Characterizing the effects of jasplakinolide on *Mesostoma ehrenbergii* primary spermatocytes to study the role of actin during chromosome movement

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ABSTRACT

It is generally thought that spindle microtubules generate forces that drive chromosome movement. However, previous results with *Mesostoma ehrenbergii* primary spermatocytes have shown that chromosomes continue to move when microtubules are depolymerized using nocodazole (NOC) (Fegaras and Forer 2018a). In NOC, half-bivalents detach from one pole and all the chromosomes move to the opposite pole. To determine what proteins may contribute to chromosome movement in the absence of microtubules and why the movement is one-sided, moving only to one pole, I targeted actin using an actin-stabilizing drug, jasplakinolide. I targeted actin because it has been found in the meiotic spindle of various other cell types. In addition, myosin has been implicated in *Mesostoma* chromosome movement (Silverio 2017), suggesting actin may be present since they work together in other systems. The purpose of my thesis was to investigate actin's possible involvement in chromosome movement and function in the spindle. Jasplakinolide treatment elicited an unusual response in the cells: jasplakinolide caused the chromosomes to keep oscillating at altered speeds and after a few minutes the precocious furrow moved and ingressed towards a single pole at random, causing one side of the cell to become larger than the other. Then the spindle pole of the larger half-cell moved along the cell periphery towards the opposite pole. Overall, my results implicate actin as being involved in chromosome movement, and suggest that additional non-microtubule spindle elements contribute to producing force for chromosome movement. By characterizing the effects of jasplakinolide, I shed light on actin's varying roles within the meiotic spindle.

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List of Abbreviations

ABPs	Actin-binding proteins
BDM	2,3- butanedione 2-monoxime
Cal A	Calyculin A
CaCl2	Calcium dichloride
DMSO	Dimethyl sulfoxide
EGTA	Egtazic acid
F-actin	Filamentous actin
G-actin	Globular actin
KCl	Potassium chloride
KH2PO4	Monopotassium phosphate
kMT	Kinetochore microtubules
MAPs	Microtubule-associated proteins
MTOC	Microtubule-organizing centres
MgSO4	Magnesium sulfate
NA	Numerical aperture
NaCl	Sodium chloride
Na2HPO4	Disodium phosphate
NP40	Nonionic polyoxyethylene
NTSC	**N**P40, **T**riton, **S**aponin, **C**holate
PBS	Phosphate-buffered saline
PIPES	Piperazine-N,N′-bis(2-ethanesulfonic acid

GENERAL INTRODUCTION

1. Cell division overview

Cell division is the process of a single cell dividing into two or more cells through either mitosis or meiosis. The type of cell division depends on the cell type; somatic cells undergo mitosis whereas reproductive cells exclusively undergo meiosis. Initially, cells enter interphase, a process of duplicating cell contents and genetic material to prepare themselves for division. Mitotic cells go through one round of equational division to separate replicated chromosomes and produce two identical diploid daughter cells. Meiotic cells, however, go through two rounds of division: meiosis-I, reductional division, and meiosis-II, equational division. During meiosis-I the chromosome number is reduced in half as a diploid cell produces two haploid cells. Thereafter, each haploid cell enters meiosis-II, where they each divide and ultimately produce four unique haploid daughter cells. Although there are some characteristic variations between mitosis and meiosis, both progress through a series of five different stages followed by cytokinesis. Generally, the stages include prophase, prometaphase, metaphase, anaphase, and telophase. I will briefly describe the conventional process of each stage as division occurs in animal cells as well as the major differences between mitosis and meiosis.

1.1 Mitosis

Prophase

The onset of prophase is when replicated DNA in the nucleus begins condensing into discrete chromosomes. Each chromosome is comprised of its replicated version; thus, one chromosome is composed of two identical chromatids that are referred to as sister chromatids. Sister chromatids are linked together by a specialized DNA sequence known as the centromere. Meanwhile, the spindle apparatus begins to form in the cytoplasm. The spindle contains a network of microtubules that function in organizing and directing

chromosomes throughout division. Spindle assembly is initiated by an organelle called the centrosome. During interphase, the centrosome duplicates and the resultant two centrosomes migrate to opposite ends of the cell during prophase; these form the poles of the spindle. The centrosomes are considered to be the microtubule-organizing centres (MTOC) as they are responsible for producing microtubules that constitute the spindle fibres (reviewed in McIntosh 2016).

Prometaphase

Prometaphase begins when the nuclear membrane disassembles, exposing the chromosomes to the cytoplasmic contents. Spindle fibres radiating from opposite poles capture and attach to the kinetochores of the sister chromatids. Kinetochores are protein complexes on the outer surface of the centromeres that mediate the bipolar attachment of the microtubules to the chromosomes (Cleveland et al. 2003). Microtubules attached to the kinetochores are referred to as kinetochore microtubules. This process of attachment is coincident with chromosome congression, which is chromosomes sliding along the spindle axis due to opposing pulling and/or pushing forces until the forces from both poles reach equilibrium. Because microtubules from each pole bind the kinetochores randomly, this periodically creates an uneven shift in the forces that cause the chromosomes to move. The forces are balanced when all the kinetochore microtubules from both poles firmly attach to the chromosomes, causing them to slide toward the spindle equator (reviewed in Maiato 2017). Whether microtubules have only attached to one side or both sides of the sister kinetochores, the chromosome still congresses.

Metaphase

Following the completion of chromosome congression, the chromosomes align midway between both poles, a position called the metaphase plate. The chromosomes separate and segregate to the poles only after all the chromosomes are situated at the plate and there is tension on each kinetochore. When there is a secure and proper bi-oriented

attachment of the chromosomes to the poles via microtubules, the kinetochore-to-kinetochore distance within sister chromatids increases. This creates high tension at the kinetochores, which signals the cell to commence anaphase (Hoyt 2001).

Anaphase

During anaphase, the bond between sister chromatids weakens due to the degradation of cohesins, the protein complexes that bind sister chromatids together. Their disassembly allows the chromatids to move towards the opposite poles. The segregation process occurs over two phases: anaphase A and B. During anaphase A the kinetochore microtubules shorten causing the sister chromatids to move towards the spindle poles; during anaphase B, the spindle elongates as antiparallel interpolar microtubules slide past each other thereby causing the spindle poles to move further apart. Cells can undergo both phases at the same time, in sequential order, either A first or B first, and in some cases, one or the other phase predominates (McIntosh 2016).

Telophase

As the chromosomes reach opposite poles, the cell progresses into telophase. At the poles, the chromosomes decondense while a nuclear membrane reforms around each set, creating two nuclei in one cell. Towards the end of telophase, filamentous actin, myosin-2, and other associated proteins assemble into a contractile ring beneath the plasma membrane. Once positioned at the cell equator the ring begins to constrict, forming the cleavage furrow (Harris 1994).

Cytokinesis

Following nuclear division, cytokinesis completes the process of cytoplasmic division to produce two equally sized daughter cells. The contractile actomyosin ring continues to

contract, tightening the cleavage furrow until the cell pinches itself in two (Harris 1994). The resultant cells then begin their own cell cycle and enter interphase.

1.2 Meiosis

Meiosis produces four genetically different haploid cells, exclusively in sex cells during their process of gametogenesis. Gametogenesis is a series of developmental phases that grow germ cells into fully mature haploid gametes - sperm and egg cells. Germ cells are the diploid precursor cells that enter meiosis-I as primary gametocytes, to produce two haploid cells that then enter meiosis-II as secondary gametocytes to produce four unique haploid cells.

Meiosis closely resembles mitosis with a few exceptions. The difference between meiosis and mitosis begins with prophase-I. After DNA replication, the genetic material condenses to form sister chromatids connected by cohesion. The two sets of sister chromatids (one paternal and one maternal) pair up with each other to undergo a crossover at site called chiasmata. This set of two chromosomes, each containing two chromatids, are referred to as homologous chromosomes, colloquially known as bivalents or tetrads. During the crossover, non-sister chromatids physically link together to reciprocally exchange DNA; this genetic recombination allows for genetic variation (reviewed in Ohkura 2015). The homologous chromosomes then line up at the metaphase plate during metaphase-I and partition from each other during anaphase-I. Meiosis-I is a reductional division process so only homologous chromosomes segregate while sister chromatids remain connected by cohesion and migrate together to the same pole. Thus, unlike mitosis, each pole receives half the number of chromosomes, and, following cytokinesis, two new haploid cells are produced. These cells generally do not enter interphase; instead, they directly enter meiosis-II. Like mitosis, meiosis-II is an equational division process; sister chromatids segregate during anaphase-II resulting in an equal number of chromosomes reaching both poles. This meiotic process described is well-known and typically observed in many organisms including humans, yeast, *C. elegans,* and many others, so it is considered to be the “conventional” form of cell division. However, this process can widely differ in many organisms.

Although meiosis in other organisms have cytological variations, the end results remain the same. For example, sex cells in mealybugs (Hughes-Schrader 1948; Bongiorni et al. 2004), plants (Bogdanov 2016), mites (Wrensch et al. 1994), and true bugs (John 1990) undergo "inverted meiosis". These cells divide in reverse order; sister chromatids segregate during meiosis-I, whereas homologous chromosomes segregate in meiosis-II. The outcome is still four unique haploid cells. All the cells described here have holocentric chromosomes, meaning kinetochores form on multiple sites along the chromosome length (Melters et al. 2012). Conversely, many species with conventional meiosis have monocentric chromosomes and heavily rely on this feature because the presence of one kinetochore and the delayed timing of its cohesion degradation allows for sister chromatids to persist in connection until meiosis-II. Bogdanov (2015) hypothesizes that specific proteins protecting cohesins from degradation such as shugoshin, are absent during meiosis-I in cells with holocentric chromosomes. Thus, it could be that inverted meiosis evolved as an adaptation to holocentric chromosomes and the absence of crucial meiotic proteins (Bogdanov 2015; Melters et al. 2012).

Spermatocytes of flea-beetles, *Alagoasa (Oedionychus) bicolor* have strikingly different meiotic features as well. These cells have ten autosomal bivalents and noticeably larger univalent sex chromosomes (X and Y) (Virkki 1971). Univalent chromosomes are sister chromatids that do not exchange genetic information during prophase-I. After nuclear membrane breakdown in prophase-I, the autosomal and sex chromosomes remain clustered in a mass. In prometaphase-I, the autosomal chromosomes move centrally, separating from the sex chromosomes that remain at the cell periphery (Wilson et al. 2003). During this time, the mitochondria form a sheath around the spindle and the moving autosomal chromosomes, excluding the sex chromosomes. Although it appears that the sex chromosomes have a separate spindle, there is only one centriole pair at each pole (Green-Marroquin et al. 2001). Earlier in prometaphase-I, microtubules from both spindle poles attach to the sex chromosomes and before anaphase-I onset the microtubules from the more distant pole detach. The univalent sex chromosomes reorient such that the sister chromatids of each chromosome become bipolarly attach to microtubules from the same pole nearest to them. Meanwhile, the autosomal bivalents align at the equatorial plate, and continue into

anaphase-I normally. During segregation, the univalents do not move at the same time as the bivalents. Instead, their poleward movement lags and eventually stops part way to the pole (Wilson et al. 2003). Meiosis-II occurs as usual; autosomal and sex chromosomes behave normally (Virkki 1971).

Monopolar spindles are another unconventional meiotic feature observed in several insect species including fungus fly, *Sciara coprophila*. Within fungus fly spermatocytes, there are a total of ten chromosomes: four maternal, four paternal, and two limiteds, which are chromosomes only present in the germline (Kubai 1982). The female- and male-derived chromosomes remain spatially distant throughout division. The distance increases as the paternal chromosomes are seemingly pushed away from the pole and from the maternal chromosomes. Unlike the paternal chromosomes that form attachments to microtubules, maternal chromosomes do not associate with kinetochore microtubules and yet they still move poleward (Kubai 1982). The limiteds remain near the pole throughout meiosis-I. In contrast, meiosis-II has a bipolar spindle, and the chromosomes conventionally follow the phases with an additional unique feature. The sister chromatids of the "X" chromosome do not segregate (Abbott and Gerbi 1981). During metaphase-II, the "X" chromosome does not reach the metaphase plate but remains proximal to one spindle pole. Consequently, only one of the resultant cells contains the "X" chromosome and is considered the functional spermatid (Abbott and Gerbi 1981).

Lastly, the spermatocytes I studied from *Mesostoma ehrenbergii* also have unique, unconventional meiotic features, which I will describe later in this section.

2. Spindle apparatus overview

The spindle apparatus is a cytoskeleton structure that sets up the framework crucial for proper segregation of chromosomes. It is a complex network, primarily composed of microtubules that work harmoniously with many other cytoskeleton components including actin and intermediate filaments. In animal cells, the formation of the spindle apparatus originates from duplicated centrosomes that migrate to opposite ends of the cell forming the two poles. Microtubules radiating from the centrosomes elongate causing the poles to further

push apart and generate the fusiform shape. Generally, there are three types of microtubules observed during cell division: (i) astral microtubules, which emanate from the poles and extend toward the cell cortex, (ii) interpolar microtubules, that extend toward the spindle equator and overlap with fibres from the opposite end in an antiparallel fashion, and lastly, (iii) kinetochore fibres, that connect the poles directly to the chromosomes by attaching to the kinetochores (Meunier and Vernos 2012; Karsenti 2001). Many meiotic processes are heavily dependent on these microtubules so I will further describe their structure and functionality.

2.1 Microtubules

Microtubule nucleation begins at the centrosomes, which act as microtubule-organizing centers (MTOC). MTOC contain γ-tubulin which joins other associated proteins to form a small tubulin complex ring that acts as a template for αβ-tubulin heterodimer polymerization (Meunier and Vernos 2012; Desai and Mitchison 1997). The assembly of αβ-tubulin heterodimers into a hollow tubular structure creates a microtubule. Due to the orientation of the tubulin dimers added in head-to-tail arrangement, microtubules develop a polarity. The end of the microtubule with α-subunit exposure is classified as the minus-end, whereas the opposite end with β-subunit exposure is the plus-end. Each end has different properties, the minus-end is the site of depolymerization that is located by the centrosomes, and the plus-end is the site of tubulin addition, where microtubules extend toward the kinetochore (Meunier and Vernos 2012). Typically, each subunit is bound to GTP when incorporated but only the GTP bound to β-tubulin subunits hydrolyze after assembly. This reaction causes a release of energy that decreases microtubule stability and leads to changes in microtubule length (Sept 2007). Throughout division, it is critical for microtubules to undergo dynamic instability, i.e., successive periods of growing and shortening. The fluctuation in length is necessary to maintain spindle structure, kinetochore attachment and contributes to chromosome movement.

Many proteins are involved in regulating microtubule dynamics during cell division including microtubule-associated proteins (MAPs), katanin and motor proteins, dynein, and

kinesin. Briefly, MAPs bind the filaments along their length and function in modulating microtubule turnover and stabilization. During interphase, MAPs primarily protect the microtubules from being severed but during division they decrease which in turn allows the severing enzyme, katanin to act on the microtubules (Heald and Nogales 2002). Katanin is an ATP-bound enzyme that becomes hydrolyzed after binding to the microtubules. This causes a conformational change in katanin, which in turn weakens the tubulin subunits and promotes depolymerization (Heald and Nogales 2002).

2.1.1 Motor proteins: Dynein & Kinesin

Dynein and kinesin are motor proteins that use the hydrolysis of ATP to release energy that fuels their 'walking' along the microtubule length. Dynein walks toward the minus-end where the MTOC is located. Typically, cytoplasmic dynein has multiple functions such as transporting cellular cargo, assists with spindle positioning, and chromosome alignment during metaphase (Raaijmakers and Medema 2014). Disruption of dynein in yeast cells (Eshel et al. 1993), and mammalian tissue culture cells (reviewed in Banks and Heald 2001) causes misalignment of chromosomes during metaphase, distorted bipolar spindles, and abnormal chromosome segregation. Additionally, during early division, dynein gets recruited to the cell cortex and pulls in aster microtubules emanating from the centrosomes to correctly position the spindle poles (Raaijmakers and Medema 2014).

Kinesin, in contrast, walks toward the plus-end of the microtubule and contributes to spindle elongation and forming spindle bipolarity. During prometaphase and metaphase, kinesin crosslinks antiparallel microtubules and slides them apart, causing the spindle to lengthen (Tanenbaum and Medema 2010). Kinesin is also involved in depolymerizing the microtubules at the minus-end during anaphase (Goshima et al. 2005).

2.2 Actin

Actin is another cytoskeleton filament that plays an important structural role in successfully completing cell division. Actin filament organization is dynamic and can change

according to cellular processes such as cell motility, vesicle transportation, and cytokinesis (Dominguez and Holmes 2011). Filamentous actin (F-actin) assembles from the polymerization of globular actin (G-actin) monomers. Similar to microtubules, individual monomers have a polarity and assemble in a head-to-tail arrangement; thus, F-actin develops an overall polarity. The two ends of a polymer can be referred to as barbed and pointed ends. Based on the electron microscope observations of Huxley (1973), when fragmented myosin was added to F-actin, they attached along the length of the filaments in a uniform direction, appearing as arrowheads (reviewed in Bonetta 2005). The pointed end of the arrowhead was classified as the minus-end of the actin filament and the barbed end of the arrowhead is the plus-end. Monomers can be added to both ends of the filament. The pointed end (minus-end) adds G-actin slowly, whereas the barbed end (plus-end) is considered the fast-growing end. The monomers bind to ATP prior to polymerization which hydrolyzes to ADP following assembly. The ATP binding site located at the pointed end remains exposed, and post assembly, ADP occupies the site which has destabilizing effects. For these reasons, the rate of dissociation is faster at the pointed end. Thus, monomer addition is preferred at the barbed end as it does not have an exposed catalytic site and contains a cap that prevents disassembly (Bray 2001).

Similar to microtubules, actin nucleation begins with a large protein complex, Arp2/3 complex, which contains two subunits resembling G-actin monomers that act as a template for actin addition (Pollard and Beltzner 2002). Arp2/3 complex also functions in developing new branches of existing actin filaments. Throughout actin filament growth, many proteins are involved in regulating the process such as profilin and thymosin. These two proteins act opposingly; profilin binds to G-actin causing the exchange of ADP to ATP which increases monomer addition at the barbed end (plus-end), whereas thymosin will bind to monomers preventing it from binding to the plus-end and decreasing growth rate (Pantaloni and Carlier 1993; Gunning et al. 2015). These proteins and many more are necessary for spatially controlling actin polymerization, which is important for coordinating actin-based motility (Svitkina 2018).

