spindle occurred when single centrosomes in metaphase sand dollar zygotes were ablated with a UV laser: the spindles remained in the same positions but the furrows that formed during late anaphase were shifted toward the pole with the ablated centrosome, sometimes shifting so far that the cell formed from the half spindle with the ablated pole excluded both sets of chromosomes (von Dassow et al. 2009). However, the furrows did not shift when single centrosomes were ablated in anaphase, so the centrosome influences the furrow position

only before the furrow is formed, unlike in our experiments in which pre-formed furrows changed positions.

With no models to rely on, we might speculate that the formation of the precocious furrow and its subsequent behaviours are a consequence of the tensegral nature of the spindle and cell (Ingber 1993, 2003a, 2003b). Ingber (1993) argued from architectural principles that tensional integrity (tensegrity) arises from interconnected tension elements and structural elements (struts), that the cytoskeleton in general is a tensegral network, that the tension and structural components in the cells arise from struts such as microtubules and intermediate filaments, and from tension elements such as the actin filament lattice (and we might add, spindle matrix components such as described in Johansen et al. 2011). The cytoskeleton can act as a mechanochemical and sensory pathway such that mechanical input is translated into local chemical signaling (e.g., Ingber 1991; Liu and Post 2000; White and Frangos 2007; Quinlan et al. 2017). That is, because the cytoskeleton acts as a tensegral network, mechanical perturbations in one part of the structure are transmitted throughout the structure to other regions. As discussed elsewhere (Forer and Pickett-Heaps 2010), the tensegral nature of the spindle and cell might give rise to precocious cleavage furrows seen in *Mesostoma* (Ferraro-Gideon et al. 2013, 2014) and to the shifting of the furrow after ablation of a centrosome (von Dassow et al. 2009). Briefly, in more "normal" cells, a stable tensegral spindle and cytoplasmic structure is achieved after nuclear membrane breakdown. The furrow does not yet ingress because the force exerted by the cell is highest during early stages of cell division, and decreases in later stages of division, as shown by the deformation of a droplet of ferrofluid (Hiramoto 1975). The furrow appears in anaphase when the structures are disassembling and changing and the tensegral 'weak spots' (e.g., White and Frangos 2007) are transmitted to the cortex (Ingber 1993). The formation of tensegrally stable structures appears to be prevented in some cases, such as *Mesostoma* spermatocytes, because of the continuous vigorous movements of bivalents throughout prometaphase (Ferraro-Gideon et al 2013, 2014; Fuge 1989, 1987) and thus precocious furrows appear in these cells during prometaphase. When there is chromosome imbalance due to a univalent excursion (Oakley 1984), the furrows move 2-3 µm in response to altered tensegral "bulk" (such as Figure 2).

From observing furrow behavior in cells treated with NOC, it appears that the movement or ingression (or not) of the cleavage furrow is related to the 'bulk' compressibility properties of the spindle. Whereas in intact spindles the 'bulk' may arise from the spindle itself, after NOC treatment the microtubules are gone and the 'bulk' arises from something else, perhaps from proteins which are known to associate with spindle microtubules such as the muscle proteins actin myosin and titin (Mogessie and Schuh 2017; Fabian et al. 2007; Forer et al. 2003) and the nuclear derived matrix proteins skeletor, megator, chromator and EAST (Johansen and Johansen 2007; Johansen et al. 2011). After NOC treatment, most of the struts have disappeared, except for microtubules in the centrosomes (Fegaras and Forer 2018) and presumably components of the matrix which have been shown to remain in the cell for some period of time after microtubules are depolymerized (Yao et al. 2012). With little bulk in one half spindle, the furrow moves halfway along the length of the cell in the direction of less bulk to regions with fewer chromosomes and is free to continue to ingress. With zero bulk (when both bivalents and univalents move to the other pole) the furrow even moves off the end of the

cell, seeming to compress what is left and pushing it into the half-spindle with all the chromosomes.

In conclusion, precocious cleavage furrows form in prometaphase *Mesostoma* spermatocytes, for reasons unknown. The furrows move up and back along the spindle axis in response to normally-occurring imbalances in chromosomes in the two half-spindles. When NOC removes spindle microtubules, the bivalent chromosomes move to one pole, sometimes accompanied by the univalent chromosomes. Shortly thereafter the otherwise arrested precocious furrows most often resume ingression and simultaneously move along the length of the cell toward the half-spindle with fewer (or no) chromosomes. When univalent chromosomes remain at the one pole, the furrow stops moving and the resultant cell looks like a shmoo, with one small portion attached to a larger portion. When the univalents have moved with the bivalents and the half spindle is empty, the furrow ingresses and moves down the length of the cell until it no longer exists. These very unusual behaviours of the furrow occur in the absence of spindle microtubules and at the present time are not understood.

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FIGURE CAPTIONS AND TABLES

Figure 1. Illustration of a *Mesostoma* spermatocyte during prometaphase, modified from Husted and Ruebush (1940). There are three bivalents (b) that span across the middle of the cell, and four univalents (u), two at each pole. The position of the precocious furrow is indicated by the arrowheads. In this example the furrow is symmetrically positioned in the middle of the cell.

