Mitotic and meiotic spindle dynamics comparison in fission yeast

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Abbreviations

DNA - deoxyribonucleic acid

MT – microtubule

MTOC –MT organizing centre

KT - kinetochore

MAP – microtubule associated protein

SAC – spindle assembly checkpoint

MI – meiosis I

MII – meiosis II

GTP – guanosine triphosphate

KT-MT – kinetochore microtubule

iMT – interpolar microtubule

aMT – astral microtubule

PEF – polar ejection force

PCM – pericentriolar material

γ-TuSC – γ-tubulin small complex

γ-TuRC – γ-tubulin ring complex

SPB – spindle pole body

NEBD – nuclear envelope breakdown

γ-TuC – γ-tubulin complex

ncMTOC – non-centorsomal MTOC

APC – anaphase promoting complex

ATP – adenosine triphosphate

AAA – ATPase associated with diverse cellular activities

NE – nuclear envelope

GDP – guanosine diphosphate

aMTOC – acentriolar MTOC

CPC – chromosomal passenger complex

YE5S – yeast extract medium supplemented with Leu, Ura, Ade, His, and Lys

ME – malt extract

GFP – green fluorescent protein

mCherry – monomeric red fluorescent protein

IP – interphase

IK – interkinesis

DNA – deoxyribonucleic acid

MBC – methyl benzimidazole carbamate

Introduction

1. Cell division

The cell is a building block of all known organisms. During its life, the cell cycles through distinct phases. A cell cycle consists of interphase, which encompasses G1, S and G2 phases, and cell division (Figure 1.1A). Generally, in G1 cells grow and prepare for DNA (deoxyribonucleic acid) replication that occurs in the S phase. In G2, cells continue to grow and prepare for cell division. In eukaryotes, two types of cell division can be distinguished: mitosis and meiosis. The role of mitosis is cell proliferation, while meiosis produces cells used for sexual reproduction. Both mitosis and meiosis follow after a single round of DNA duplication – cells in mitosis divide once, but cells in meiosis divide twice. The imperative task of cell division is to separate the duplicated genetic material precisely into daughter cells.

1.1. Mitosis

Mitosis is a cell division type in which two identical daughter cells are produced from a single parent cell, retaining the parental ploidy (Figure 1.1B). It is an essential process whose purpose is proliferation – whether it be increasing the number of cells, e. g. growth and maintenance of a multicellular organism, or reproduction, e. g. vegetative reproduction of plants. Therefore, it is essential that chromosome segregation is error-free, as inaccuracies may lead to aneuploidy that could result in catastrophic consequences such as cancer, developmental defects, or cell death (Holland and Cleveland, 2009; Thompson, Bakhoum and Compton, 2010).

To achieve flawless chromosome separation necessary for ploidy maintenance, cells build a mitotic spindle (Gatlin and Bloom, 2010; Heald and Khodjakov, 2015). The spindle is a macromolecular apparatus that consists of microtubules (MTs), motor proteins and non-motor proteins (David J. Sharp, Rogers and Scholey, 2000; Manning and Compton, 2008). A mitotic spindle is typically

formed in prophase, when centrosomes, the major MT organizing centres of the cell (MTOCs), commence nucleating MTs (Rale, Kadzik and Petry, 2018). Growing MTs are attached via the minus-end to the centrosome, and with the distal plus-end they explore the area around them in search for other MTs or kinetochores (KTs), specialized protein structures located at the centromeric region of DNA that serve as a platform for MT-mediated attachment of chromosome to the spindle.