2.2.1 Motor protein: Myosin

Actin-based motility depends on interactions between actin filaments and its motor protein, myosin. Myosin, like kinesin and dynein, walks along actin filaments powered by ATP hydrolysis. Their interaction results in a contractile form of movement that contributes to the cell's ability to move as a whole and/or intracellular components such as muscle contraction, cytokinesis, and membrane transport (Lodish et al. 2000). Presently, myosin has a large subfamily of 18 different classifications based on their location and functionality. The varying myosins can generally be categorized as conventional or unconventional myosin. Myosin-2 is a conventional myosin, commonly known for producing muscle contractions and non-muscle contractions like cytokinesis, cell migration, and cell-shape changes (Tuxworth and Titus 2000). Overall, the mechanism for contractility in all scenarios is relatively the same. Briefly, myosin-2 has a head, neck, and tail domain that assembles into a bipolar thick filament (Wilson et al. 1992). The head portion has an actin-binding site and ATP binding site. Prior to actin attachment, the head is ATP-bound and only binds to actin after hydrolysis of ATP to ADP and inorganic phosphate (Pi). ADP+Pi complex remains attached to myosin-2 as it attaches to actin and thereafter ADP+Pi is released which causes the head to change positions, subsequently dragging the actin filament. Myosin-2 dissociates from actin when ATP binds to the head again and the cycle repeats itself. Myosin-2 moves toward the barbed end (plus-end), pulling the actin filament in the opposite direction so actin is moving toward the pointed end (minus-end) (Wilson et al. 1992). The direction of myosin movement is determined by the polarity of actin filaments; myosin filaments are flexible and can attach to actin in different directions according to actin's orientation to ensure the filaments pull together (Bray 2001).

Interestingly, an unconventional myosin, myosin-10 has a considerably important role in spindle assembly, spindle positioning, and was also found to act as a crosslinker between actin and microtubules (reviewed in Titus 2004; Woolner et al. 2008). Although F-actin and microtubules have separate cytoskeletons and distinct roles in meiosis, there is evidence of crosstalk during spindle positioning and cytokinesis (Rodriguez et al. 2003). During early prophase, spindle positioning occurs as the astral microtubules radiating from the

centrosomes interact with cortical actin, directing the duplicated centrosomes to opposite ends of the cell. In some species like budding yeast, the linkage between cortical actin and microtubules is mediated by myosin and dynein, localized at the plus-end of astral microtubules (Pearson and Bloom 2004). In *C.elegans*, actin arrays that are located at specific cell cortex sites interact with cytoplasmic dynein-dynactin complexes which in turn capture astral microtubules to anchor the spindle pole (Rodriguez et al. 2003). With respect to cytokinesis, a number of studies (Sawai 1992; Danilchik et al. 1998; Conrad et al. 1992) have shown microtubules disrupted either before or during anaphase, prevents cleavage furrow progression or causes regression. When midzone microtubules are disrupted or a physical barrier is inserted between the cell cortex and the midzone, microtubules and actin crosstalk is presumably interrupted and cytokinesis ceases (Raich 1998).

Actin and microtubule interactions have also been implicated in chromosome movements (Tsvetkov et al. 2007). However, whether actin filaments are required for or are even present in chromosome movements has long been controversial. There is an incomplete understanding of the mechanism underlying chromosome movements during anaphase. Many models solely consider microtubules as the force producers, driving chromosomes to their respective poles. The prevailing models include the Flux model and Pac-Man model, which will be further discussed in the following section along with the other theorized force producers of chromosome movement.

3. What are the force producers of chromosome movement?

3.1 Flux Model

The Flux model postulates that chromosomes move to the spindle poles because kinetochore fibres depolymerize at the minus end (Desai et al. 1998) (Figure 1a). Typically, microtubules polymerize at the plus-end located by the kinetochores and depolymerize at the minus-end located by the spindle pole. During metaphase, the subunits polymerize and depolymerize at similar rates, therefore achieving a steady-state dynamic that maintains a constant microtubule length; this process is known as treadmilling (Margolis and Wilson

1981). However, this equilibrium shifts during anaphase A as the rate of polymerization declines relative to the depolymerization rate, subsequently shortening the kinetochore fibre and pulling the chromosome toward the pole (Buster et al. 2007). Coupling this process is microtubule poleward flux. Some consider flux as the movement of monomers in the MTs from one end to the other, while others refer to flux as MTs moving (translocating) to the poles through sliding mechanisms (Khodjakov and Kapoor 2005). Overall, this model proposes that chromosome movement is a summation of depolymerization at the minus-end that coincidentally produces a force required for microtubule flux, which in turn exerts a pulling force on the chromosomes, dragging them poleward (Rogers et al. 2005).

Many studies on the Flux model revealed three important components that function in microtubule assembly/disassembly and flux. Experiments using *Drosophila* identified the first component as a minus-end destabilizing kinesin-13 protein, KLP10A. Along with removing tubulin subunits from the minus-end, KLP10A facilitates poleward flux (reviewed in Buster et al. 2007). The second component identified was the CLASP protein, Mast/Orbit, which is responsible for promoting polymerization at the plus-end and is downregulated during anaphase onset (Maiato et al. 2004). Lastly, experiments using *Xenopus* eggs discovered Eg5, a kinesin-5 protein as a critical component in driving flux. Eg5 is localized at the plus-end and produces flux and traction force by cross-linking microtubules to slide them poleward (Miyamoto et al. 2004).

Conversely, when *Drosophila* embryos were microinjected with KLP10A inhibitory antibodies the results point out anomalies to the Flux-model. The loss of KLP10A function should result in improper segregation as poleward flux would be prevented. However, chromosome movement during anaphase A decreased by 40% resulting in lagging chromosomes but continued poleward movement (Rogers et al. 2005). Because the chromosomes did achieve segregation, other microtubule-based mechanisms are considered responsible for these movements, more specifically the Pac-Man mechanism.

3.2 Pac-Man Model

The Pac-Man model postulates chromosomes move during anaphase A due to the kinetochore "chewing" its way to the spindle pole along microtubules (Rieder and Salmon 1994) (Figure 1b). Unlike the Flux model, the poleward force is generated at the plus-end by the kinetochore. Microtubules depolymerize at the kinetochore while remaining attached, subsequently pulling the chromosomes along to the pole. Gorbsky and colleagues (1987) proposed this mechanism based on their experimental observations. Cells were microinjected with fluorescently labeled tubulin subunits, and, after their incorporation, regions on the kinetochore fibres were photobleached. Thereafter, it was observed that while chromosomes continued poleward during anaphase, the photobleached regions nearest to the spindle pole, remained stationary (Gorbsky et al. 1987, 1988). Because the photobleached region did not move poleward and disappear, this indicated microtubule disassembly was occurring at the kinetochore end and that microtubules are stationary. Furthermore, when Nicklas (1989) cut kinetochore microtubules extending between the chromosomes and pole, the chromosomes continued to move poleward, presumably due to the depolymerization at the plus-end. Overall, the Pac-Man model proposes that anaphase chromosome movement is due to force generated at or by the kinetochores.

Some studies have suggested that the Flux and Pac-Man model function simultaneously in segregating chromosomes during anaphase A (Sharp and Rogers 2004; Zhang et al. 2007). Experiments conducted by Sharp and Rogers (2004) revealed two microtubule destabilizing kinesin enzymes, KLP59C and KLP10A, work coincidently at opposing ends of kinetochore microtubules to move chromosomes poleward. As proposed by the Flux model, KLP10A facilitates microtubule disassembly at the minus-end by the spindle pole. Whereas, in accordance with the Pac-Man model, KLP59C works on disassembling microtubules at the plus-end by the kinetochore. Inhibition of both KLP59C and KLP10A blocked poleward microtubule movement, more specifically 40% of the rate was reduced due to the Flux model and the remaining 60% was due to the Pac-Man model. The results demonstrate that both Flux and Pac-Man mechanisms concurrently function to move chromosomes poleward. Overall, these models theorize microtubules and their motor

proteins are the sole components responsible for chromosome-to-pole movement during anaphase.

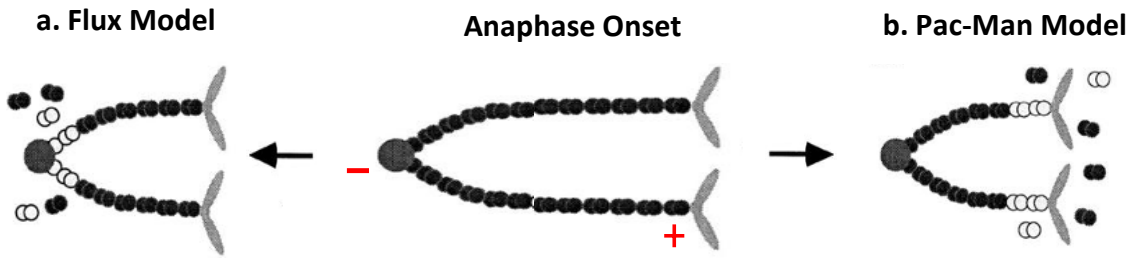

Figure 1. Illustration of two models exemplifying chromosome poleward movement during anaphase A, modified from Desai *et al*. (1998). Shown here is one half-spindle and kinetochores of two half-bivalents attached to the spindle pole via kinetochore microtubules (kMTs). Minus-end by the centrosome is indicated by red (-) and plus-end by the kinetochore is indicated by red (+). Lighter subunits on kMTs mark location of depolymerization. (a) Flux model, poleward flux occurs when kMTs disassemble near the pole. (b) Pac-Man model, chromosome-to-pole movement occurs due to disassembly at the kinetochore.

3.3 Findings that negate microtubule-based models

While microtubule-based models have been widely accepted, other studies have demonstrated contrary results, highlighting the discrepancies of these models. For example, in diatoms microtubules do not insert at the kinetochore but instead attach to a "collar", an amorphous matrix ring that encircles each half-spindle (Pickett-Heaps et al. 1996). Additionally, chromosomes move past the minus end of the spindle during late anaphase. These occurrences are inconsistent with the Pac-Man model that postulates chromosomes are attached to kinetochores and the force required for poleward movement is generated at the kinetochore.

Another inconsistency was observed by Zhang and Nicklas (1996). When chromosomes and their kinetochores were removed from grasshopper spermatocytes via micromanipulation, the kinetochore microtubules continued shortening at a rate similar to that of normally attached microtubules. This indicates that kinetochores are not required to

generate force and that other components in the spindle may be contributing to chromosome movement.

Furthermore, when kinetochore microtubules were cut by UV microbeam irradiation, the chromosomes and their kinetochore stubs (i.e., the leftover microtubules that remained attached at the kinetochores) continued moving poleward (reviewed in Forer et al. 2015). The chromosomes in crane-fly spermatocytes were observed to migrate at the same rate as their movement prior to irradiation, while in grasshopper spermatocytes and newt fibroblasts, they accelerated. In contrast to the Flux and Pac-Man model, these results suggest that kinetochore microtubules may be limiting the rate at which chromosomes move poleward.

Lastly, when Fegaras and Forer (2018a) added a microtubule depolymerizing drug, nocodazole to *Mesostoma* spermatocytes, all the chromosomes selectively moved to one pole. After drug addition, the chromosomes stretched out towards their respective pole, then half-bivalents from one pole detached and moved toward the opposite pole at a rate much faster than normal oscillations or segregation speeds. The continual movement of chromosomes in the absence of microtubules further suggests that chromosomes do not solely rely on microtubules to generate force.

The Flux and Pac-Man model describe mechanisms that are inadequate and fail to explain all chromosome movement during anaphase A. Based on the inconsistencies presented in the microtubule-based models, it is worthwhile to consider other non-microtubule components in the spindle. The force that causes chromosomes to move may likely arise from contributions of the spindle matrix or actin and its motor protein, myosin.

3.4 Spindle Matrix Model

The spindle matrix model theorizes that although microtubules are central to chromosome movement, they are not the direct force producers; instead, it is the spindle matrix that produces the force by interacting with kinetochore microtubules and their motor proteins (Pickett-Heaps and Forer 2009). Researchers hypothesized the involvement of the spindle matrix based on the results of earlier experiments (Pickett-Heaps et al. 1996; Spurck et al. 1997) that showed chromosomes continued moving and even accelerating

poleward after kinetochore microtubules were severed. This outcome led Johansen and Johansen 2007 and Pickett-Heaps and Forer 2009 to consider microtubules as “governors”, rigid fibres that resist movement; they suggested that the rate of chromosome movement to the pole is governed by the rate of depolymerization of kinetochore microtubules and that the force for movement is not produced by microtubules. In addition, the poleward acceleration of the chromosome suggests depolymerization is a consequence of force rather than being the force generator; presumably, the spindle matrix does the pulling and it is this force that acts on the fibres, controlling their disassembly (Pickett-Heaps and Forer 2009). The spindle matrix model interpretation accounts for other experimental observations as well. For example, after kinetochore microtubules were cut, the kinetochore stubs often changed orientation so that they were no longer directed towards the pole. However, chromosomes continued moving to their respective poles, possibly because the matrix forces were directed poleward (Forer et al. 2015).

Previous experiments with diatoms (Pickett-Heaps 1991; reviewed in Pickett-Heaps and Forer 2009) showed they have a “central spindle”, two inter-digitated half-spindles, and a collar (the matrix) that extends between the kinetochores and poles. When the kinetochore microtubules were cut, the central spindle appeared to collapse. This suggests there was a force that compressed the spindle such as the matrix and that the matrix is an elastic structure, required for proper chromosome segregation (Johansen and Johansen 2007). Furthermore, when all half-spindle microtubules were severed in newt and PtK cells, the spindle pole of each side moved inwards to the equator (Spurck et al. 1990). This further suggests an elastic force exists, presumably the matrix that acted on the spindle poles moving them inwards. Overall, these data indicate that a system other than microtubules, such as the spindle matrix generates force that acts on microtubules, thereby governing the direction and speed of chromosome movement.

The spindle matrix model has long been debatable for reasons mainly because no one has described its components or its morphology - it is only based on physiological data. However, there has been growing evidence of its composition and function that can provide insight into its involvement in chromosome movement. The spindle matrix is considered to be an elastic gel-like substrate that stabilizes the spindle and constitutes many nuclear-

derived proteins (Johansen et al. 2011). Matrix proteins that have been identified include Skeletor, Chromator, EAST, Megator, and many more (reviewed in Forer et al. 2015). These proteins are localized from pole to pole of the spindle and interact with microtubules, chromosomes, and other spindle components. For example, Skeletor forms its own fusiform spindle in early prophase that persists through anaphase and acts as an organizational guide to microtubules that form their spindle shortly after. When microtubules were depolymerized by nocodazole, the Skeletor spindle remained intact but contracted around the chromosomes, implying the matrix has underlying interactions with the mitotic spindle and remains stretched out by microtubules (Walker et al. 2000; Yao et al. 2012). Chromator is another matrix protein that has been found to directly interact with Skeletor (Rath et al. 2004). Chromator is localized on the chromosomes in a pattern that overlaps with Skeletor. When Chromator was depleted via RNAi, the microtubule spindle malformed and chromosomes did not properly segregate (Rath et al. 2004). This suggests Chromator may act as a bridge between microtubules and the spindle matrix (Yao et al. 2012). In addition to matrix proteins, there are other non-microtubule components like actin and myosin that are likely involved in force production.

3.5 Actin and Myosin in chromosome movement

Actin and its associated motor protein, myosin, are the cell's other major cytoskeleton components evidenced in the spindle and implicated as force producers for chromosome-to-pole movement (Silverman-Gavrila and Forer 2000; Forer et al. 2007). Through a variety of methods, actin and myosin have been colocalized in the spindle and observed to be in close association with kinetochore fibres in varying cell types (reviewed in Forer et al. 2003; Fabian and Forer 2007; Maiato and Ferras 2017). In addition, some researchers theorize that actin and myosin produce force since many different actin and myosin inhibitors have resulted in altered anaphase chromosome movement and spindle malformation. For example, when crane-fly primary spermatocytes were treated with anti-actin drugs, cytochalasin D and latrunculin B, chromosome poleward movement slowed or stopped during anaphase (Forer and Pickett-Heaps 1998). When the drugs were applied earlier during prophase, a few

bivalents were prevented from attaching to the spindle. These unattached bivalents either drifted to the poles or cytoplasm and remained motionless for the rest of division. Similar results were observed when Silverman-Gavrila and Forer (2001) treated crane-fly spermatocytes with anti-myosin drugs, 2,3- butanedione 2-monoxime (BDM) and ML-7. The application of BDM during anaphase caused chromosome poleward movement to slow down, stop or move back toward the spindle equator. Myosin was also inhibited earlier during prometaphase by ML-7 and similar to early actin inhibition, the chromosomes were prevented from attaching to the spindle.

Furthermore, Silverman-Gavrila and Forer (2000) showed actin and myosin inhibition also block poleward flux of tubulin in kinetochore microtubules. However, these results were indirect, relying on measurements of formation of acetylated tubulin. To directly test if actin and myosin are involved in tubulin flux, Forer et al. (2007) used actin and myosin inhibitors to block the elongation of kinetochore stubs during metaphase in crane-fly spermatocytes. When microtubules are severed by UV microbeam irradiation, chromosomes and their associated kinetochore stubs continue to move poleward. According to previous experiments, kinetochore stubs elongate at a constant velocity by tubulin flux at the kinetochore end (Wilson and Forer 1989). To determine if flux derives from actin and myosin, Forer and colleagues (2007) created kinetochore stubs, and added actin and myosin inhibitors. The inhibitors blocked elongation and poleward movement of kinetochore stubs. These results were observed in PtK cells, as well; severed microtubules stopped moving in the presence of actin and myosin inhibitors (Sheykhani et al. 2013b). Together, their results confirm that actin and myosin are required for producing flux.