Figure 2. a-c. (a) and (c) are two frames from a video sequence of one spermatocyte in prometaphase. The coloured outlines emphasize the cleavage furrow position. (a) Symmetrically positioned cleavage furrow. There are two univalents at each pole. (b) Asymmetric furrow, smaller at the top because there is one univalent at that pole and three at the bottom pole. (c) The cell outlines were superposed to show that the cleavage furrow shifted along the length of the cell, moving towards the half-cell with fewer chromosomes. (a') and (b') are diagrammatic representations of the univalent distributions in cells with similarly asymmetric cleavage furrows.

Figure 3 a-l. A sequence of images from one cell treated with 20 µM NOC. After the addition of NOC, chromosomes detach from the top pole and quickly move towards the bottom pole, after which the precocious furrow moves toward the pole with fewer chromosomes (the top pole) while simultaneously ingressing. Arrows point to positions of chromosomes, (b) points to a bivalent, (u) points to a univalent, and black arrowheads point to the furrow. (c-e) the univalents stay at the top pole while the bivalent kinetochores move towards the bottom pole after which the furrow begins to ingress and moves towards the top pole (f-k). (l) shows a reconstituted nucleus at the top pole and one forming at the bottom pole, both of which appear similar to inter-meiotic nuclei in regularly dividing spermatocytes shown in Fegaras and Forer (2018). The furrow still is seen, although it has regressed somewhat from earlier. The cell remained in NOC throughout this sequence.

Figure 4 i-ii. Chromosome movements in two different *Mesostoma* spermatocytes after the addition of NOC. Arrows indicate the positions of a bivalent (b) and univalents (u). i) a-c. (a) Immediately after the addition of 5µM NOC the bivalents stretch towards the two poles, then (b) the bivalents detach from the bottom pole and move towards the top pole. The univalents do not move with the bivalents, as seen in (c), and remain at the bottom pole. ii) d-f. After the addition of 10µM NOC, the bivalents stretch towards both poles (d) then both bivalents and univalents detach from the top pole simultaneously in (e) and begin movement towards the bottom pole. Both the bivalents and univalents complete movement to the bottom pole(f). There are no chromosomes left behind at the top pole.

Figure 5 a-c. Three different spermatocytes treated with NOC. The red outlines are seconds after drug addition; the blue outlines are the final furrow positions after the furrow completes its movement and/or width changes. (a)The arrows indicate the positions of the chromosomes. Partial movement, regression of furrow width. Chromosomes do not detach. (b) Full movement, ingression of the furrow. Both bivalents and univalents move into the bottom half cell. (c) Halfway movement, ingression of the furrow. The bivalents move towards the top half cell, while univalents remain behind. The furrow has moved toward the bottom pole, forming a cell that looks like a "shmoo".

Table 1. The three ways the furrow may move in relation to the three ways the furrow changes width, after the addition of various concentration of NOC. Numbers represent numbers of cells.

Conc. Of NOC	Partial movement			Full Movement			Halfway Movement		
	Ingression	Regression	Partial	Ingression	Regression	Partial	Ingression	Regression	Partial
5 µM	1	2	4	1	0	0	8	0	3
10 µM	1	2	6	4	0	0	6	0	2
20 µM	3	4	3	3	0	0	6	0	2
Totals	5	8	13	8	0	0	20	0	7
Totals			26			8			27

Figure 6. Comparison of furrow movement, ingression and order of movement vs. ingression in *Mesostoma* spermatocytes treated with various concentrations of NOC. The total cell count is 61 for both furrow movement and width, and is 28 for order of movement vs. ingression because only the cells which had both ingression and full or halfway movement were included.

Figure 7 a and b. The rate of cleavage furrow ingression in anaphase of control cells is similar to precocious furrow ingression in cells treated with NOC. (a) After anaphase commencement the furrow ingressed at a rate of 0.66 µm/min and did not change position. The diagram in the upper right corner shows how the furrow positions and widths were determined from the video frames. The positions of each side of the cleavage furrow was plotted (black arrowheads) in comparison to a fixed point at the spindle pole (red X). The distance between arrowheads represents the furrow width, and the distance between the arrowhead and the X represents the furrow width. (b) Cell treated with 20 µM NOC; time zero is at the addition of NOC. After chromosomes detached and moved to one pole, the furrow ingressed at a rate of 0.94µm/min while simultaneously moving towards the half-cell with fewer chromosomes at a rate of 1.74 µm/min. Both the furrow ingression and movement stopped at roughly the same time, about 5 minutes after perfusion with NOC.

Table 2. Summary of time averages concerning furrow movement and furrow width, as recorded in *Mesostoma* spermatocytes treated with various concentrations of NOC. Times are given in minutes : seconds. Table includes a comparison of the three different types of movement and the three ways the furrow changes width.