In addition, Sheykhani *et al.* (2013a) showed reduced levels of myosin phosphorylation associated with kinetochore microtubules of those chromosomes that slowed or stopped moving in myosin inhibitors. This indicates myosin phosphorylation directly affects anaphase force production. Silverio (2017) also studied the role of myosin phosphorylation on chromosome movement in *Mesostoma ehrenbergii* spermatocytes. Cells were treated with several myosin inhibitors, individually or in combination, and an enhancer that hyperactivates myosin, calyculin A (CalA). Each inhibitor targets a different myosin phosphorylation pathway. The results showed altered bivalent oscillations from both single

and combined drug treatments. However, combined drug treatment of inhibitors did not completely stop bivalent oscillations, therefore suggesting there are other redundant myosin phosphorylation pathways that work to activate myosin and thereby continue force production.

On the contrary to inhibitors, Fabian *et al*. (2007) hyperactivated myosin using CalA to treat crane-fly spermatocytes. When CalA was added during anaphase, chromosomes accelerated toward the poles. Promotion of F-actin, however, did not produce the same results when crane-fly spermatocytes were treated with actin-stabilizing and polymerizing drug, jasplakinolide (Xie and Forer 2008). This drug binds to three monomer subunits along the actin filament length, preventing its depolymerization. The monomers within the filament cooperatively interact, and thus, jasplakinolide stabilizes regions extending beyond its binding site. When Xie and Forer (2008) treated cells with jasplakinolide during anaphase, chromosome movement slowed, stopped, or, rarely, accelerated. Interestingly, the effect resembled that of actin inhibitory drugs. The reasons for this remain unknown however, a possible explanation could be that actin depolymerization is required for movement and jasplakinolide prevents this by stabilizing F-actin, akin to the mechanism in lamellipodial protrusions (Vallotton et al. 2004). Alternatively, jasplakinolide could be blocking sites on actin that are crucial binding sites for other proteins (Xie and Forer 2008).

Similarly, when actin was enhanced in mammalian oocytes by Mogessie and Schuh (2017), the results showed slowed and lagging chromosome movement as well as errors in segregation. Mogessie and Schuh (2017) artificially increased actin by overexpressing a spindle actin-stabilizing domain linked to a microtubule-binding domain, which resulted in an increase of kinetochore fibre bundling. On the other hand, disrupting or depleting actin resulted in a decrease of kinetochore fibres. These complementary results suggest that actin promotes kinetochore fibre formation. However, enriching spindle actin also caused a decrease in tubulin turnover and microtubule flux, subsequently leading to slowed chromosome movement, and defects in chromosome alignment and segregation. As previously seen with jasplakinolide and within this study, actin promotion causes effects similar to those of actin inhibition. While the results imply

actin is essential in properly aligning chromosomes, driving their poleward movement, and promoting kinetochore fibres, there is some ambiguity in how actin functions. Actin appears to be involved in various ways, but it is not entirely understood what actin does during anaphase, except that it somehow participates in forming kinetochore fibres, aligning and moving chromosomes.

Overall, the summation of data suggests that actin and myosin are crucial components within the spindle that work either in conjunction with the spindle matrix, microtubules or independently to produce force for chromosome poleward movement. Now that I have described normal cell division and the different possibilities of force being produced for chromosome movement, I will describe the peculiarities of *Mesostoma* to give a better understanding of my experiments.

4. Study system: *Mesostoma ehrenbergii*

I studied chromosome movement in primary spermatocytes of aquatic flatworms, *Mesostoma ehrenbergii*. This organism is interesting and advantageous to study for various reasons. These hermaphroditic flatworms are transparent with translucent lobed testes that are lined along either side of the pharynx. Due to their position, the testes are easily extracted for experimental use. The testes are asynchronous and the cells within are usually at different stages of spermatogenesis, which could pose to be a difficulty in finding cells specifically in first division. However, the cytological features of the primary spermatocytes such as their "dumbbell" appearance, three large bivalents, and four univalents make it easy to locate and observe chromosome movement (Figure 2a).

Mesostoma are reared at minimal cost and readily available in abundance. Some offspring are born live while some remain as dormant eggs before hatching. The two forms are known as viviparous offspring (S eggs) and diapausing eggs (D eggs), respectively. *Mesostoma* reproduce in one of two ways, they either first form S eggs, deliver their babies and thereafter form D eggs or they exclusively produce D eggs (Hoang et al. 2013). Generally, it takes about 2.5 weeks for the babies to enter the adult stage which is when I select them for dissection. The testes appear plump and clearish-white in colour,

which indicates many of the cells are at the dividing phase (Hoang et al. 2013), and the chances of finding a primary spermatocyte increase. I do not dissect animals with S eggs because their progeny are needed to continue the next generation of stock.

4.1 Meiosis in *Mesostoma ehrenbergii*

To give an overview of *Mesostoma* spermatocytes, during prometaphase the univalents reside at the poles while the bivalents extensively oscillate between the two poles and progress into anaphase, without ever aligning at a metaphase plate (Ferraro-Gideon et al. 2013a). Univalents often move between poles to presumably achieve non-random assortment before entering anaphase via distance segregation. These cells also form precocious cleavage furrows in early prometaphase, which ingress and remain arrested until anaphase begins. Telophase and cytokinesis progress normally. I will now describe these unconventional processes in more detail.

4.1.1 Bivalent oscillations

The three bivalents are metacentric as seen in chromosomes with normal meiosis. Bivalents form bipolar attachments in prometaphase and orient themselves such that the homologous chromosomes are not perpendicular to the spindle fibres but are oriented parallel to the spindle pole axis (Figure 2a). The bivalents continuously move toward and away from each pole along the spindle pole axis; this movement is referred to as oscillating. Throughout prometaphase, their oscillations can shift between in-phase or out-of-phase movements (Ferraro-Gideon et al. 2013a). When one kinetochore moves towards its respective pole and the other kinetochore simultaneously moves away from its pole, the chromosome is observed to be moving as a whole unit. The chromosomes stretch when both kinetochores move towards their respective poles at the same time and shorten when both move away from their respective poles at the same time. The bivalents persistently oscillate for 1-2 hours until anaphase onset (Ferraro-Gideon et al. 2013a). Typically, the chromosomes travel significantly faster towards a pole, 6.2 μm/min, compared to moving away from a pole,

5.2 µm/min. The amplitude of its oscillation, which is the distance between the pole and the furthest point a kinetochore travels away from the pole, averages 4.0 µm and the period, i.e., duration of a kinetochore moving away and then back to the same pole, averages 92.5 s (Ferraro-Gideon et al. 2014). Plotting the chromosome movements by tracking kinetochores creates a sawtooth wave pattern as previously described by Ferraro-Gideon *et al.* 2014 (Figure 2b).

Ferraro-Gideon *et al.* (2014) found that bivalents can periodically exchange poles, wherein half-bivalent kinetochores detach and switch positions. Each kinetochore reorients to the opposite spindle pole and then proceeds to oscillate. Because reorientations occur after bivalents achieve correct attachment to each pole, this hints there might be coordinated positioning of the half-bivalents. That is, reorientation occurs so certain half-bivalents go to the same pole, and this suggests half-bivalents might segregate non-randomly.

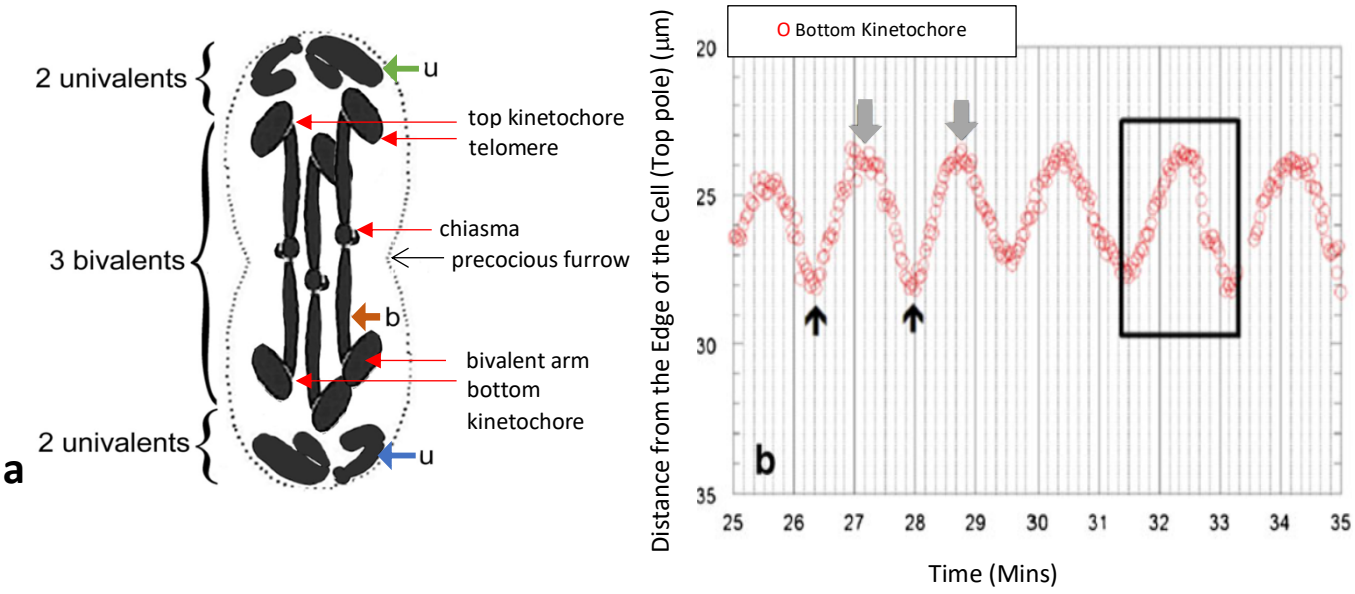

Figure 2. *Mesostoma* primary spermatocyte and chromosome movement graph. Image (a) modified from Fegaras and Forer (2018b) and (b) modified from Ferraro-Gideon *et al.* (2014). (a) The large metacentric bivalents (orange arrow) are positioned in the middle of the cell and extend across the length of the cell. There are two univalents residing at each pole, one acrocentric (blue arrow) and one metacentric (green arrow). Thin black arrow marks the position of the precocious furrow. Red arrows indicate different structural parts of a bivalent. (b) Graphical representation of a bottom kinetochore moving throughout prometaphase. Black box indicates one oscillation, gray arrows indicate the peak and black arrows indicate

the trough of an oscillation. Amplitude is represented as the distance between trough to peak and duration between two troughs or two peaks represent the period of one oscillation.

4.1.2 Distance segregation

During prophase, the four univalents reside at the spindle poles, remain there throughout prometaphase, and segregate via distance segregation during anaphase. As defined by Hughes-Schrader (1969), distance segregation is when partner chromosomes are unpaired and do not physically connect but still segregate to opposite poles. Prior to anaphase, univalents occasionally undergo 'excursions' as described by Oakley (1983). She described the four univalents as two pairs. For example, consider X1X2 and Y1Y2 as the two pairs. The pairs are morphologically different from each other, and members of the same pair are identical; two are metacentric and two are acrocentric (Figure 2a). During prometaphase-I, individual univalents can move between the poles, in what is referred to as excursions. Oakley found that univalents can be imbalanced prior to anaphase – e.g., all three at one pole and the fourth univalent at the opposite pole (Figure 3). But by anaphase onset, the univalents evenly distribute to have one of each kind at each spindle pole. Excursions were presumed to occur to correct an imbalance of univalents at the poles or to correct two of the same kind being at the same pole. However, the excursions happened more often than necessary to correctly have one of each univalent at each pole in a random assortment. That is, sometimes univalents of one pair exchanged poles even after achieving a correct distribution. For example, X1 would exchange with X2 after there was already one X chromosome and one Y chromosome at each pole. Thus, Oakley (1985) suggested the univalents non-randomly segregate. She concluded that neither of those movements would occur if all that was required for random assortment was one member of each pair at each pole.

4.1.3 Precocious cleavage furrow

Conventionally, cleavage furrows form in late telophase after proper nuclear division is achieved and furrows ingress fully to complete cytoplasmic division during cytokinesis. In *Mesostoma* spermatocytes, furrow formation and ingression are usually observed during prometaphase. A precocious cleavage furrow appears at the spindle equator early in prometaphase, arrests after slightly indenting, and resumes ingression to deepen when nearing anaphase (Forer and Pickett-Heaps 2010). The precocious furrow, however, often moves during prometaphase when there is chromosomal imbalance; the univalents residing at the poles occasionally move between the two poles, creating an unequal distribution of chromosomes at both ends (Oakley 1985). The furrow moves along the cell length according to the chromosome imbalance, typically shifting 1-2 μm towards the pole with fewer chromosomes (Fegaras and Forer 2018b) (Figure 3). Thus, the furrow periodically appears asymmetrical throughout prometaphase (Forer and Pickett-Heaps 2010). The furrow becomes symmetrical nearing anaphase, as the univalents arrange themselves to have one of each kind at each pole. The furrow usually completes ingressing only after anaphase completion, at roughly 1 μm/min until the cell is fully cleaved (Forer and Pickett-Heaps 2010; Fegaras and Forer 2018a).

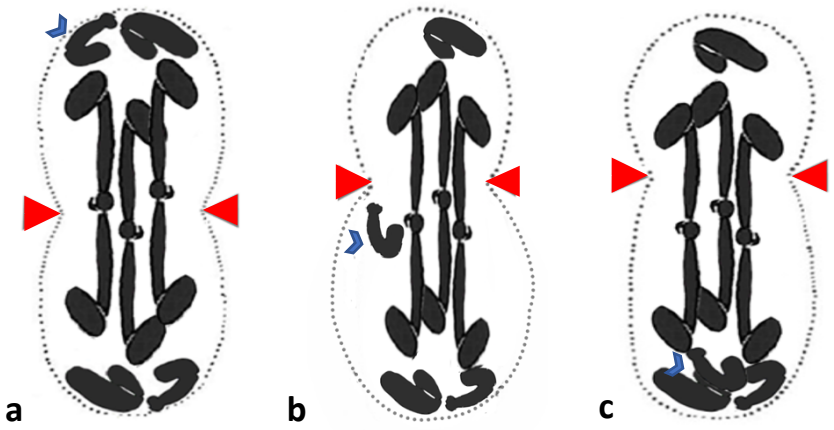

Figure 3. Precocious cleavage furrow position changes throughout prometaphase. Image modified from Fegaras and Forer (2018b). Red arrowheads indicate furrow position. Blue bracket indicates a univalent position. Furrow is symmetrically positioned (a) when there is two univalents at each pole. When a univalent excursion occurs (b) there is an unequal

distribution of univalents at each pole and the furrow position shifts along the cell length towards the half-cell with fewer chromosomes, becoming asymmetrically positioned (c).

5. Study Objective

Previous results with *Mesostoma* have shown chromosomes continue to move when microtubules are depolymerized using nocodazole; half-bivalents randomly detach from one pole and migrate to the other (Fegaras and Forer 2018a). To determine whether actin is needed during chromosome movement in the absence of microtubules and why the movement is one-sided, I targeted actin using jasplakinolide. My primary focus is to target actin because it has been found in the meiotic spindle and is implicated in chromosome movement of various other cell types, as discussed above. In addition, Silverio (2017) found that myosin inhibition and enhancement alter chromosome oscillations in *Mesostoma* spermatocytes and thus, I expect similar effects from targeting actin since they act together in other systems.

Jasplakinolide addition has previously caused chromosome movements to slow, stop, or, rarely, accelerate in crane-fly spermatocytes (Xie and Forer 2008). Thus, my initial hypothesis was that when added to *Mesostoma* primary spermatocytes, jasplakinolide would similarly cause chromosome movement to be altered. However, initial jasplakinolide results were unusual in that all chromosomes seemed to move to one pole bringing with them the other spindle pole. Thus, my focus shifted to describe in detail the movements caused by jasplakinolide and to verify if the whole spindle pole consistently moves toward the opposite pole. By characterizing the effects of jasplakinolide, I aim to gain a greater understanding of actin in relation to the mechanisms controlling chromosome movement and how it might function in the spindle.

Characterizing the effects of jasplakinolide on *Mesostoma ehrenbergii* primary spermatocytes to study the role of actin during chromosome movement

INTRODUCTION

Microtubules and their associated proteins are generally considered to be the main components that constitute the spindle and that are responsible for producing the forces that move chromosomes during cell division. At present, the prevailing views are that kinetochore microtubules pull chromosomes to the poles either because microtubules depolymerize at the pole (Flux model) or at the kinetochore (Pac-Man model) (reviewed in Khodjakov and Kapoor 2005). While there is compelling data that microtubules are critical elements, several other studies indicate poleward chromosome movement might be because of non-microtubule elements such as the spindle matrix or actin-myosin (reviewed in Forer et al. 2015).

The spindle matrix model proposes that microtubules have a more passive function and that the spindle matrix plays a major role in configuring the spindle and driving chromosomes poleward (Johansen and Johansen 2007; Pickett-Heaps and Forer 2009). For example, when kinetochore fibres are severed with a UV microbeam during anaphase, chromosomes continue to move poleward. In crane-fly spermatocytes, chromosomes continued moving at normal rates whereas, in grasshopper spermatocytes and newt fibroblasts the chromosomes moved at higher speeds than before (reviewed in Forer et al. 2015). Similarly, when microtubules are depolymerized by nocodazole in *Mesostoma* spermatocytes, chromosomes still move: partner half-bivalents at one pole detach and rapidly move to the opposite pole (Fegaras and Forer 2018a). Together, these results imply microtubules limit the speed of chromosome movement since chromosomes move faster without normal microtubules. This also indicates that another force-producing element within the cell (e.g., the spindle matrix) generates poleward movement. Additionally, spindle poles move towards each other when all kinetochore microtubules of a half-spindle are severed (Spurck et al. 1990), suggesting that force is still being generated when microtubules are absent, most likely from the spindle matrix. While there is some ambiguity regarding spindle matrix function, we know it consists of a variety of nuclear-derived proteins (e.g., Skeletor, Chromator, Megator and EAST) that form a spindle-like structure and work independently from the microtubule-based spindle (reviewed in Johansen et al. 2011). Other

non-microtubule components like actin-myosin could be acting within or in conjunction with the spindle matrix to move chromosomes poleward (e.g., Forer et al. 2008).

The involvement of actin and its motor protein, myosin, in producing force has been debatable. However, actin and myosin have been identified in the spindle of a variety of cells, including humans (reviewed in Forer et al. 2003; Mogessie and Schuh 2017) and growing evidence suggests they play an important role during chromosome movement. Many studies that have inhibited or enhanced actin and myosin, demonstrate altered chromosome movement. For example, when crane-fly spermatocytes are treated with drugs that depolymerize F-actin (e.g., cytochalasin D and latrunculin B) or block myosin activity (e.g., BDM, ML7, and Y27632), chromosome movement slows or stops during anaphase, and if added prior to anaphase, results show improper chromosome attachment to the spindle (Forer and Pickett-Heaps 1998; Silverman-Gavrila and Forer 2001; Fabian and Forer 2005; Sheykhani et al. 2013b). Actin and myosin inhibition have also shown to block tubulin flux of kinetochore microtubules (Silverman-Gavrila and Forer 2000; Forer et al. 2007), further suggesting they are major contributors in producing force. In complementary experiments, myosin enhancer calyculin A caused chromosomes to accelerate poleward when added during anaphase (Fabian and Forer 2007). When actin was enhanced, it caused an increase in kinetochore fibre bundles, suggesting actin promotes kinetochore fibre formation; actin enhancement also caused chromosome movement to slow and caused errors in chromosome alignment and segregation (Mogessie and Schuh 2017). Similarly, when actin is stabilized by jasplakinolide, chromosome movements slow, stop, or rarely, accelerate (Xie and Forer 2008). These data indicate actin promotion and/or stabilization alter chromosome movement in a similar fashion to actin inhibition treatment. Presently, it's unknown how actin functions during chromosome movement. In order to elucidate how actin may function in the spindle, it is helpful to understand how these proteins function in other cytoskeletal systems.

The aim of my study was to target actin to determine its involvement in moving chromosomes in *Mesostoma* spermatocytes. Fegaras and Forer (2018a) observed that chromosomes in *Mesostoma* spermatocytes move in the absence of microtubules, presumably due to force production by other elements including but not limited to actin and myosin. Because myosin has been implicated in moving chromosomes in *Mesostoma*

(Silverio 2017), and actin and myosin work together in other systems, I expect chromosome movements will be altered when actin is targeted with jasplakinolide.

Jasplakinolide stabilizes filamentous actin (F-actin) and prevents depolymerization by binding to 3 monomer subunits and interfering with the disassembly process (Bubb et al. 2000; Pospich et al. 2020). The effects of this drug have been varying as many different cells have shown different dose and time-dependent responses. In previous studies, higher doses and longer treatment of jasplakinolide induced actin aggregation (Lazaro-Dieguez et al. 2008) and promoted polymerization of actin (Holzinger and Meindl 1997). In lower concentrations and shorter treatment periods, jasplakinolide slows, stops or accelerates chromosome movements in crane-fly spermatocytes (Xie and Forer 2008). For my thesis, I initially hypothesized that chromosome oscillations in *Mesostoma* would similarly be altered by treating cells with low concentrations of jasplakinolide. I treated live *Mesostoma* spermatocytes in prometaphase-I with jasplakinolide and the initial experiments unexpectedly showed all chromosomes moved to a single pole bringing with them the other spindle pole. The focus of my work then extended to characterize the unusual effects of jasplakinolide and confirming whether the whole spindle pole consistently moves to the opposite pole. By doing this, I aim to unravel actin's role in chromosome movement and how it might function in the spindle.

MATERIAL AND METHODS

Animal care

Mesostoma were reared in the lab as outlined by Hoang *et al.* (2013). The animal stock was initially started from worms obtained from Lake Rondeau, Ontario by Hebert and Beaton (1990). The dormant eggs from these worms hatch under anaerobic conditions. Thus, the eggs were placed in sealed, water-filled plastic jars with algae and kept in the dark at room temperature for several days. Thereafter, the jars were placed under light and aerobic conditions by removing some water in the jars. The new babies were fed brine shrimp or *Daphnia*. To maintain a constant supply of animals, the babies of the subsequent generations

get split into new jars, each containing 5 animals per 200 ml of dechlorinated water. The plastic jars were kept at 27°C and in a light:dark cycle of 16h:8h within an incubator (Environmental Growth Chambers Model: TC-1). Additional stock was kept in a small fish tank at room temperature. The animals were fed daily with brine shrimp.

Live cell preparations

To make preparations of live spermatocytes, adult *Mesostoma* with resting eggs and large testes were selected for extraction. Prior to dissecting, the flatworms were rinsed three times in *Mesostoma* Ringer's solution (61 mM NaCl, 2.3 mM KCl, 0.5 mM CaCl2 and 2.3 mM phosphate buffer, pH 6.8). Then the testes were extracted by using a pulled glass needle (pulled from 5 or 10 micropipettes). The contents were mixed with 2.5 µL of 20 mg/mL fibrinogen (Calbiochem) dissolved in Ringer's solution and thinly spread out on a flamed coverslip. Thrombin (2.5 µL) was quickly added on top to form a fibrin clot, as formerly described (Forer and Pickett-Heaps 2005; Ferraro-Gideon et al. 2014; Fegaras and Forer 2018a). The coverslip was then inverted onto a drop of Ringer's solution on a perfusion chamber. The coverslip was sealed on the perfusion chamber with wax, made from a 1:1:1 mixture of vaseline: lanolin: paraffin, to prevent leakage.

Drug Treatment

Live cells were examined by phase-contrast microscopy. Initially, each prep was scanned for spermatocytes in prometaphase-I. Once located, the live cells were recorded in real-time using a 100X oil immersion lens (NA 1.3). Control cells were perfused with dimethyl sulfoxide (DMSO) 1000x diluted in *Mesostoma* Ringer's solution. The stock solution of jasplakinolide was made up in DMSO to be 1000x the final concentrations used to treat the cells. The stock concentration (1000x) was used to make the final concentrations: 10 nM, 250 nM, 500 nM, and 750 nM of jasplakinolide made up in DMSO. Thereafter, 5µL of the desired concentration of jasplakinolide was dissolved in 5 mL of *Mesostoma* Ringer's solution and used to perfuse experimental cells. DMSO is a solvent used to solubilize drugs and has no effect on *Mesostoma* spermatocytes at 0.1% concentration (Fegaras and Forer

2018a; Forer and Pickett-Heaps 2010). The cells were recorded for a minimum of 10 minutes prior to drug addition and for at least 20 minutes after. The cells were usually washed out at varying times after the cell stopped responding, with 10-15 mL of *Mesostoma* Ringer's solution.

Immunofluorescence

The immunofluorescence procedure followed was as described by Fegaras and Forer (2018a). The cells were first lysed, fixed, and then stained with primary and secondary antibodies to stain for tubulin. Experimental cells targeted for staining were recorded live for 5-7 mins during prometaphase-I before jasplakinolide was perfused. In order to immediately lyse and fix the cell to capture the cell's response to jasplakinolide at the desired moment, lysis buffer containing 0.25% glutaraldehyde was perfused into the chamber to immediately lyse and fix the cell. The lysis buffer called NTSC contains 3% **N**P40, 2% **T**riton X-100, 2% **S**aponin, 0.5% **C**holate (Sodium Deoxycholate), 5% DMSO, 100 mM PIPES, 10 mM EGTA, and 5 mM MgSO4. Roughly after 2 mins or less in NTSC containing 0.25% glutaraldehyde, the coverslips were placed in a new Petri dish with NTSC without glutaraldehyde, for 1-2 hours. The coverslips were then rinsed with phosphate-buffered saline (PBS) 1x stock (1.31 M NaCl, 0.051 M Na2HPO4, 0.016 M KH2PO4; final pH 7.0) for 5 mins. Control cells were lysed and fixed by placing the coverslip in a Petri dish for < 2 mins containing NTSC and 0.25% glutaraldehyde. This step was repeated once more and then the coverlsips were put in sodium borohydride (1mg/1mL) or 0.05 M glycine for 10 mins to neutralize the free aldehyde groups. The coverslips were rinsed in PBS twice, for 5 mins each. Finally, the coverslips were stored in a Petri dish submerged in a 1:1 mixture of PBS: Glycerol and kept at 4°C until they were ready to be stained.

Microtubules were stained with anti-tubulin primary antibody, YL ½ rat monoclonal (1:1000 or 1:500), followed by secondary antibody, Alexa 488 goat-anti-rat immunoglobulin IgG (1:100) (Invitrogen). Coverslips that were stored were floated cell side down in PBS for 1-2 hours to remove glycerol. They were rinsed in PBS twice again, for 10 mins each, and quickly splashed with 0.1% Triton X-100 in PBS to aid in spreading the antibodies.

Approximately 85 uL of the primary antibody diluted in PBS, was added to each coverslip. These preps were incubated for 24 hr at room temperature. The same procedure was followed the next day for secondary antibody addition and the preps were incubated in the dark to prevent the fluorochromes from being photobleached. After the staining period was over, the coverslips were rinsed in PBS twice, 10 mins each, and splashed with PBS: Glycerol before being mounted on a slide by Mowiol (Osborn and Weber 1982). The coverslips were placed cell side down on top of Mowiol, which contained 0.2g/L paraphenylene diamine, an anti-fading agent (Fabian and Forer 2005), and then left to dry in the dark for 24-48 hr. Thereafter, the slides were stored in the dark at 4 °C until they were ready to be viewed with the confocal microscope. Cells were viewed through the LSM 700 Zeiss Observer confocal microscope, using a Zeiss EC Plan-NEOFLUAR 40X oil immersion objective (NA, 1.3) or 60X oil immersion objective (NA, 1.35). Z-stacks were captured using an analysis software for digital microscopy, Zen Blue.

Data Analyses

The recorded videos of live cells on DVD discs were time-lapsed into movies using an online program, VirtualDub (www.virtualdub.org). Analysis of chromosome movements was done using the in-house program WinImage (Wong and Forer 2003) and movement graphs were constructed using the commercial program SlideWrite. WinImage was used to analyze: 1) chromosome movements by tracking the kinetochore distance from a fixed point on the edge of the cell (near a pole) and 2) furrow movement by tracking the cleavage furrow distance from a fixed point and the width between the two furrow points. The data collected were used to construct movement graphs on SlideWrite to plot distances versus time. These graphs were used to calculate the velocities, amplitude, and periods of the kinetochore movement, as well as the furrow ingression and movement rate. Student's t-test and Welch's t-test (two-tail) were completed using Excel to determine if there were significant differences before and after the drug treatment for different parameters and between the different drug concentrations. Images taken from the time-lapsed movies and captured from the confocal microscope were further processed using Image J (available at http://rsb.info.nih.gov/ij/) and Adobe's Photoshop C3.

RESULTS

Control cells

Chromosome movement

Meiosis-I in *Mesostoma ehrenbergii* primary spermatocytes is illustrated in Figure 1. During early prometaphase-I, the four univalents reside at the poles while the three bivalents oscillate between the two poles. The bivalents keep oscillating and never form a metaphase plate. They oscillate for the entire prometaphase, up to 2 hours, and directly enter anaphase mid-oscillation. Within any given cell, each bivalent oscillates independently and partner kinetochores of any individual bivalent vary in their speeds and direction of movement.

Chromosome movements are shown graphically in Figure 1B in which kinetochore positions are plotted as distances between the kinetochores and a fixed point (designated near a pole). The plot resembles a saw-tooth wave pattern, from which the average amplitude, period and speeds of a kinetochore moving towards and away from its respective pole were determined (Table 1). These parameters vary between partner kinetochores of one bivalent and from the other bivalents within the same cell. In my sample of two control cells, the amplitude, the total distance between the trough and peak, averages about 5.3 μm and the period, i.e., duration of a kinetochore moving away and then back to the same pole, averages 105.7 s (Table 1). With respect to speed, the chromosomes travel faster towards a pole at a rate of 7.0 μm/min (range: 4.4 – 8.4 μm/min) compared to moving away from a pole at 5.9 μm/min (2.6 – 7.6 μm/min) (Table 1). The data for my control cells are comparable to non-treated cells previously described by Ferraro-Gideon *et al.* (2013) and Fegaras and Forer (2018a).

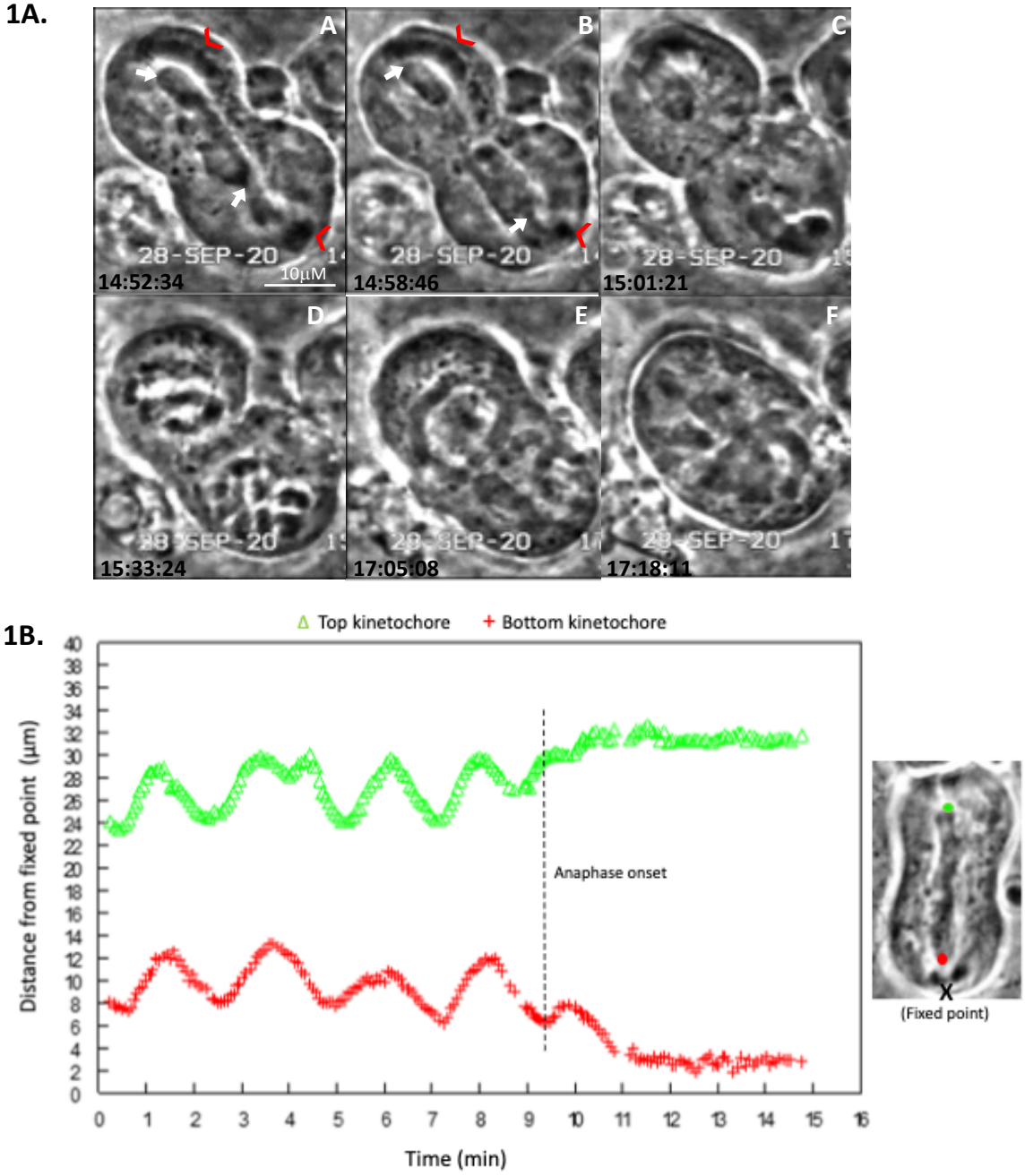

Figure 1. (**1A**) Image sequence of *Mesostoma* spermatocyte control cell. White arrows indicate the partner kinetochores of the bivalent and red brackets indicate the univalents that reside at the poles. In (A) the cell is in prometaphase –I and shortly after the cell enters anaphase-I, (B-C) the bivalents disjoin mid-oscillation, and the half-bivalents move backwards to their respective poles. In (D) chromatids in each half-cell aggregate and form a new nucleus. More than an hour later the chromosomes rearrange into hollow circles (E). In (F) the chromosomes are oscillating in prometaphase-II. Times are shown in hr:min:sec. (**1B**) Chromosome movement graph of one bivalent in the control cell. The top and bottom kinetochore distance was measured from a fixed point (bottom pole) throughout

oscillation. Average speeds were determined through lines of best fit for each slope before anaphase. The distance between a trough and peak represents the amplitude and the duration between two consecutive peaks or troughs, represents the period of one oscillation. The black dash line indicates when the bivalents disjoined; anaphase was complete within 2 minutes.

Precocious Furrow

Cleavage furrows appear at the start of prometaphase; each furrow precociously develops and slightly ingresses at the spindle equator. The position of the furrow shifts when the distribution of chromosomes at each pole changes. The precocious cleavage furrow moves along the cell length according to the chromosome imbalance and thus, the furrow periodically appears asymmetrical throughout prometaphase. By the onset of anaphase, the univalents equally distribute themselves to have one of each kind at each pole, after which the furrows become symmetrical. After anaphase, the furrow usually resumes ingression and cleaves the cell.

The rate of furrow ingression and movement along the length of the cell were determined by plotting distance versus time as described in Fegaras and Forer (2018b). The ingression of the furrow was measured by tracking the change in the furrow's width, which was considered the cell's diameter at the exact position of the furrow. Furrow movement was determined by measuring the change in distance between the furrow position and a fixed point at one end of the cell, until anaphase was complete.

Second division

The second division process in *Mesostoma* spermatocytes is not well described and has only been explored in a few cells. The two cells I followed into second division (Figure 1 D-F), formed two nuclei up to 30 mins after anaphase completion. The chromatids aggregated before turning into two hollow circles that were each enveloped by a membrane, forming a nucleus, akin to the observations of Fegaras and Forer (2018a). These nuclei rotate back and forth in place and after one or more hours prometaphase-II commences. A new spindle forms and the chromosomes oscillate.

Experimental cells

Jasplakinolide caused chromosome movements to be altered in *Mesostoma* primary spermatocytes in a consistent sequence of responses (Figure 2). After drug addition, chromosomes continued oscillating at reduced speeds and after a short period the pre-anaphase cleavage furrow started to ingress. While ingressing, the furrow moved toward one pole, causing one side of the cell to become larger than the other. The spindle pole of the larger half-cell then began to move along the periphery of the cell in a unidirectional manner toward the opposite pole. This spindle pole appeared to slide at an angle away from its original position and moved in the same direction as the furrow. During this movement, the spindle pole in the smaller half-cell did not move. Thereafter, the furrow stopped ingressing and moving usually before or at the same time the spindle pole stopped moving.