Conc. Of NOC	# of cells	Chromo-some velocity (µm/min) ± SD	Movement			Width		
			How long after chromosome detachment did furrow begin to move? ± SD	Time furrow spent moving along cell length ± SD	Movement type (partial; halfway; full) indicated by # of cells	Time after chromosome detachment when furrow began to change width. ±SD	Time furrow spent changing width ± SD	Width change type (ingress; regress; partial) indicated by # of cells
5 µM	19	15.5 ± 9.07	01:51 ± 00:47	05:45 ± 03:39	7; 11; 1	02:04 ± 00:56	05:32 ± 03:36	10; 2; 7
10 µM	21	35.1 ± 24.2	02:17 ± 01:27	06:41 ± 02:56	9; 8; 4	02:55 ± 01:31	06:55 ± 03:40	12; 2; 7
20 µM	21	57.8 ± 35.1	01:46 ± 01:58	04:46 ± 02:39	10; 8; 3	01:54 ± 01:55	07:12 ± 05:41	12; 4; 5

Figure 8 a-**h.** The furrow both moved and ingressed in NOC and completely cleaved the cell after wash-out of NOC. Arrows point to the positions of bivalent kinetochores; the black arrowheads point to the furrow. After addition of 20 µM NOC (b), the chromosomes stretch out towards the poles (c). A few minutes after NOC addition the bivalents detach from the bottom pole (d) and move towards the top pole (e). The univalents remain at the bottom pole. The furrow moves towards the bottom pole while ingressing (e-g). After NOC is washed out (g), the furrow cleaves the cell into two unequally sized daughter cells (h). The top larger cell contains the bivalents and the univalents that were originally present at the top pole, and the smaller bottom cell contains only the univalents.

Supplementary video 1: cell treated with 20µM NOC between 1259:54 and 13:00:01, when the images become out of focus. The bivalents oscillated up and back to the two spindle poles initially. After NOC was added, the oscillations ceased as the bivalents stretched out. The bivalent kinetochores facing the upper pole detached at around 13:01:16 and moved into the bottom half-spindle, leaving the univalents behind at the top pole. After these movements the cleavage furrow slowly ingressed and simultaneously moved toward the upper pole, forming a cell with two unequal parts, that looks like a ‘shmoo’.

Supplementary video 2: cell treated with 20μM NOC between frames 91-95, when the image was out of focus. [The time between each frame is 2 seconds.] As in video 1, after NOC the chromosomes stretched out; all bivalent kinetochores detached from the upper pole, starting at about frame 115, and moved to the bottom half-spindle together with the univalent from the upper pole. Subsequent to the chromosome movements the cleavage furrow ingressed and moved toward the upper pole, ending up at the cell periphery.