I treated cells with 10 nM, 250 nM, 500 nM, and 750 nM of jasplakinolide to determine if the response of the cell changes with different concentrations. 10 nM of jasplakinolide had no effect on the cells. Cells treated with 250 nM, 500 nM or 750 nM all reacted similarly; because there was no significant difference, the data for all three concentrations were pooled and analyzed collectively. Hence, the effects of treatment were not dose-dependent, and the threshold concentration is somewhere between 10 nM and 250 nM.

Of the 25 cells studied, two cells entered anaphase after drug addition. One cell underwent anaphase while the spindle pole was moving along the cell membrane and the other entered anaphase <1 min after jasplakinolide addition, consequently, their chromosome movements could not be accurately studied. However, both cells still showed unusual responses in terms of their furrow and one-sided spindle pole movement, thus they were included in those analyses. For chromosome movement analyses, 35 kinetochores from 23 cells were followed as all three bivalents were seldom in the same focal plane; kinetochores from only 1-2 bivalents could be followed at the same time for the duration of the experiment.

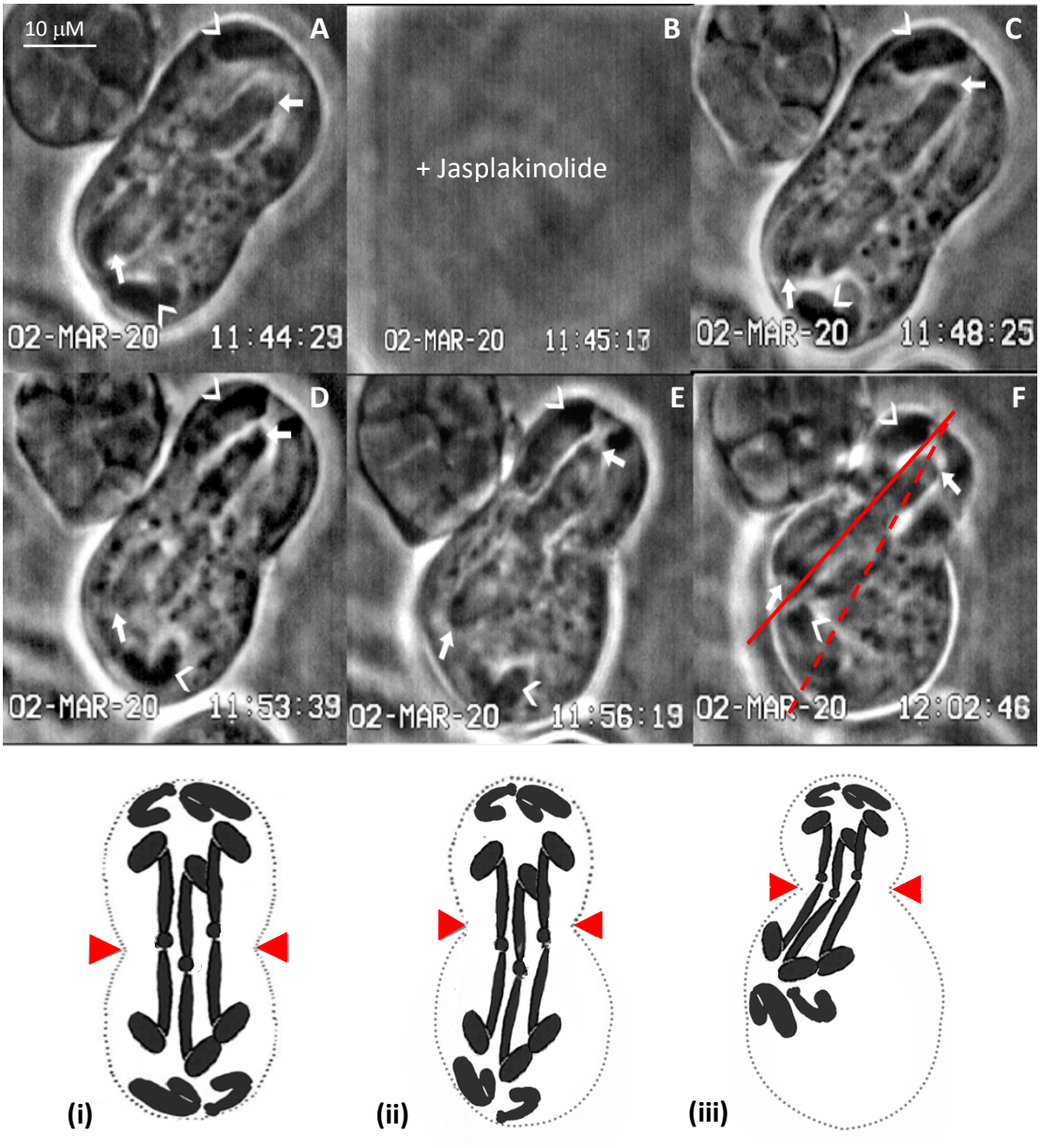

Figure 2. Image sequence of a cell treated with jasplakinolide. White arrows indicate the position of the bivalent's partner kinetochores and white brackets indicate the univalents. The red dash line indicates the original spindle axis and the solid red line indicates the axis after the bottom pole stops moving. (A) The cell is in prometaphase-I. (B) Jasplakinolide addition. (C) Bivalent still oscillates. (D) The furrow is constricting. (E) The furrow continues to ingress while it moves toward the top pole because of which the bottom half-cell becomes larger than the top half-cell. The bottom pole moves along the cell periphery in a clockwise direction toward the top pole. (F) Furrow movement and ingression stopped at roughly the same time the bottom pole stopped moving. Times are shown in hr:min:sec. (i-iii) Diagrammatic representation of the cell's response. Red arrowheads indicate furrow position.

Chromosome movement

Shortly after drug addition, chromosome movement altered. The bivalents continued oscillating in the presence of jasplakinolide at an overall reduced speed before spindle pole movement began (Figure 3). Many of the half-bivalents (25/35) were slowed to varying extents, speeds decreasing by 11-56% of their initial speeds; those that decreased by 10% or less were not considered significant changes (Figure 4). The range of decreased speed may be a function of their initial speed. Both partner kinetochores and kinetochores of different bivalents within the same cell were affected differently by jasplakinolide. In some instances, kinetochores (5/35) moved at increasing rates toward and away from their respective pole while their partner kinetochores and the other kinetochores within the cell moved at decreasing rates. Altogether, kinetochores with slowed oscillations moved at significantly reduced speeds both toward and away from their respective pole. These kinetochores moved the same relative distance as prior to treatment, albeit for slightly longer durations (Table 1).

In all cells, bivalents oscillated on average three times (range: 1-6 oscillations) before the one spindle pole started to move along the cell membrane. When half-bivalents of the large half-cell and its spindle pole began moving toward the opposite pole, the bivalents usually stopped oscillating. In some cells (5/23), however, bivalents continued to oscillate on average two times as the direction of oscillation changed angle (Figure 3), with significantly decreased speeds, amplitude and period (Table 1). When the angle changes stopped, oscillations either (i) completely stopped, e.g., both partner kinetochores become relatively motionless, or (ii) partially stopped, e.g., the small half-cell's kinetochores persisted oscillating while their partner kinetochores remained motionless.

Table 1. Comparison of average velocity of each kinetochore toward and away from their respective poles, amplitude and period between non-treated (control cells) and experimental cells before and after jasplakinolide addition. Only kinetochores with slowed movements were included. Values are mean ± standard deviation.

	Non-treated cells	**Before Jasplakinolide**	**After Jasplakinolide**	
			Immediate	*During rotation*
Speed (µm/min)				
Toward pole	7.0 ± 1.5	6.8 ± 2.1	5.9 ± 1.9*	4.30 ± 1.5**
Away from pole	5.9 ± 1.0	6.5 ± 2.0	5.7 ± 2.0*	4.7 ± 1.5**
Overall	6.5 ± 1.5	6.6 ± 2.1	5.8 ± 2.0*	3.9 ± 1.5**
Amplitude (µm)	5.27 ± 0.85	4.51 ± 1.51	4.61 ± 1.56	2.64 ± 0.95**
Period (sec)	105.74 ± 30.8	108.75 ± 41.22	122.32 ± 55.27*	75.04 ± 16.27**
Number of cells, kinetochores	2, 4	23, 30	23, 30	5, 10

* Indicates values are significantly different with $p < 0.05$, ** $p < 0.01$

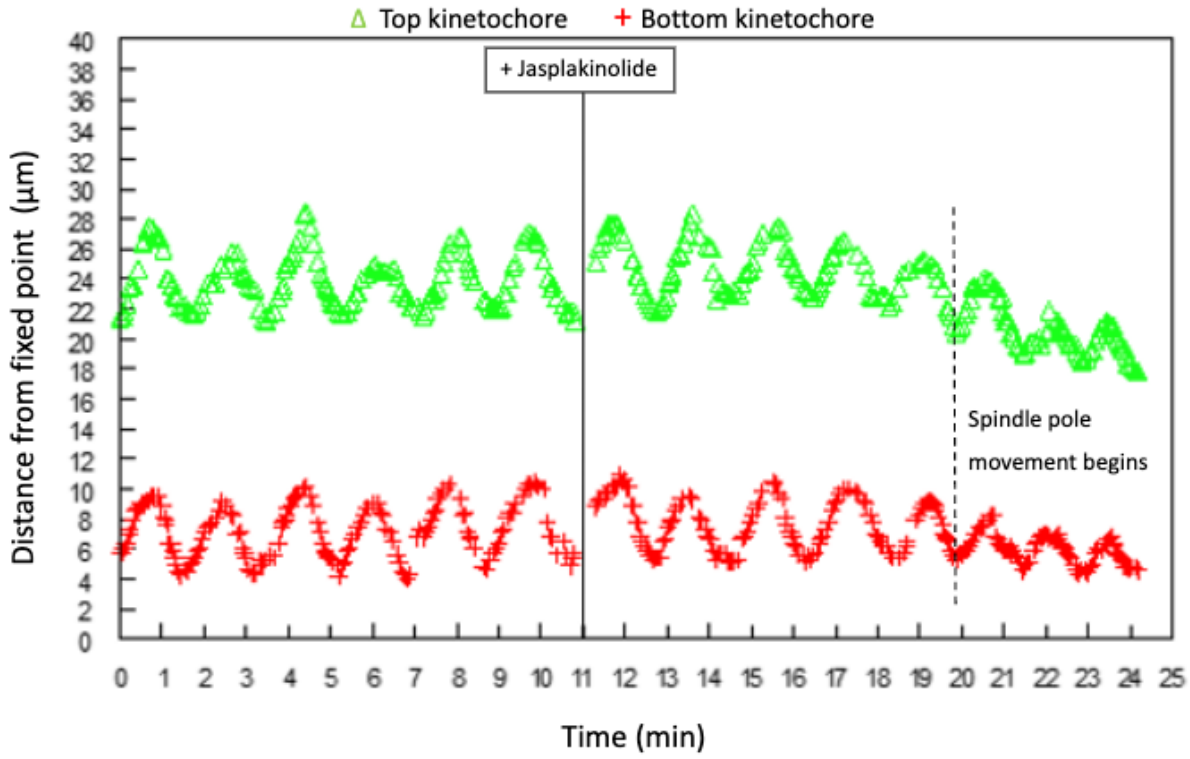

Figure 3. Chromosome movement graph of one bivalent in a *Mesostoma* spermatocyte treated with jasplakinolide. Oscillations of partner kinetochores were tracked before and after jasplakinolide addition (indicated by bold line) until bivalent stopped moving. Average velocity toward and away from a pole, amplitude and period were measured for each oscillation. After five oscillations post-drug treatment, the spindle pole began moving (indicated by dash line) wherein the bivalent persisted oscillating with a decrease in velocity, amplitude and period for approximately 4 mins until the kinetochores ceased movement. The top kinetochore concurrently shifted toward the bottom pole while oscillating; the bottom kinetochore continued oscillating at its original position.

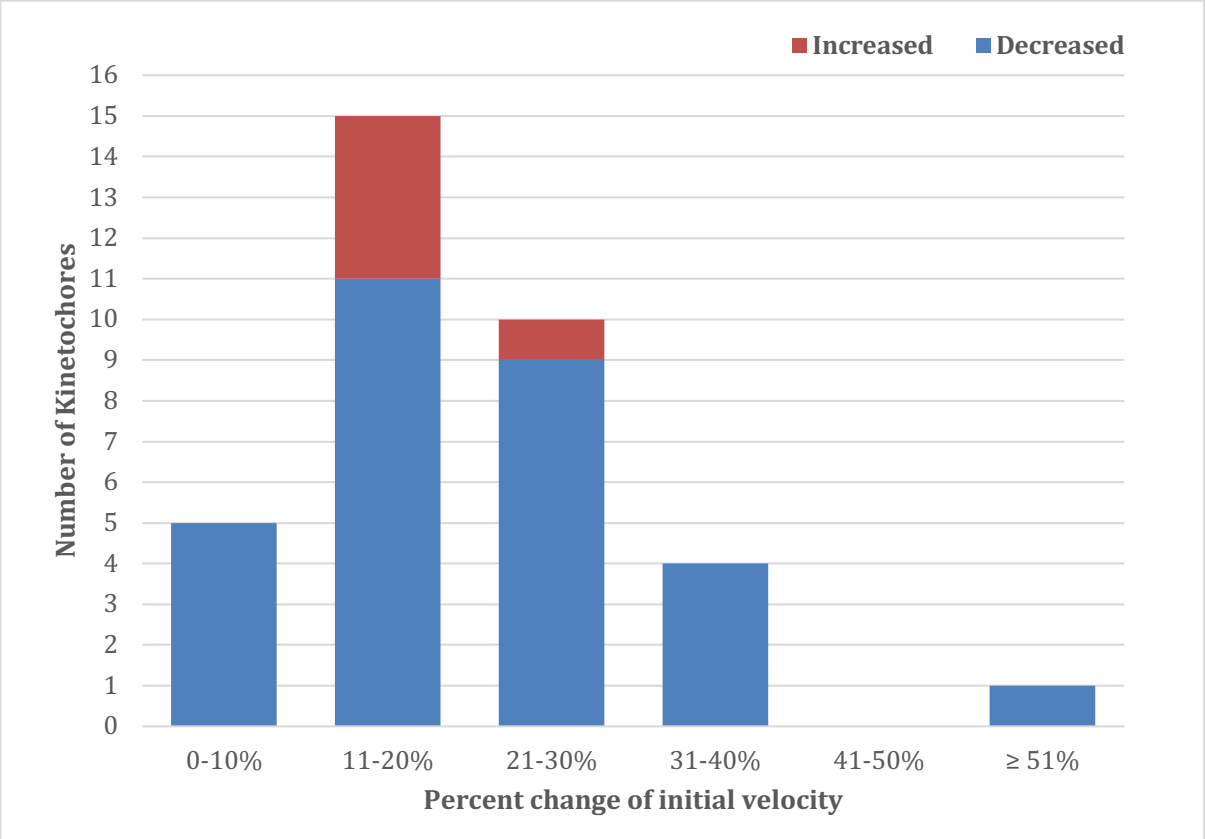

Figure 4. Distribution of percent change in kinetochore initial velocity. The extent of kinetochore velocity change, decreasing (*N*=30) or increasing (*N*=5), when oscillating after jasplakinolide treatment and before spindle pole movement began.

Furrow ingression and movement

After jasplakinolide addition, the furrow remained in its original, pre-drug treatment position for a short period of time (~ 6 mins) until it began ingressing and/or moving towards a random pole (Figure 5). The order of ingression and/or movement varied between each cell. Sometimes the furrow started ingressing before moving poleward or conversely, the furrow began moving before ingressing. Most often, the furrow ingressed at the same time it moved along the length of a cell toward a pole (Table 2). Because of furrow movement poleward, one end of the cell became larger than the other. This was observed in all cells except for one. That particular cell did not have a furrow prior to treatment and did not form a full furrow after jasplakinolide. In all cases, the furrow moved toward a pole at random,

regardless of the chromosomal distribution in the cell. The pole it ultimately shifted toward was always the "static" pole, i.e., the spindle pole that did not alter its orientation. The furrow often began moving before the onset of spindle pole movement (of the large half-cell) and even after the half-bivalents moved toward the static pole, the furrow arrested near the static pole. This furrow movement is different than that occurs in non-treated cells, in which the furrow moves toward the pole that has fewer chromosomes. In cells treated with jasplakinolide, the furrow remains at the pole to which the majority of the chromosomes shift, as shown in Figure 5D.

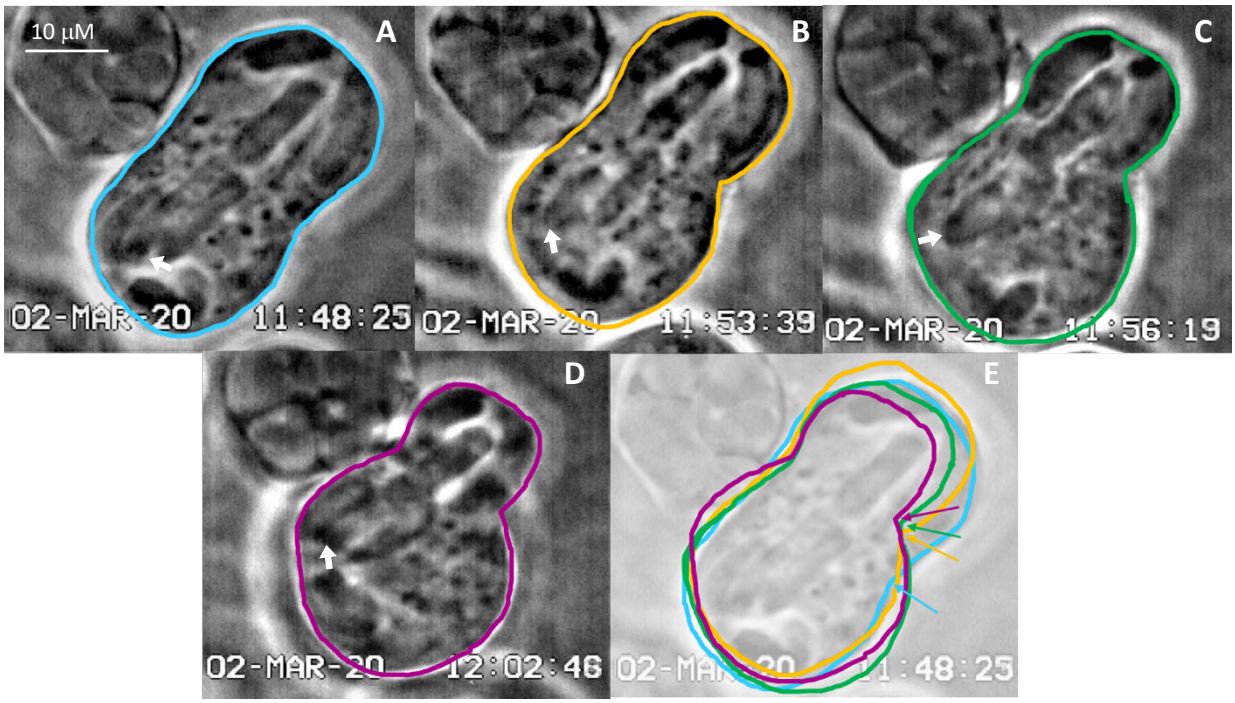

Figure 5. The movement of the precocious cleavage furrow after jasplakinolide addition. The colored outlines represent the cell shape and furrow position. The white arrow indicates the half-bivalent's kinetochore position as the bottom pole moves toward the static pole. After drug perfusion, (A-D) the furrow ingresses while simultaneously, moving toward the top pole. As the furrow moves and constricts, the bottom cell enlarges while the top cell gets smaller. Furrow ingression and movement halted at (D). (E) A superimposed image of the colorized outlines from A-D show changes in the cell morphology, furrow position, and width. Times are shown in hr:min:sec.

Table 2. Summary of average times regarding the furrow's response to jasplakinolide treatment. Values are mean ± standard deviation.

Initial furrow response (Order of events)	Number of Cells	How long after drug addition did the furrow begin to ingress and/or move? (mins)	For how long did the furrow ingress and move along the cell length? (mins)	
			Duration of furrow width decreasing	Duration of furrow position shifting
Same time	14	6.72 ±1.10	3.70 ± 0.78	3.82 ± 1.03
Ingression	8	7.62 ± 2.71	4.56 ± 0.97	3.74 ± 1.06
Movement to pole	2	4.7 ± 0.42	2.82 ± 0.69	2.3 ± 0.33
Overall	24	6.69 ± 1.69	3.86 ± 0.95	3.65 ± 1.07

The extent of furrow ingression (measured as the change in furrow width) and movement also varied between cells. The duration of both events roughly occurred for 3-4 mins (Figure 6; Table 2), after which the furrow arrested in an asymmetrical position. At this position, the furrow sometimes continued ingressing; however, it never fully cleaved the cell. Most cells (14/24) ceased ingression after the furrow width decreased by 30-40% of their initial width. On average, the furrow ingression speed was 1 ± 0.5 μm/min (s.d) (*N*=24), which was similar to the speed in control cells, 1.1 ± 0.7 μm/min (s.d) (*N*=2). The furrow moved along the length of the cell at roughly 1.5 ± 0.7 μm/min (s.d). The furrow position shifted on average 4 ± 1.9 μm (s.d) poleward, about 2x more than the usual distance moved in control cells due to chromosomal imbalance at the poles.

I looked to see if there was any correlation between the symmetry of the furrow pre- and post-drug addition. Could the furrow symmetry before treatment possibly indicate which pole it ultimately shifts toward? At the time of jasplakinolide addition furrows were either symmetric or asymmetric. After drug perfusion, the symmetric furrows (*N*=17) were likely to move to either of the poles; I could not discern any indication of which pole they would move to. On the other hand, 6/7 asymmetric furrows consistently moved to the nearest pole after jasplakinolide addition. Based on this outcome, asymmetric furrow movement post-drug addition does not seem random; rather, furrows seem to move to the nearest pole. Further, this could also indicate which spindle pole is likely to move since the furrow and

rotating spindle pole usually move in the same direction. Symmetric furrows, however, move to a single pole at random due to some unknown factor and so furrow movement direction and the one-sided pole movement in these cells cannot be predicted.

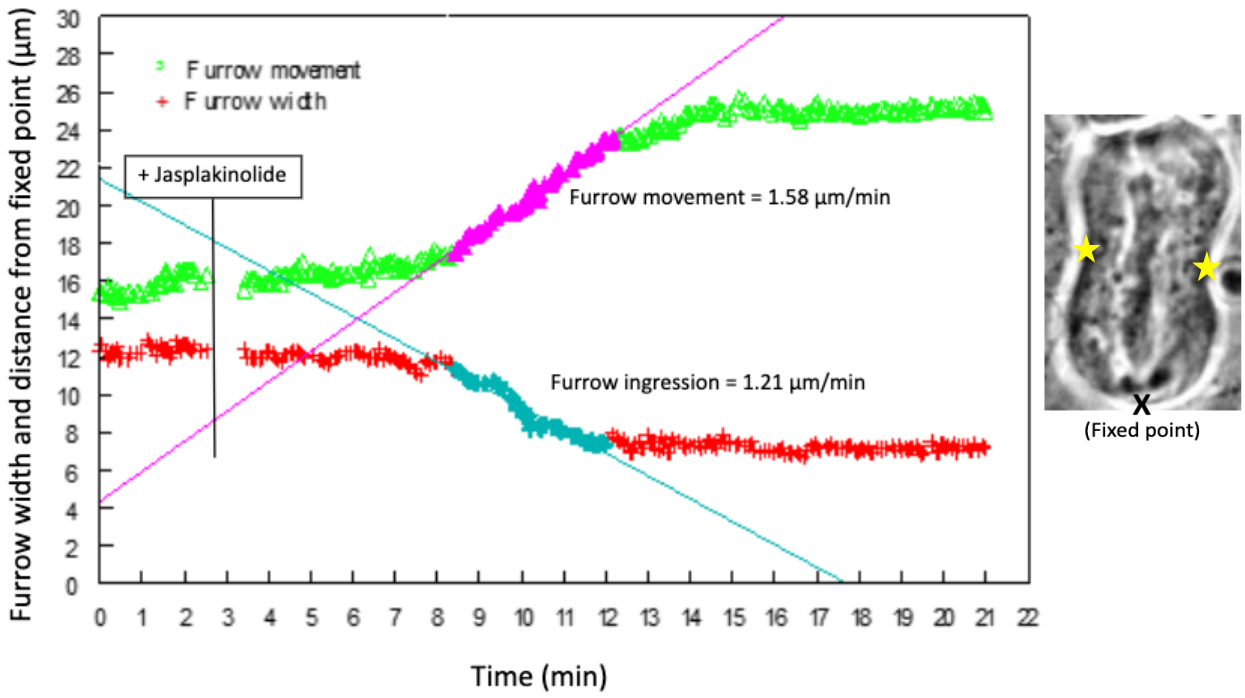

Figure 6. Illustration of furrow movement and ingression after jasplakinolide treatment. Furrow movement was measured by tracking the position of the furrow (represented by yellow stars) relative to the fixed point at the edge of the cell (marked by X). Furrow ingression was measured by tracking the change in width (distance between two yellow stars). The furrow began ingressing and simultaneously moving roughly 5 mins after drug addition. The furrow completely stopped ingressing about 10 mins after drug addition and its poleward movement slowly came to a stop, shortly after. Lines of best fit show rate of furrow width decreasing and position shifting.

Spindle pole movement

The last response observed was the movement of a single spindle pole along with its associated kinetochores, toward the opposite pole. Because all the half-bivalents in each cell moved poleward and arrested at the same time, it seemed they moved as a connected group and so it was presumed that their respective spindle pole also moved. The kinetochores and pole of the larger half-cell consistently moved in the same direction as the furrow - toward the pole in the smaller half-cell. The onset of this movement also occurred at roughly the same time the furrow was ingressing and/or shifting positions. In a few instances, however, the spindle pole (5/25) moved 1-2 mins after the onset of the furrow's change in disposition. The manner in which the half-bivalents and spindle pole moved differed amongst the cells as well as the duration and speeds of their movement (Table 3). There were two main types of movements categorized as (1) rotation, where the pole slid along the curvature of the cell membrane as it moved poleward and (2) no rotation, where the pole moved along its original spindle axis, straight towards the pole located in the small half-cell (Figure 8). Sometimes the bivalents continued to oscillate while rotating as previously mentioned and shown in Figure 3; this was considered a subtype of movement (Table 3*i*). Most frequently the poles and their associated half-bivalents were seen to only rotate poleward. While rotating, the bivalents appeared to bend as the kinetochores moved at an angle away from their original orientation and arrested in a diagonal position (Figure 7). On average, the poles (N=18) were positioned about 18 ± 4.29° (s.d), away from their initial spindle axis. The spindle poles at the small half-cell also appeared to move during this time and it occurred to a much lesser extent (≤ 7°), which I considered insignificant since such small movements are also seen in control cells.

With respect to the duration and speed of the one-sided movement, the spindle poles (N=25) on average moved for durations ranging from 2.04 to 7.52 mins and their kinetochores moved at speeds averaging at 3.3 ± 1.2 min/μm (s.d). Those cells that responded with rotation often moved for a longer period of time and at slower speeds. Cells with bivalents that continued oscillating while rotating spent significantly more time moving compared to those cells that had no rotational movement (Table 3). In accordance with

duration, half-bivalents moving in a straight line moved 1.4x faster than rotating half-bivalents.

Table 3. Summary of the parameters analyzed and compared against the three types of spindle pole movements in response to jasplakinolide. Values are mean ± standard deviation.

Type of movement	Number of cells	Time from drug addition to spindle pole movement (min: sec)	Duration of spindle pole movement (min: sec)	Angle of spindle pole rotation in large half-cell (°)	Rate of kinetochore movement (µm/min)
I. Rotation	13	6.97 ± 1.35	4.16 ± 1.49	19.26 ± 4.24	2.8 ± 1.3*
i. Rotation + oscillations	5	7.91 ± 2.42	5.77 ± 1.22*	15.86 ± 3.78	-
II. No rotation	7	6.71 ± 0.97	3.35 ± 1.14*	-	3.9 ± 0.8*

* Indicates values are significantly different with $p < 0.05$

Note: rate of kinetochore movement for bivalents that oscillated while rotating could not be determined as these kinetochores were constantly in motion and it would not accurately reflect the velocity of rotational movement.

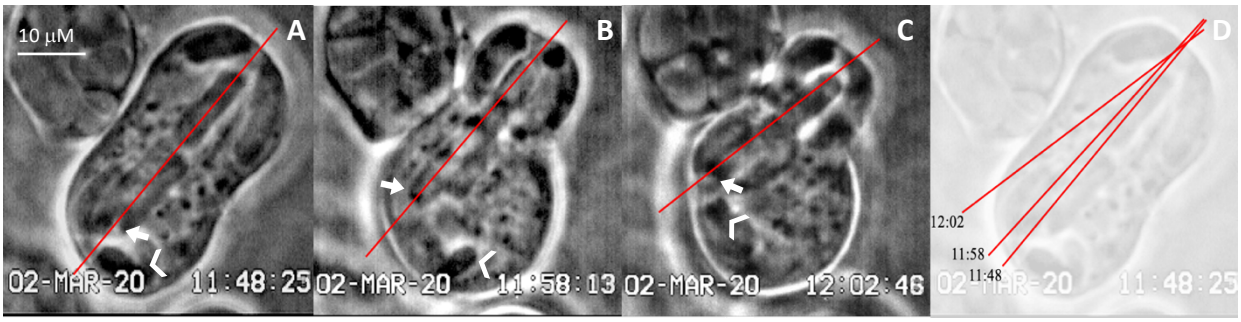

Figure 7. Image sequence of spindle pole movement within a cell treated with jasplakinolide. White arrow indicates the position of the bottom half-bivalent and white brackets indicates bottom univalent position. Red line represents the spindle axis. After drug is added (A) the bivalents oscillated along the original spindle axis. (B) The bottom spindle pole in mid-rotation toward the opposite pole; the univalent has not started moving. (C) Spindle pole rotation stopped at an angle of approximately 14˚ from the original axis and the univalent has now positioned itself by the pole. (D) Images A-C are superimposed and show the changes in pole movement in a timely succession. Top pole in small half-cell minimally shifted during the rotation. Times are shown in hr:min:sec.

In addition, univalents moved as illustrated in Figure 7 and Figure 8, although not always at the same speed or at the same time the bivalents moved. Sometimes they appeared to be dragged along by the moving half-bivalents. Univalents were not tracked often since they were usually in a different focal plane than the bivalents, and thus it was only presumed they also consistently moved in each cell. Nonetheless, their movement further suggested the whole spindle pole moves and for this reason, further investigation was done to accurately understand if the spindle pole and all its associated kinetochores were moving together.

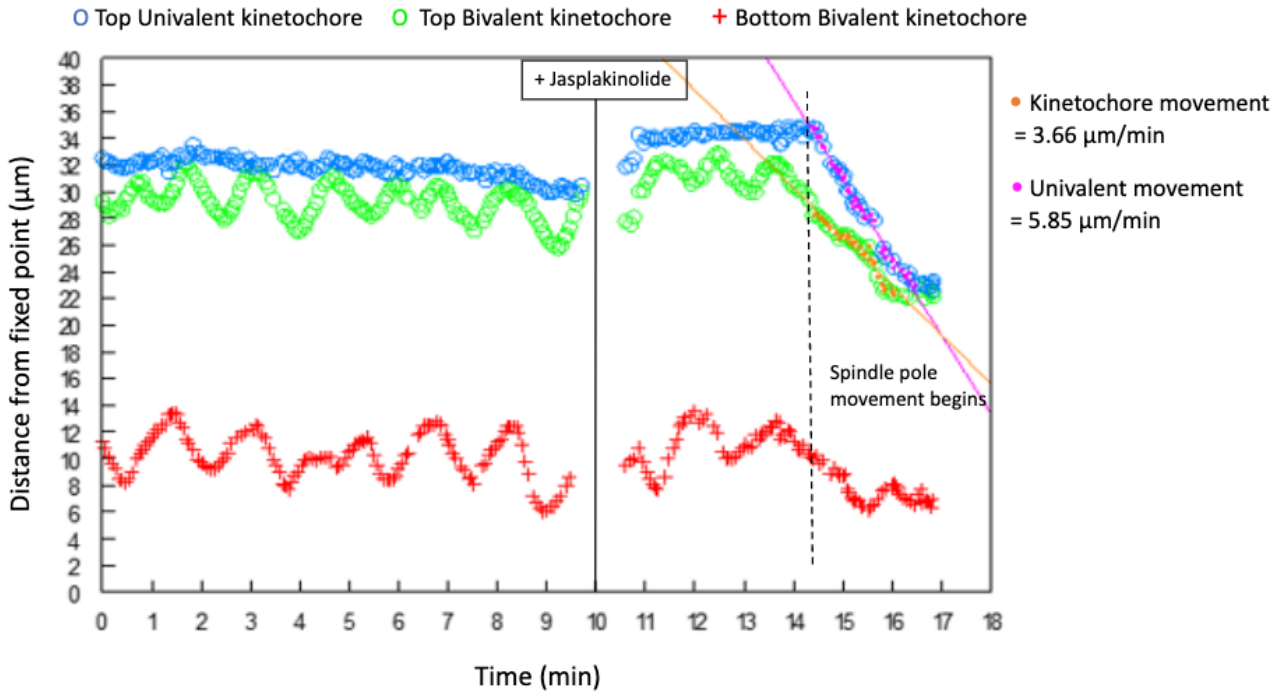

Figure 8. Chromosome movement graph of one univalent and bivalent in a *Mesostoma* spermatocyte treated with jasplakinolide. Spindle pole movement began around 4.10 mins after drug addition and the univalent (blue) moved at the same time. The top half-bivalent and univalent did not rotate, but these chromosomes moved straight down toward the bottom pole along their initial spindle pole axis. Lines of best fit show the bivalent (orange) and univalent (pink) kinetochores of the top pole move at varying rates. Both halted at roughly the same time, around 1.30 mins after moving approximately 6 µm and 12 µm, respectively, towards the bottom pole. The bottom kinetochore also moved toward its respective pole however, movement continued thereafter.

The spindle pole cannot be identified using phase-contrast microscopy, but it can be detected through a confocal microscope via immunofluorescence. Hence, to test this presumption I stained jasplakinolide-treated cells for tubulin. Because the spindle pole is the site at which microtubules radiate, its change in position after jasplakinolide addition can be visualized and localized by using anti-tubulin antibodies. In control cells (Figure 9A), the microtubules during prometaphase-I of *Mesostoma* spermatocytes looked as described previously (Fegaras and Forer 2018a). The centrosomes at each pole were the most densely stained and appeared quite bright relative to the radiating microtubules. Stained kinetochore fibres appear thicker and shorter than non-kinetochore fibres since they attach to nearby kinetochores of the bivalents and univalents. Cells treated with jasplakinolide were lysed and stained when the moving kinetochores were in mid-rotation and after the kinetochores completely stopped moving. Unlike the control cells, the centrosomes of the moving kinetochores were no longer oriented along the same axis line as their opposite pole. Instead, the centrosome of the moving pole shifted orientations and was positioned diagonally relative to the other pole as illustrated in Figure 9B-C, for both mid-rotation movement and when rotation completely halted. Despite the cells being poorly stained because of some technical issues, I was able to draw this conclusion based on the examination of weak staining observed in other cellular contents on the same slide. Nonetheless, from the apparent bending of the bivalents and shifted orientation of the centrosome, immunofluorescence confirms that the half-bivalents concurrently move with the univalents and spindle pole.