Fig 1

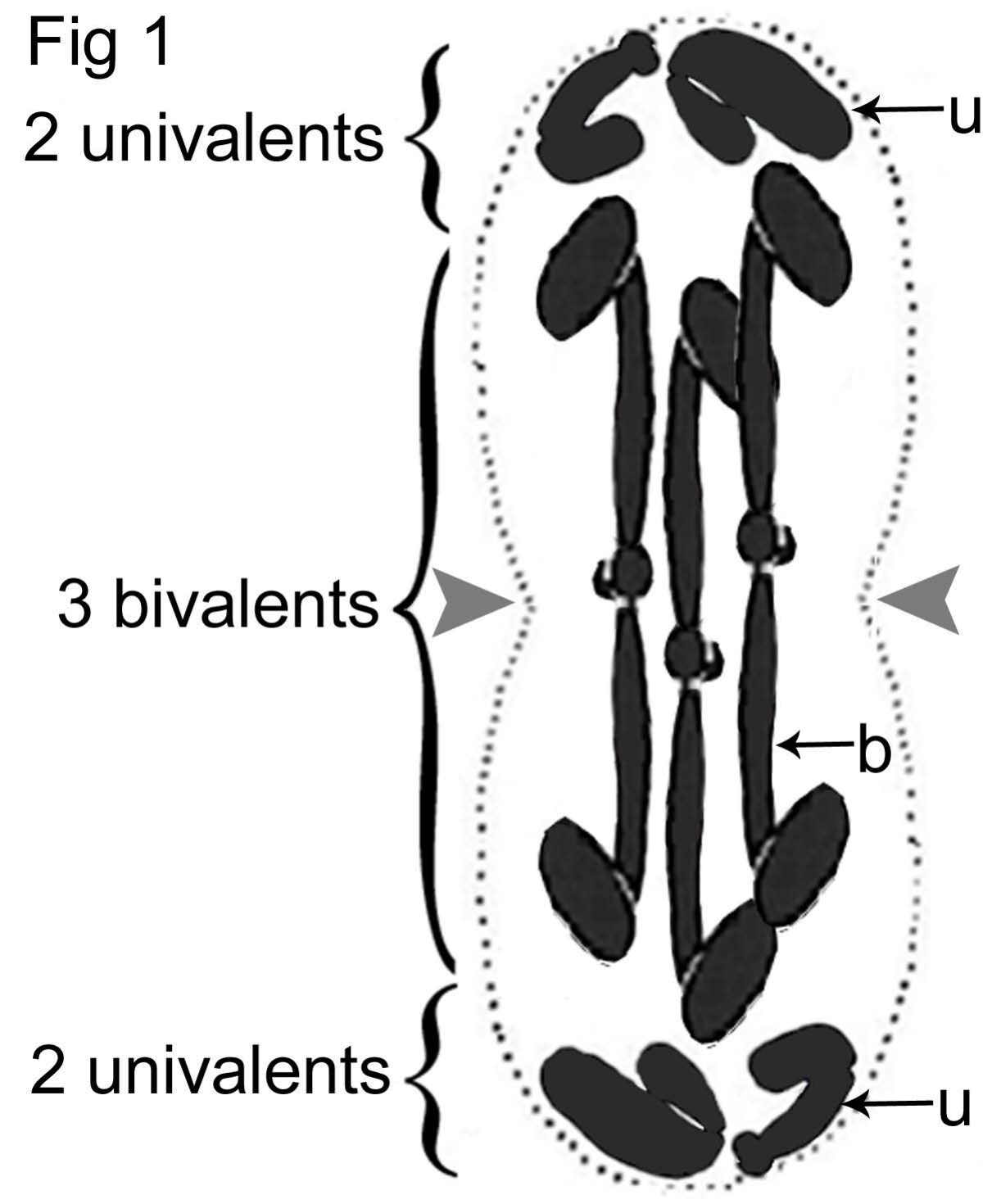
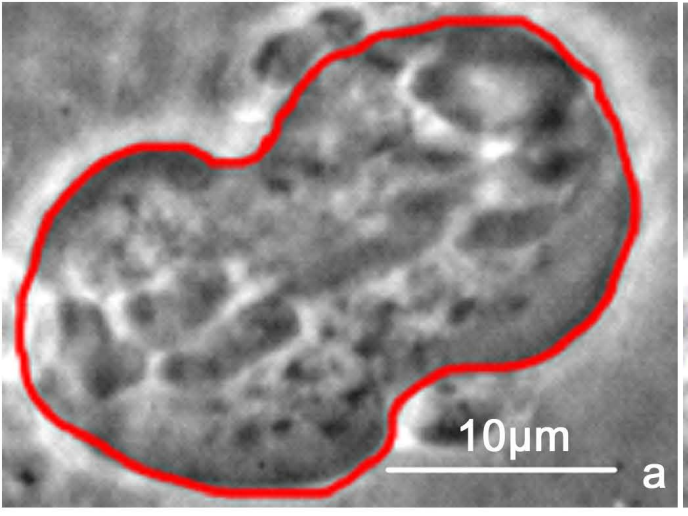
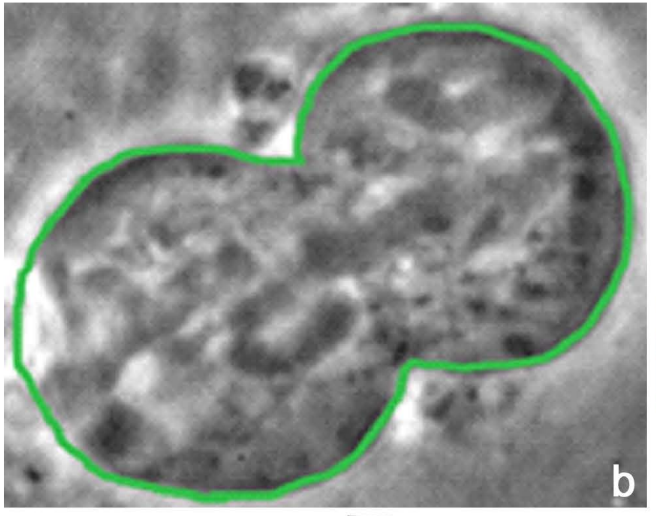
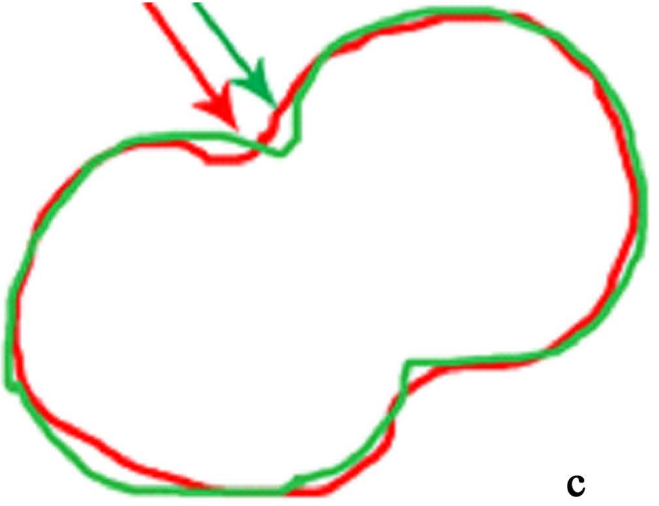


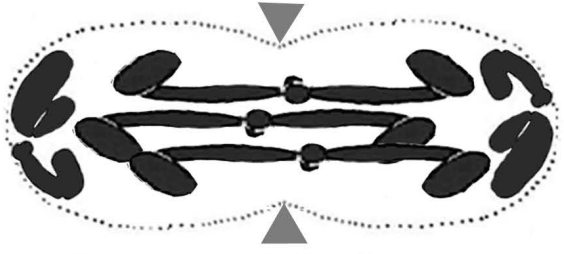
Fig 2.

Precocious furrows shift in response to univalent imbalances

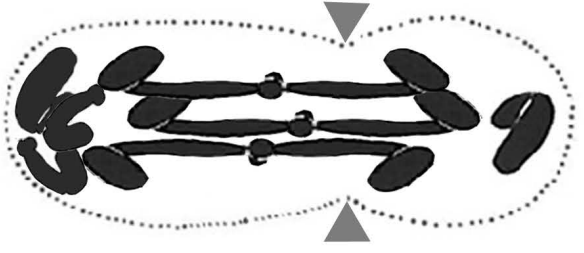








Symmetric furrow a`



Asymmetric furrow b`

Fig 3.

During the addition of NOC bivalents (b) move to one pole then the furrow moves while ingressing towards the pole containing only univalents (u)

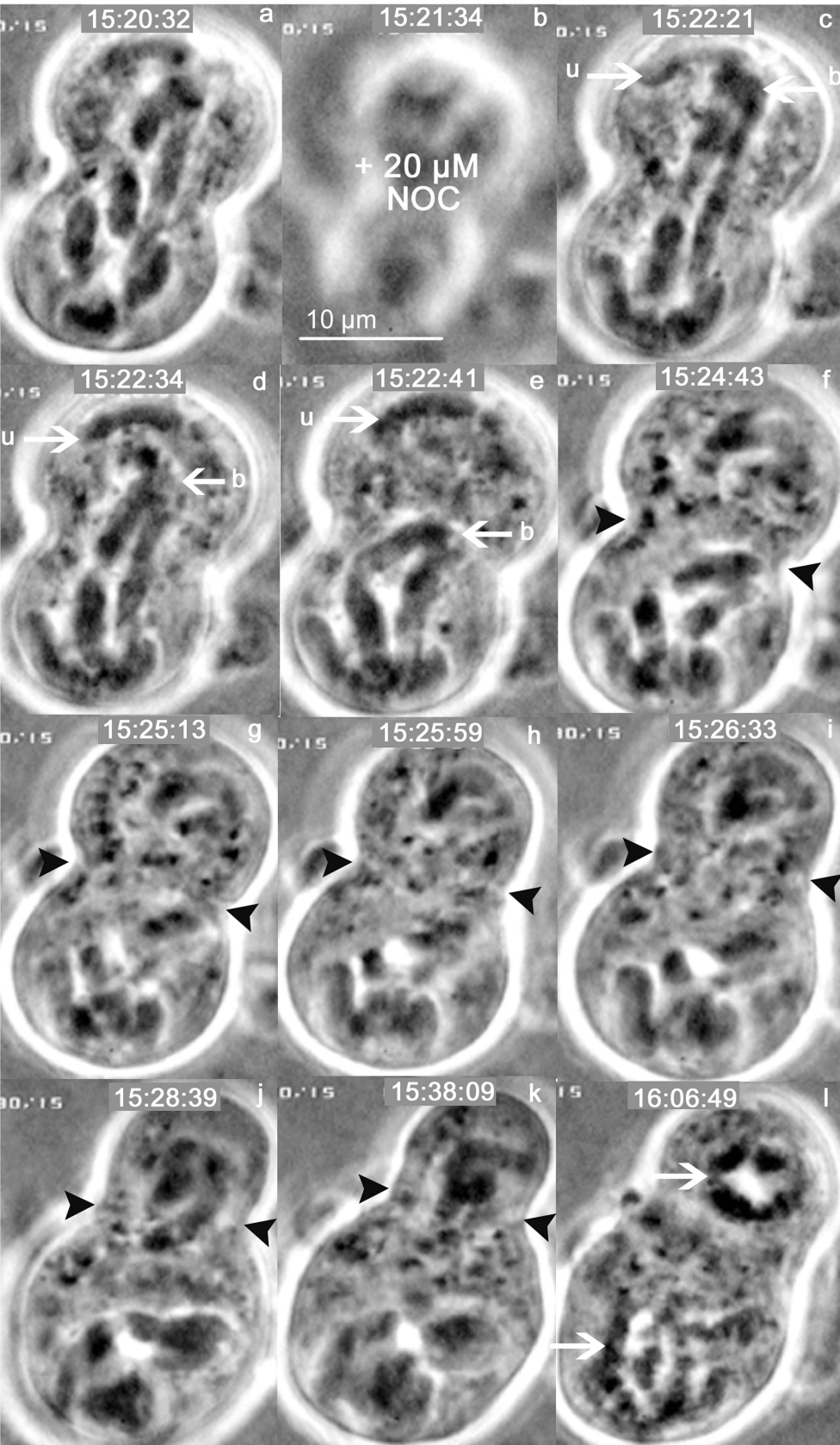
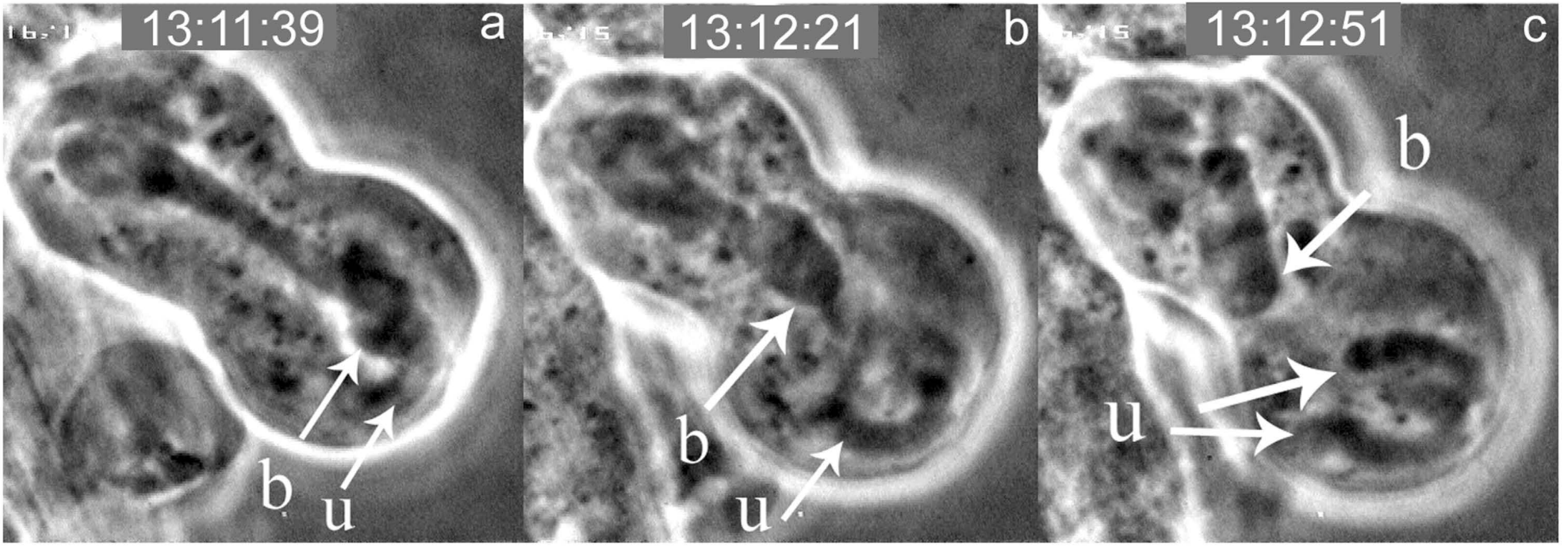