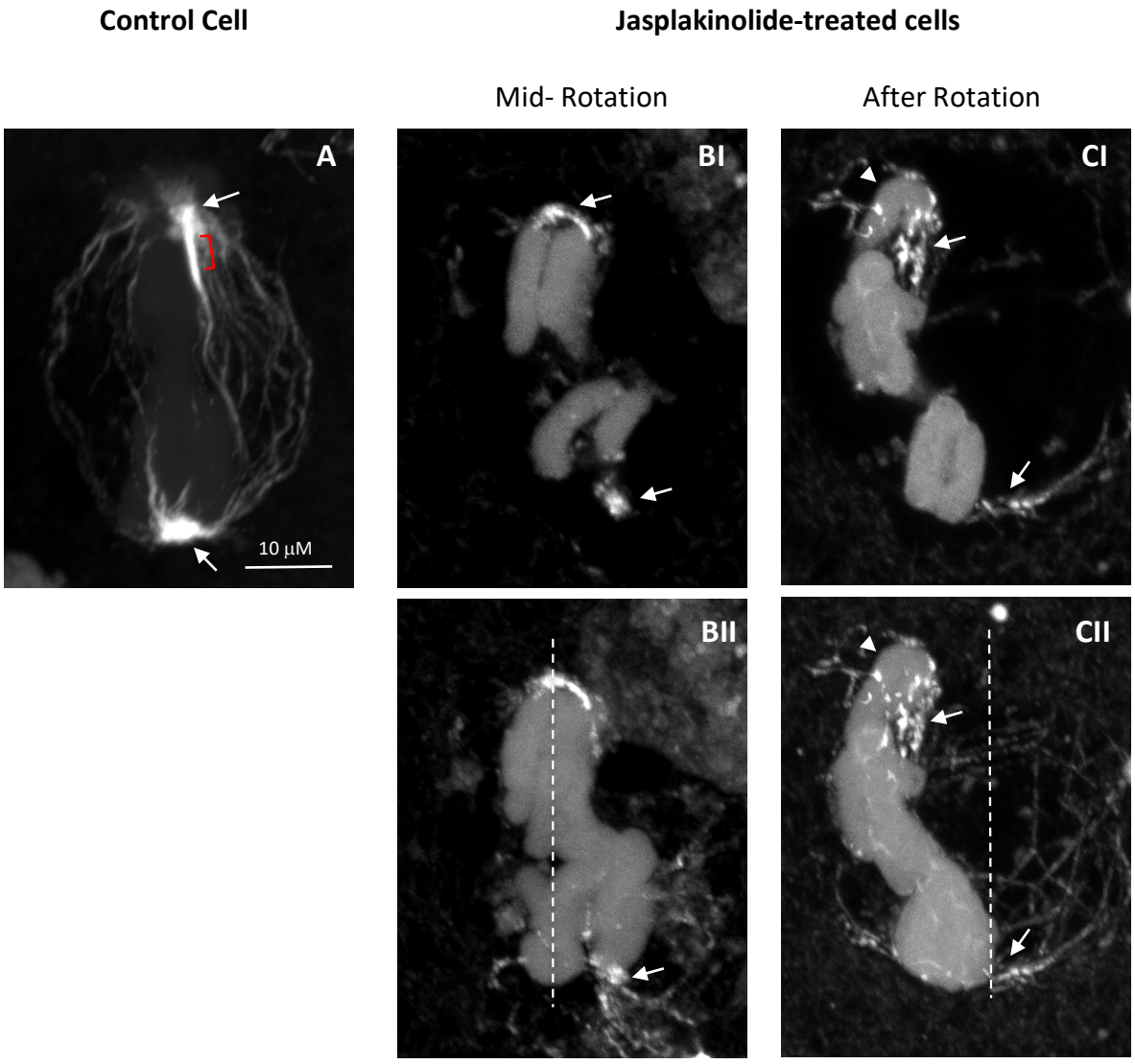

Figure 9. Illustrates control cell and jasplakinolide-treated cells stained for tubulin. The white arrows indicate the centrosome position at the top and bottom pole. (A) Centrosomes in the control cell are brightly stained and both the non-kinetochore and kinetochore (indicated by red bracket) microtubules are distinct. Chromosomes are in dark grey extending between the two centrosomes. The top and bottom pole are aligned, and the spindle pole axis is a straight line. (BI) and (CI) images are of a few slices of their Z-stack while (BII) and (CII) images are of the complete Z-stack (all slices). In (B) the cell was stained when the bottom pole was in mid-rotation and in (C) the cell was stained only after the top pole completely stopped rotating. Both poles are discernable in (BI) and (CI). Dash line in (BII) and (CII) represent the estimated location of the original spindle pole axis and the single white arrow points out the centrosome location of the rotating pole. Both moving poles moved in a counterclockwise direction from their original spindle pole axis and were diagonally positioned relative to the anchored pole. Chromosomes are in light grey and can

be seen bending in (BII). All chromosomes, including the univalent (indicated by white arrowhead) moved with the centrosome in (C) and (B). Albeit not obvious here, the univalents can be seen in individual images of the (B) Z-stack. DIC images were not included as they were not available using LSM-700 ZEISS.

Washout

In order to confirm that the cell's response to jasplakinolide was not due to cell death, I did two experiments. I washed out jasplakinolide at different times, ranging from 10 to 45 mins after addition, and I followed cells that were not washed out for 2 hours or more (Figure 10) to determine if the cells were viable. In all six of the cells that I followed, they seemingly progressed into meiosis-II. The spindle poles and chromosomes did not return to normal positioning and the bivalents did not continue oscillating, nor did they undergo anaphase. Instead, the cells began changing in a similar manner to control cells transitioning from meiosis-I to meiosis-II. Soon after washout or roughly 30 mins after the spindle pole halted movement in cells that were not washed out, the furrow regressed, and the cells gradually became circular. It was unclear how the chromosomes transitioned but it usually took an upward of 45 mins for the chromatids to aggregate and form two new nuclei. These nuclei appeared to move back and forth in fixed positions, and thereafter chromosomes were seen to oscillate along an axis that was perpendicular to their meiosis-I axis, much like control cells. Altogether, these events indicate the cells were functioning normally; the responses to jasplakinolide thus were not due to lethal damage to the cells and can be attributed to the effects of stabilizing actin.

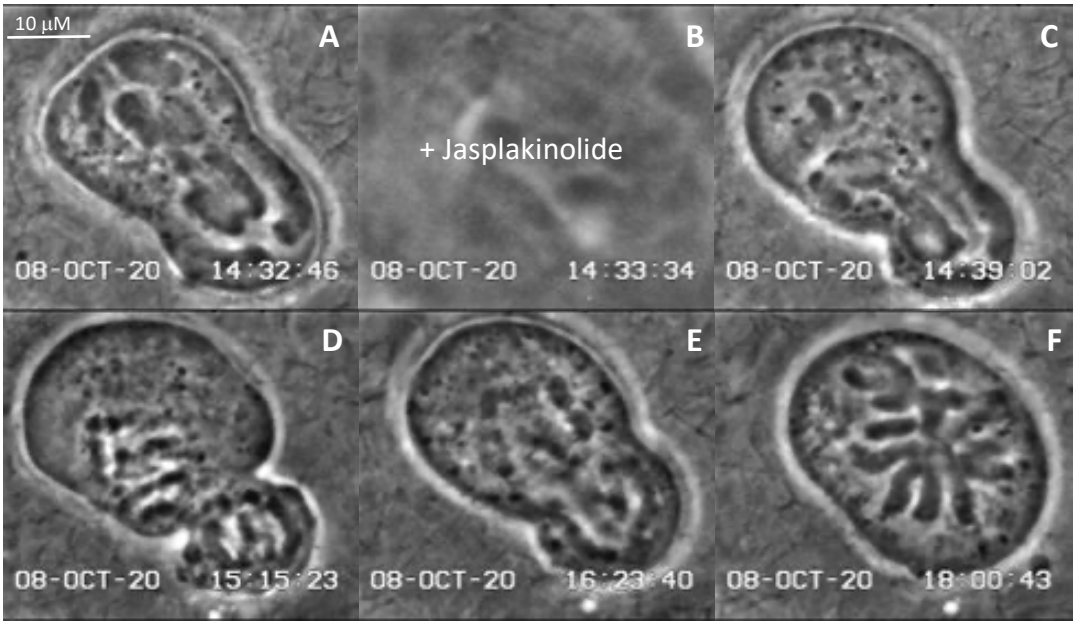

Figure 10. Image sequence of jasplakinolide-treated cell recovering without washout. After drug perfusion (B), the spindle pole rotated and the furrow ingressed and moved toward the bottom pole (C). Without washing out the cell, the chromosomes separated into two chromatid aggregates (D) around 35 mins after furrow and pole movements halted. More than an hour later, two hollow circles formed (E), the furrow completely regressed, and the cell subsequently entered prometaphase-II (F). In (D-F) the cell closely resembles the behavior of control cells transitioning from meiosis-I up to prometaphase-II. Times are shown in hr:min:sec.

DISCUSSION

The objective of this study was to investigate the unusual response of *Mesostoma* primary spermatocytes to jasplakinolide treatment in order to better understand actin's involvement in force production of chromosome movement and function within the spindle. My experiments targeted actin during prometaphase-I by treating live cells with jasplakinolide, a compound that stabilizes actin filaments but that also can induce polymerization of new filaments and can bundle existing filaments (Bubb et al. 2000; Holzinger and Meindl 1997; Terada et al. 2000; Holzinger 2001). Overall, chromosome movements in the cell were altered. In addition, and unexpectedly, jasplakinolide also caused one spindle pole and its half-bivalents to move along the cell periphery towards the opposite pole, often around the same time the precocious furrow recommenced ingression and moved toward the same pole. The major findings of my study elicit questions such as, how does actin work during chromosome movement? why does the furrow ingress, shift and reside near a single spindle pole? why is there a unidirectional movement of the large half-cell spindle pole toward the opposite pole? and why do this pole and the furrow move in the same direction? I now discuss these issues, and present possible explanations.

Chromosome movement

When jasplakinolide was added, more than 70% of half-bivalents moving towards and away from their respective pole immediately slowed (Figure 4). Partner kinetochores responded differently from each other and from other kinetochores within the cell (Figure 4). Most of the kinetochores slowed to varying extents; rarely, a few kinetochores (5/35) sped up even when their partner kinetochores were slowing down. The immediate effect of jasplakinolide on chromosome movements was similar to the results previously described by Xie and Forer (2008). They found that when jasplakinolide was added to crane-fly spermatocytes during anaphase, chromosome movements mostly slowed, but also stopped or rarely accelerated. Partner half-bivalents responded similarly to each other, but each pair responded differently from the other chromosome pairs in the same cell (Xie and Forer 2008). Each half-bivalent in one pair responded the same presumably because of the

signaling and/or tethers between the separating half-bivalents that coordinate their movement (Xie and Forer 2008; Forer and Berns 2020). Tethers, elastic components extending between the arms of separating partner half-bivalents have also been found in *Mesostoma* spermatocytes (Forer et al. 2017; Fegaras-Arch et al. 2020). In many animals, tethers are elastic in early anaphase and become inelastic as anaphase progresses. When half-bivalent arms were cut in prometaphase or early anaphase, the arm fragments moved past the cell equator toward their partner at the opposite end of the cell (Forer et al. 2017). The presence of tethers in *Mesostoma* suggests the movements of partner half-bivalents may also be coordinated such as in crane-fly spermatocytes. However, Fegaras-Arch *et al.* (2020) note that sometimes the arm fragments did not move when cut during prometaphase, presumably because the tethers were inelastic or not present. In addition, Ferraro-Gideon *et al.* (2013) note partner half-bivalents in *Mesostoma* non-treated cells behave independently, which implies coordination between the partner half-bivalents is not common in normal cells. For these reasons, it seems unlikely that coordination from the tethers is related to the varying movements in jasplakinolide treatment. Thus, it is still perplexing as to why responses varied between partner half-bivalents and between other half-bivalents in the same cell when given the same drug.

The varying responses observed could be due to differences in the individual kinetochore fibres. Some studies have shown that there are differences in how kinetochore fibres initially form. The first microtubule of kinetochore fibres may originate from the spindle pole or from the kinetochore (Rieder 2005; Maiato et al. 2004). It has also been shown that some microtubules forming throughout division are nucleated along the sides of preexisting kinetochore microtubules (Petry et al. 2013). The number of microtubules within individual kinetochore fibres also varies. For example, during metaphase in HeLa cells, a single kinetochore fibre may have 13 to 22 microtubules, while PtK1 cells in metaphase can have 20 to 30 kinetochore microtubules within a single fibre (McEwen et al. 1997; 2001). The number of microtubules in each kinetochore fibre increases as cell division progresses (Tolic 2017). In addition, researchers have indicated different kinetochore fibres within a PtK1 spindle have varying flux rates (Cameron et al. 2006; Tolic 2017). Thus, considering many differences exist between individual kinetochore fibres, it could be surmised that such

differences played a part in causing *Mesostoma* chromosomes within a single cell to have varying responses to jasplakinolide.

Furthermore, Cameron *et al.* (2006) and Xie and Forer (2008) suggested that the differences in kinetochore fibres may be due to multiple force-producing mechanisms that work according to the spatial and temporal contexts within the cell. Along with these researchers, I suggest there are varying force-producing mechanisms that perhaps all work independently, concurrently or in combination with each other. There may also be some redundant force producers that function as per the presence or absence of certain factors like motor proteins or spindle matrix proteins. Thus, I presume different kinetochore fibres within the same cell had variable responses to the same drug as a result of multiple force-producing factors working and interacting at different times and/or certain areas within the cell. If kinetochore fibres were heavily dependent on force producers that required actin, this would explain why some half-bivalents slowed to great extents when jasplakinolide was added. Conversely, if actin is not relied on by other force producers utilized by certain kinetochore fibres, then this would explain why some half-bivalent movements were trivially affected. Alternatively, if actin is required and its normal activity was perturbed, the cell may have adapted to utilize other redundant force producers, which insignificantly changed chromosome movement speeds. It could also be that actin counters or limits other force producers that normally cause rapid movements. Hence, when jasplakinolide was added, kinetochore fibres may have relied on those force producers; therefore, causing half-bivalents to move at faster rates.

The conditions of the cell during spindle formation could also contribute to which force-producing mechanisms were more likely to be utilized. For example, in human oocytes spindle actin gradually forms throughout division, becoming more prominent as the cell enters and completes anaphase (Mogessie and Shuh 2017). Thus, it may be that the low density of actin earlier in meiosis only affects a few kinetochore fibres or that kinetochore fibres solely rely on other force producers for movement until there is an adequate amount of spindle actin present. Lastly, because bivalents did not completely stop moving in the presence of jasplakinolide at any time between early prometaphase to anaphase as seen by

Xie and Forer (2008), this further suggests that bivalent oscillations in *Mesostoma* use multiple force-producing mechanisms, many of which are not heavily reliant on actin.

In addition, Mogessie and Shuh (2017), have indicated actin promotes kinetochore fibre formation: adding anti-actin drug cytochalasin D caused a decrease in the density of kinetochore microtubules and artificially promoting actin polymerization increased kinetochore microtubules. This line of thinking could also be applied to *Mesostoma* cells. It seems likely that the gradual growth of the spindle actin is related to the number of kinetochore microtubules in a single fibre increasing as meiosis progresses (Tolic 2017). Hence, in accordance with Mogessie and Shuh, I assume the regions with more actin would correspondingly be found near the mature kinetochore fibres during prometaphase. The kinetochore fibres of that region were consequently impacted to a greater extent by jasplakinolide than the other fibres that consist of fewer microtubules.

It is worthwhile to look at how actin may function on a molecular level to elucidate how jasplakinolide impacted bivalent oscillation speed and period. Many studies have shown actin is dynamic and regularly needs to disassemble and assemble to create movements such as in protrusions of lamellipodia, filopodia (Crammer et al. 1999), dendritic spines (Pirta et al. 2009) and chromosome movements within starfish oocytes (Lénárt et al. 2005) and mammalian oocytes (Li et al. 2008; Mogessie and Shuh 2017). As such, actin turnover may also occur in *Mesostoma* cells. However, if actin solely functioned in this manner then jasplakinolide should have caused bivalent oscillations to stop. Hence, in addition to perturbing actin turnover, I think jasplakinolide blocked actin-binding proteins (ABPs), myosin and other spindle matrix proteins from binding to actin, thereby altering bivalent oscillation speed and period.

When proteins bind to actin, they induce conformational changes to actin's cytoskeleton (Harris et al. 2020). This process is critical as the torquing, tension and bending of actin influences protein interactions with the filaments by directing crucial proteins to different regions of the actin cytoskeleton for its specific function, increasing specificity for proteins to bind, and allowing for stronger bonding to myosin (Harris et al. 2020; Wang et al. 2019). The same could apply to the actin filament network in *Mesostoma* spermatocytes. If the force producers for kinetochore fibres use actin, then jasplakinolide may have blocked

proteins (e.g., actin-binding proteins, spindle matrix proteins or myosin) thereby hindering conformational changes of spindle actin. This would in turn have perturbed the spatial organization of those proteins and their specific function required for chromosome movement. For example, myosin may need to be localized near the poles to create contractile motion with actin required for chromosome poleward movement but without conformational changes to spindle actin, myosin may not move, or its localization may lag, delaying force production. Because bivalent oscillations continued albeit at altered speeds, my findings suggest that jasplakinolide does not bind to the same sites as myosin but does affect its interaction with actin. Future studies should investigate what proteins along with myosin interact with actin and the extent these proteins may influence force production.

Furrow ingression and movement

Upon jasplakinolide addition, the chromosomes continued moving and soon after the pre-existing furrow began ingressing and moving along the cell length toward a specific pole (Figure 5). In normal *Mesostoma* cells, the furrow slightly ingresses in prometaphase, arrests and then recommences ingressing in anaphase to complete cleaving the cell. The furrow only moves during prometaphase when there is an uneven chromosomal distribution between the two poles, and always shifts toward the pole with fewer chromosomes. My findings are puzzling because in jasplakinolide-treated cells the furrow moves to a single pole regardless of the chromosomal distribution and arrests by the pole that is associated with the majority of the chromosomes.

To determine what may have caused this response, it would be helpful to understand the mechanisms that normally coordinate precocious furrow ingression and furrow movement. However, these events are not completely understood nor explained by standard models. The standard models say that furrow formation and ingression are induced by microtubules directly or indirectly interacting with the cell cortex during the metaphase-to-anaphase transition. Some consider spindle asters (i.e., astral microtubules) are responsible for positioning the furrow at the spindle equator by signaling the cortex, while others consider the central spindle (i.e., interzonal microtubules) responsible (Shannon et al. 2005;

D'Avino et al. 2005; Barr and Gruneberg 2007). Although the proposed mechanisms could somewhat be applied to *Mesostoma* cells, they do not account for furrow formation as early as prometaphase and none can explain the furrow shifting along the length of the cell. On the other hand, the mechanism of ingression is widely accepted to result from the actomyosin contractile ring in many species including *Mesostoma* (Fegaras and Forer 2018b; Wolfe and Gould 2005; Cheffings et al. 2016).