Fig 4.

i) All bivalents (b) move to one pole, univalents (u) do not move



ii) All bivalents (b) and univalents (u) move to one pole

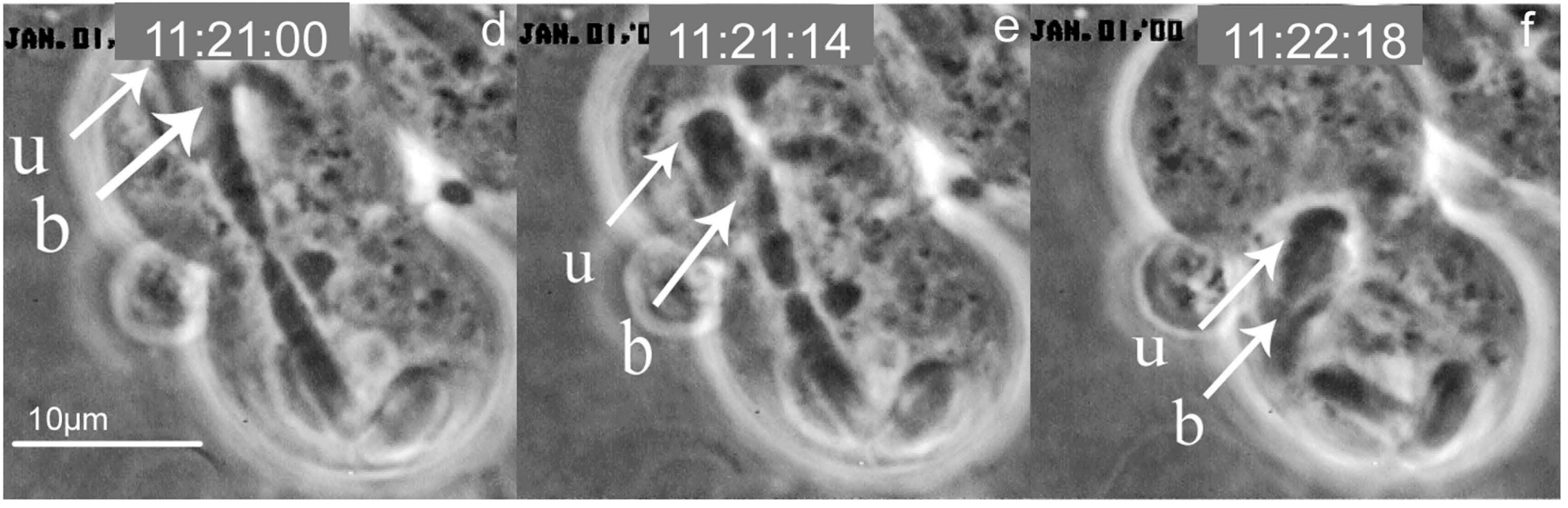


Fig 5.

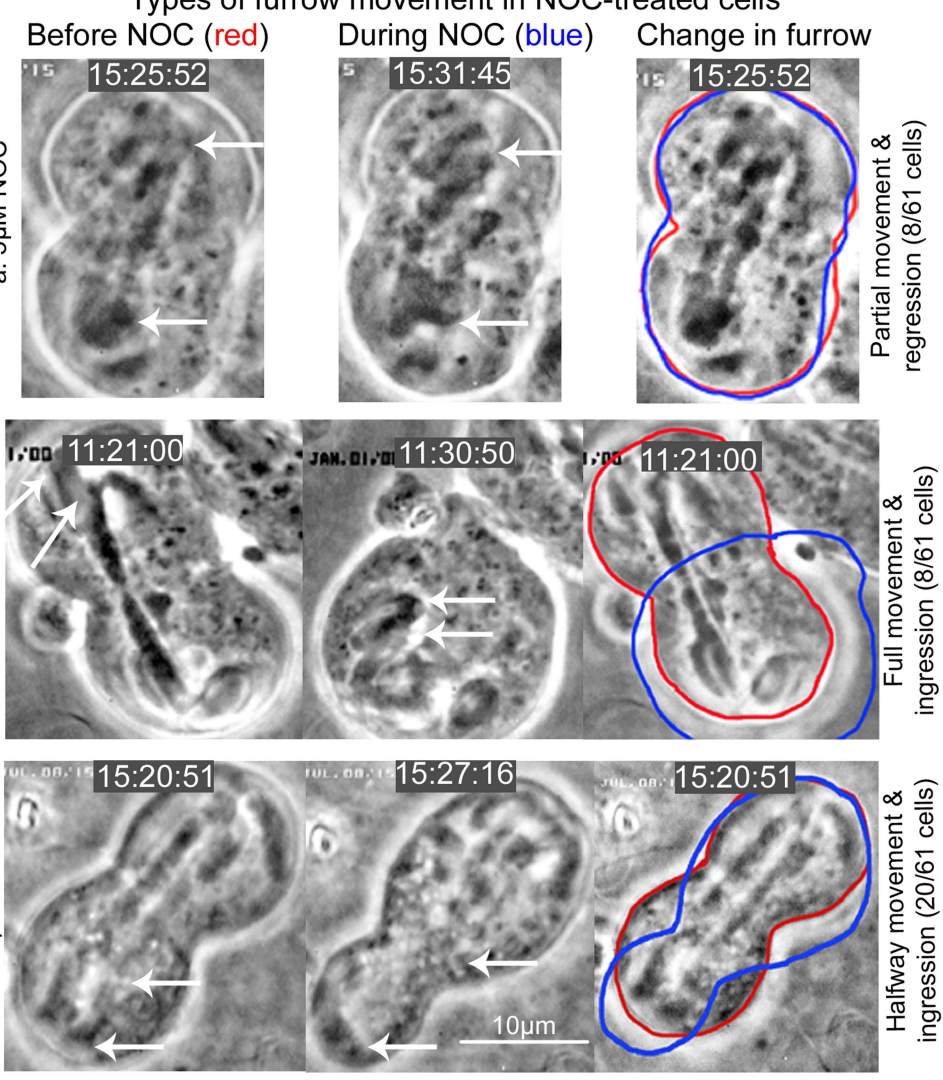

Types of furrow movement in NOC-treated cells
Before NOC (red)
During NOC (blue)
Change in furrow
a. 5μM NOC
15:25:52
15:31:45
15:25:52
Partial movement & regression (8/61 cells)
b. 10μM NOC
11:21:00
11:30:50
11:21:00
Full movement & ingression (8/61 cells)
c. 20μM NOC
15:20:51
15:27:16
15:20:51
10μm
Halfway movement & ingression (20/61 cells)

Fig 6. Furrow movement and width in *Mesosotma* spermatocytes treated with varying concentrations of NOC