The ingression of the furrow induced by jasplakinolide is a common response seen in previous studies (Mishra et al. 2013; Mendes Pinto et al. 2013; Terry et al. 2017). Furrow constriction occurs in part because of actin depolymerization of the actomyosin contractile ring but when jasplakinolide was used to block actin depolymerization, the furrow continued to ingress and the actin density in the contractile ring was insignificantly affected (Mishra et al. 2013; Mendes Pinto et al. 2013). Based on my results (Table 2), this suggests that actin depolymerization is not necessary for ring contraction and that the furrow should ingress irrespective of jasplakinolide. Alternatively, jasplakinolide could have bound to a site on actin that did not interfere with the binding site of myosin-2, the specific type of myosin within the actomyosin ring (Tuxworth and Titus 2000). Hence, the ring was able to continue contracting despite actin stabilization. Furthermore, furrow ingression rates were found to be similar to the ingression rates in control cells after anaphase (pg.44), which suggests that the actomyosin mechanism was not affected by jasplakinolide. Thus, furrow ingression induced after drug addition was likely not related to the contractile ring and more likely due to perturbed interactions between the cell cortex and the spindle that normally induce ingression.

Along the lines of this thought, jasplakinolide may have disrupted interactions between spindle components and the cell cortex that caused the furrow position to change. In other experiments, the precocious furrow in *Mesostoma* proceeded to move after microtubules were depolymerized and shifted towards the pole with fewer chromosomes (Fegaras and Forer 2018b) and when a single centrosome was irradiated in sand-dollar embryos, the furrow shifted toward the aster-less pole (von Dassow et al. 2009). These experiments show in the absence or disruption of microtubules the furrow position changes, suggesting microtubules are not required in positioning the furrow, unlike the views of

standard models. In another study, when *Mesostoma* kinetochore fibres of one half-spindle were irradiated, the furrow moved either toward or away from the irradiated site (Ferraro-Gideon 2013). The furrow did not move, however, when non-spindle components were irradiated such as the chromosomes, univalents or the cytoplasm. Thus, Ferraro-Gideon (2013) suggested that signaling exists between the spindle components and the furrow since the furrow only shifted when spindle components were altered. Along with Ferraro-Gideon (2013), I predict spindle components may be signaling the furrow to move based on the spatial and temporal context within the cell. Jasplakinolide addition must have altered the spindle components somehow by a direct and/or indirect manner thereby stimulating signals that cause the furrow's position to shift.

In addition to signaling, the furrow movement, as well as ingression, may be due to perturbations of the cell's structural tensegrity by jasplakinolide. As described by Ingber (1993) a cell is considered to have tensional integrity (tensegrity) that arises from an integrated network composed of supportive structural elements (e.g., microtubules, intermediate filaments), tension components (e.g., actin filament lattice) and elastic fibres (e.g., actomyosin and titin). All the elements are held together by a cell matrix and work cohesively to maintain a balanced tensegral structure. The structure remains under continuous tension and local perturbations are transmitted to other areas by adjustments throughout the overall structure (Ingber 1991,1993). That is, mechanical input in one area (e.g., force, compression, deformation) are transmitted through the structure and transcribed into local signaling in another area. Discussions in other literature (Forer and Pickett-Heaps 2010; Fegaras and Forer 2018b) consider that the spindle is a tensegral structure – MTs as struts, and matrix and actin-myosin as tensors in combination with the spindle matrix (discussed in Johansen et al. 2011) are tensegral as well and plausibly what promotes the precocious furrow and its movement along the cell length due to chromosomal imbalance within *Mesostoma* spermatocytes. Although this is speculation, it might explain why the furrow moves and ingresses a short period after jasplakinolide addition. It could be that the tensegral constituents reorganized in response to jasplakinolide, subsequently forming structural tension in different areas and redistributing the spindle components. This may have led to more proteins and tension in one half-spindle which signaled the furrow to move and

ingress towards one pole and remain at that pole irrespective of the chromosomal distribution in the cell.

The tensegral spindle might also explain why the asymmetric furrows before drug addition moved toward the nearest pole. Presumably, the structural tensegrity was already imbalanced for asymmetric furrows before jasplakinolide addition. Thus, the furrow moved in the same direction post-drug treatment and jasplakinolide alterations merely accentuated the cell's preexisting condition. As for the condition of cells with symmetric furrows prior to drug addition, the tensegral spindle was presumably balanced as the chromosomes were not unequally distributed like the cells with an asymmetric furrow. Therefore, the direction of furrow movement seems to relate to the cell's condition prior to drug treatment and its random movement towards a pole might primarily be based on the altering structural tension and distribution of the components responding to jasplakinolide. Future studies should investigate which spindle components are crucial for changing the furrow's position and which are responsible for inducing ingression and if they are the same or influence each other. This would also help determine why furrow ingression and movement often started and stopped at the same time (Table 2). Different components can be altered individually or in combination and localized using confocal microscopy to determine their relation to furrow behaviour.

Spindle pole movement

As confirmed by immunofluorescence, jasplakinolide causes a single spindle pole and its associated chromosomes to move toward the opposite pole, at roughly the same time or after the furrow ingresses and moves. This spindle pole and the furrow always moved toward the same pole – the "static" pole (Figure 7). To determine what may be causing this unidirectional movement of a single spindle pole, it would be helpful to first understand whether the chromosomes were solely responding to jasplakinolide and dragging the spindle pole along as a result, or if the spindle pole was altered and consequently pushed the chromosomes. Based on my findings the answer to this question is unclear since the univalents sometimes moved at different speeds and times compared to the bivalents and

from the time-lapsed movies and immunofluorescence images the chromosomes appeared to move before the spindle pole (Figure 9CII).

Various possibilities could account for the movement; one such feature could be the elastic tethers that extend between the arms of a bivalent. When microtubules were depolymerized by nocodazole in *Mesostoma* cells, chromosomes detached from one pole and rapidly moved to the opposite pole (Fegaras and Forer 2018; Fegaras and Forer 2020). The authors assume tension exists between the two arms of a bivalent due to the elastic tethers and this tension may contribute to the chromosomes moving to one pole. Perhaps this could explain the one-sided movement observed in jasplakinolide-treated cells. Disrupting actin's normal activity could have altered the phosphorylation of tethers which may have caused an increase in tension between the arms. Subsequently, the half-bivalents may have moved to their partners to relieve this tension. The moving half-bivalents would have pulled their respective spindle pole along as the connectivity between their kinetochores and the pole were intact. The type of spindle pole movement (Table 3) may be due to the amount of tension produced: the higher tension could have caused the kinetochores to move at a faster speed in a linear direction instead of moving along the cell periphery. While this may be possible it's unlikely since one cell showed the same one-sided movement even after the chromosomes segregated during anaphase. Thus, in the absence of tension between the partner half-bivalents, one spindle pole and its segregated chromosomes moved along the cell periphery to the opposite pole. Tethers may, however, contribute to the movement if present during prometaphase; this will have to be further investigated. I also observed bivalents sometimes continued oscillating during the unidirectional movement (Table 3*i*). If the movement was due to perturbations to the chromosomes, I would expect oscillations to completely stop during movement. Together these data suggest the movement was likely initiated by perturbations at the spindle pole and not at the chromosomes.

Spindle rotation has previously been seen in a study by Thaiparambil *et al.* (2012). In most cells, spindles are positioned and anchored in the cell by the interaction of the astral microtubules and the cell cortex (O'Connell and Wang 2000; Shaw et al. 1997). Thaiparambil *et al.* (2012) speculated when the interactions between astral microtubules and the cell cortex are impeded by actin bundles, the spindle becomes misoriented. They

confirmed this by using jasplakinolide (500 nM) to induce actin bundles in HeLa cells after which the spindle rotated. In other experiments, disruption between astral microtubules and the cell cortex by different drug treatments or change in cell shape by micromanipulation causes spindle repositioning in normal rat kidney (NRK) cells (O'Connell and Wang 2000). The results indicated the cell constantly monitors the geometric relationship between the cell cortex and spindle to determine the type and extent of movement. It also confirmed that spindle positions are not predetermined; rather, the spindle pole is able to interact anywhere on the cell cortex via astral microtubules. This could apply to jasplakinolide-treated cells: when the furrow concurrently moved and ingressed it caused one side of the cell to become larger than the other, and thus, the spindle pole of the larger half-cell may have moved in response to the change in cell shape. The spindle pole consistently moving towards the same pole as the furrow and often at the same time, also implies there must be some coordination between the moving spindle pole and the furrow and further indicates the cell has tensegrity. As previously suggested, jasplakinolide addition may have caused alterations to the tensegral structure constituents which might have perturbed the cell cortex thereby altering the spindle pole orientation. The same mechanism that altered the furrow may have been involved in affecting the spindle poles, this might explain why the furrow and spindle pole had similar sidedness in response to jasplakinolide. Thus, I presume cells use the tensegral structure and molecular signals to continuously monitor and adjust the spindle pole relative to the cell cortex.

The constriction of the furrow and its movement towards one pole may have restricted the small half-cell spindle pole from moving and why the large half-cell pole moves to greater extents. However, the one-sided movement was also observed in one cell that didn't have a furrow pre- and post-drug treatment. In the absence of a furrow, one spindle pole moved toward the opposite pole that remained relatively "static". Additionally, the extent of furrow ingression varied amongst the cells, some cells only ingressed 10-20% in which case the width decreased by 2 μm or less. These measurements were similarly seen in control cells when the furrow becomes asymmetric during chromosomal imbalance (Fegaras and Forer 2018b). Thus, there was space for the small half-cell pole to move yet

their movements were minimal to none. For these reasons, I presume there is a difference between the two poles.

During cell division, the centrosome duplicates and each centrosome moves to opposite ends of the cell, forming the spindle poles (Wade 2009). Other studies have shown the two centrosomes are not necessarily equal: one centrosome is older than the other and some proteins such as cenexin exclusively bind to the old centrosome. The old centrosome is also responsible for anchoring microtubules in interphase and properly orienting the spindle during mitosis (reviewed in Meraldi 2015). Experiments using luminal epithelial cells showed cenexin depletion results in decreased astral microtubule lengths, instability of astral microtubules at the minus-end, and spindle misorientation (Hung et al. 2016). Perhaps in *Mesostoma*, the spindle pole that moved was the younger centrosome that lacked cenexin. Thus, if jasplakinolide-induced actin bundles accumulated by the poles, the younger centrosome was more likely to be affected presumably due to having less stable and/or shorter astral microtubules. The actin bundles would have led to weakening the anchored centrosome and allowing for the spindle pole to move as a result. Future experiments should stain the centrioles and cenexin to test this speculation. If the younger centrosome was the spindle pole that consistently moved, then cenexin staining should be consistently localized by the "static" pole, e.g., the older centrosome, and absent by the moving pole.

Sometimes the moving spindle pole slid along the cell membrane to the opposite pole and sometimes it moved linearly, straight towards the opposite pole (Table 3). The different types of movement could be due to the density of actin bundles at the moving pole. Within a dividing cell, the centrosomes can also function as actin filament organizing centers (Farina et al. 2016). Briefly, each centrosome is able to polymerize filaments from actin monomers, in a manner similar to microtubule polymerization from tubulin dimers. If there are different amounts of actin monomers at each pole at the time of jasplakinolide addition, this could affect how the spindle pole moved. For example, if there were a higher amount of actin monomers present near one pole this would have increased the likelihood of actin bundles forming and interfering with the cortex capturing astral microtubules from the spindle pole and thus the pole moved linearly. If there were smaller amounts of actin bundles then the astral microtubules of the moving pole were likely captured along one side of the cell cortex,

causing the pole to move along the cell periphery. However, the extended movement of one spindle pole to the other suggests other forces were involved in continuously moving the pole.

In addition to the potential differences at the poles, I presume other forces within the cell were involved in propagating the one-sided movement. According to the spindle matrix model, the spindle matrix, its proteins and other components, including actin and myosin, generate force that moves the chromosomes poleward (Forer and Pickett-Heaps 2009; Johansen et al. 2011; Johansen and Johansen 2007). When microtubules were disrupted in diatoms chromosomes moved poleward during metaphase (Pickett-Heaps and Spurck 1982) and in *Mesostoma* spermatocytes all chromosomes moved towards a single pole (Fegaras and Forer 2018a). Both sets of authors suggested that the spindle matrix propelled the chromosomes poleward. My findings are similar except that both the spindle pole and its associated half-bivalents moved poleward together. Thus, I similarly presume the underlying spindle matrix acted on the spindle pole and chromosomes to propel them to the opposite pole in response to jasplakinolide. Fegaras and Forer (2018a) suggested the one-sidedness could be because the matrix is polarized toward one pole. By extending this thought, I surmise when actin was stabilized the matrix relied more so on its other components that may have different directionality of producing force (e.g., bidirectional vs unidirectional). Some matrix components could be unidirectional and produce force towards one pole. Perhaps, actin works in a unidirectional manner and when the components that produce counter-forces were affected by jasplakinolide, the overall forces within the cell were no longer balanced. Alternatively, actin may work bidirectionally and its stabilization might have altered other components that work unidirectionally, thus altering the overall balance of forces. Therefore, I assume an imbalance of forces acting on the spindle pole contributed to the one-sided movement. For example, spindle matrix proteins like Megator, Skeletor, EAST and Chromator could each have different directionalities within the cell. Future studies should possibly test this by using different enhancers and inhibitors that target these proteins in jasplakinolide-treated cells. If perturbing these proteins alters spindle pole and/or furrow movement, then it would indicate that the matrix and certain specific proteins play a role in moving the pole and/or furrow after jasplakinolide treatment.

Concluding remarks

The main conclusion from my experiments is that actin plays an important role in chromosome movements, spindle orientation as well as in furrow positioning. Immediately after jasplakinolide addition, bivalent oscillations were altered. Based on these results, I conclude actin is involved in generating force that contributes to chromosome movement; actin's dynamic behaviour plays a role in regulating bivalent oscillation speed and period. In response to jasplakinolide, the pre-existing furrow recommenced ingression and shifted along the length of the cell toward one spindle pole. The spindle pole of the larger half-cell moved toward the opposite pole, often at the same time the furrow moved and always in the same direction as the furrow. These events have not been previously noted in experiments using jasplakinolide. My results suggest actin depolymerization is not required for furrow ingression; it is, however, involved in furrow positioning. My results imply there is a relation between the furrow position and spindle pole as they often move at the same time and in the same direction. I suggest the two are coordinated and perturbations that caused the furrow to move were connected to moving the single spindle pole. While the movement of chromosomes to a single pole has been commonly noted in previous experiments with *Mesostoma* (Fegaras and Forer 2018a; Silverio 2017; Ferraro-Gideon 2013), the movement of one spindle pole to the opposite “static” pole is novel. My data indicates the movement of one half-spindle was likely initiated by perturbations at the spindle poles. However, since only one spindle pole moved this may have been due to differences between the two poles. Little is known about the differences between the poles and my results may serve as a base for more conclusive work in differentiating the poles in the future. Furthermore, the extended movement of one spindle pole and type of movement suggests other spindle matrix components contributed to moving the pole and these proteins may have different directionalities in producing force within the cell (e.g., bidirectional vs unidirectional). My thesis is a step forward in determining the underlying mechanisms of chromosome movement and evidencing spindle sidedness. Overall, the reasons for these unusual behaviors are inconclusive and further studies need to be conducted to increase our understanding of these movements and their purpose.

Significance

Actin's involvement in chromosomal segregation has long been debated in the field. While there is extensive evidence that microtubules are essential for chromosomal movement, others have argued that poleward chromosome movement may be aided by non-microtubule spindle components such as actin and myosin (reviewed in Forer et al. 2015). My findings shed light on actin's involvement in producing force for chromosome movement and how actin may be utilized in various ways within the spindle thereby adding evidence to non-microtubule-based theories like the spindle matrix model. Further, elucidating which components are involved in chromosome movement is important for determining whether the current models of chromosome segregation are widely applicable. The data presented here not only increase our understanding of the normal functioning within *Mesostoma* meiotic cells but potentially many other meiotic cell types including humans. Cell division is the single most important process across all species and understanding this process accurately will have great biological, evolutionary and clinical implications.

Limitations & Future directions

Future experiments should look at localizing actin in the jasplakinolide-treated cells. To get a better picture of how the cells are affected by jasplakinolide, I would like to stain for actin. Staining for actin was a limitation in my study as actin is most commonly stained by fluorescently labelled phalloidin, which binds to the same actin sites as jasplakinolide (Pospich et al. 2019). There are alternative methods to visualizing actin that one can consider such as using nanobodies directed against actin, like the Actin-Chromobody (Melak et al. 2017). Because one spindle pole moves toward the other, it would be of interest to stain for the centrioles and cenexin. From this, we would see how jasplakinolide could be directly or indirectly affecting them and investigate differences between the poles.

Another limitation of my study was not knowing if jasplakinolide interacts with other cellular components and if the other interactions played a role in causing the unusual effects. To test whether the results elicited by jasplakinolide were due to stabilizing actin and not

because of side effects of jasplakinolide, I would treat cells with another actin-stabilizing agent. Like jasplakinolide, miuraenamide A can both promote F-actin polymerization and stabilization (Wang et al. 2019). Both the compounds function similarly, except they bind to different sites on actin. Thus, miuraenamide A may block different crucial binding sites, which may result in the cells responding differently. If the cells respond differently to both drugs, this could potentially indicate which effects of jasplakinolide were due to blocking certain binding sites. As well, if miuraenamide A elicited the same response as jasplakinolide, I could then try to stain for actin using phalloidin.

My findings indicate that in addition to actin, there are other spindle elements that contribute to bivalent oscillations and plausibly the one-sided movement. To gain some insight into what components may be involved, I would use pharmaceutical drugs to inhibit and enhance different spindle matrix proteins such as Skeletor, Megator, Chromator, EAST and others, in jasplakinolide-treated cells. Staining for these proteins would then allow me to see if and when their localization within the spindle changes after jasplakinolide addition. In addition to indicating what caused one pole to move versus the other, this would directly increase our understanding of their functionality within the spindle and in relation to actin, which at the current moment is not fully understood.

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