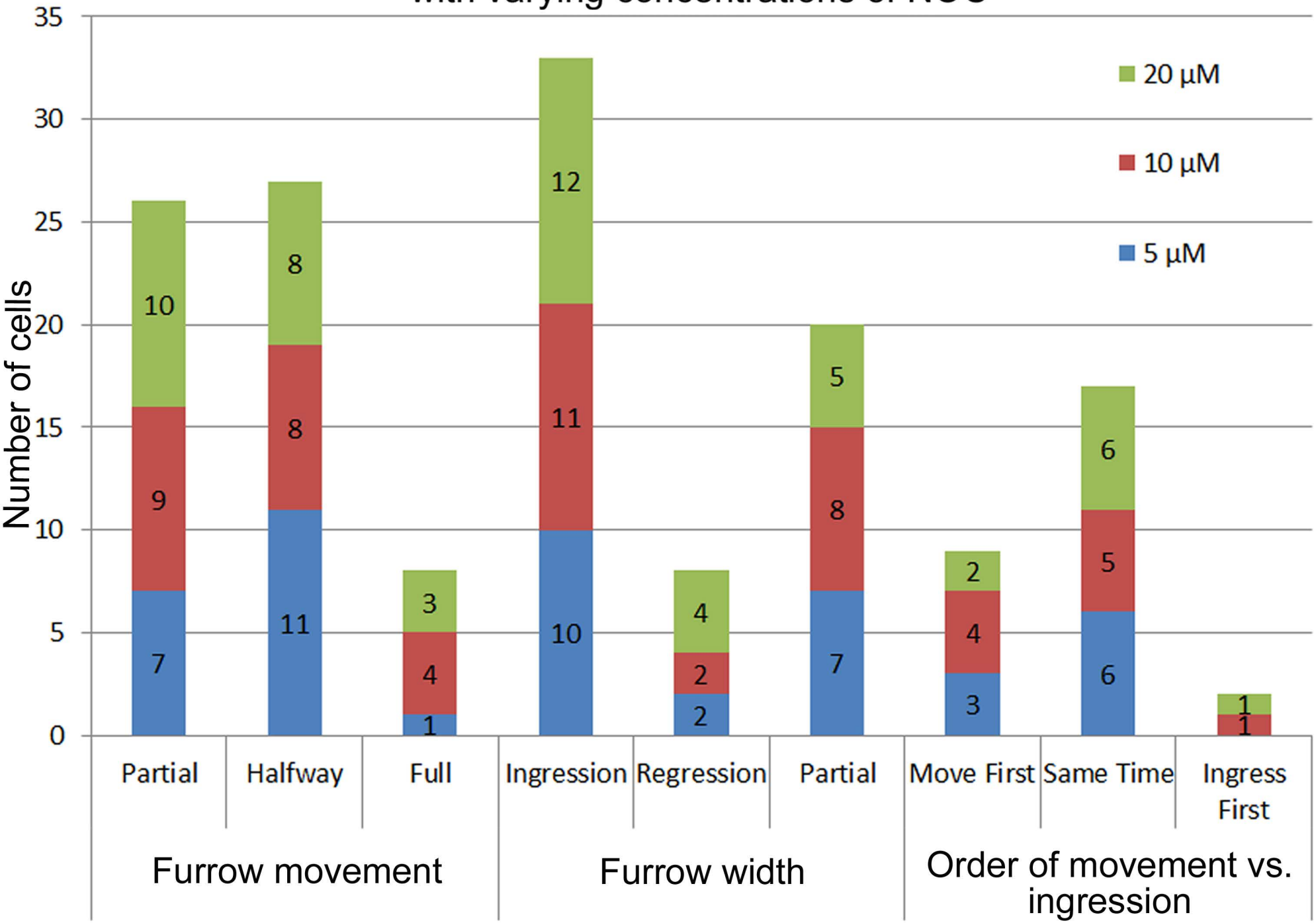


Fig. 7

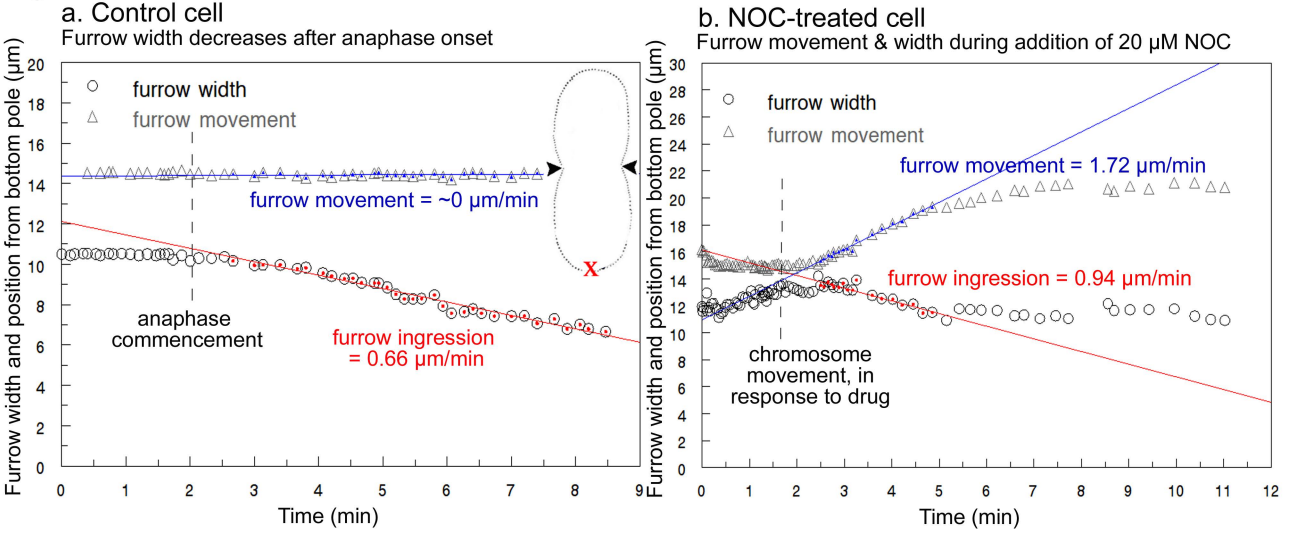

a. Control cell
Furrow width decreases after anaphase onset
furrow width
furrow movement
furrow movement = ~0 μm/min
anaphase commencement
furrow ingression = 0.66 μm/min
X
Furrow width and position from bottom pole (μm)
Time (min)
b. NOC-treated cell
Furrow movement & width during addition of 20 μM NOC
furrow width
furrow movement
furrow movement = 1.72 μm/min
furrow ingression = 0.94 μm/min
chromosome movement, in response to drug
Furrow width and position from bottom pole (μm)
Time (min)

Fig 8.
Furrow remains ingressed in NOC and cleaves the cell after wash-out