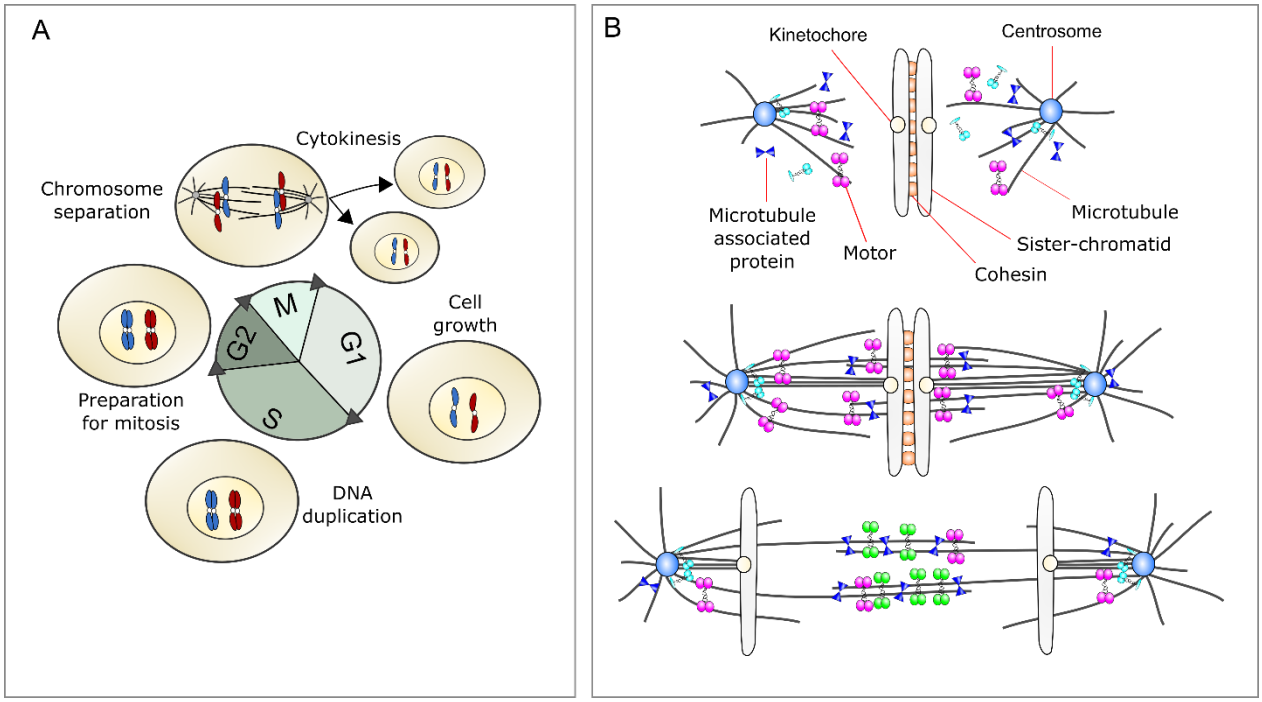

Figure 1.1. Vegetative cell cycle and mitosis. (A) In vegetative state, cells cycle between interphase (phases G1, S, and G2) and mitosis (phase M). **(B)** A scheme depicting centrosomal mitotic spindle assembly and elongation. The opposite centrosomes nucleate microtubules (MTs) that interdigitate and are crosslinked by motors and MT associated proteins (MAPs). MTs from opposite poles attach the kinetochores (KTs) and the chromosomes are positioned to the spindle equator. Once the spindle assembly checkpoint is silenced, the cohesin between sister-chromatids is removed and the sister-chromatids are segregated to the opposite spindle poles.

Spindle bipolarity is established when interdigitating MTs from the opposing centrosomes are cross-linked via motors and MT associated proteins (MAPs) into antiparallel arrays and slid apart

(Tanenbaum and Medema, 2010). Chromosomes, which are attached to the spindle MTs via the KTs, congress to the metaphase plate and align through a complex interplay of forces that maintain a steady-state spindle length (Goshima *et al.*, 2005; Dumont and Mitchison, 2009). Once all sister-KTs are attached to the MTs emanating from the opposite spindle pole (the chromosomes are bi-oriented), the spindle assembly checkpoint (SAC) is satisfied (Musacchio and Salmon, 2007). This activates separase (Peters, 2006), which then cleaves cohesin, a protein complex that connects sister-chromatids, thereby allowing the chromosomes to be pulled to the opposite spindle poles in anaphase A.

Two mechanisms that govern chromosome movements can be discerned in anaphase A: Pac-Man and poleward flux. In the Pac-Man mechanism, the MTs attached to the KTs (KT-fibre) depolymerize at the plus-end, and shrink towards the spindle pole, simultaneously transporting the chromosomes to the poles (Gorbsky, Sammak and Borisy, 1987). In poleward flux, the chromosome is translocated toward the spindle pole by depolymerization of the KT-fiber near the spindle pole (Mitchison, 1989). Expeditious spindle elongation follows in anaphase B that further separates the genetic material away from the site of cytokinesis. Mitosis ends with two daughter cells that have the same chromosome number as the mother cell.

1.2. Meiosis

Meiosis is a type of cell division that produces cell(s) with half of the starting genetic material. These cells are used in sexual reproductions where they can fuse and produce a diploid zygote. Meiosis consists of two consecutive divisions following one round of DNA duplication. Unlike mitosis, the homologous chromosomes pair in prophase I of meiosis and undergo homologous recombination (Page and Hawley, 2003). This process gives rise to new DNA combinations and promotes genetic variability, and could be the purpose of sexual reproduction. Accurate

chromosome segregation in both meiotic divisions is imperative. In humans, chromosome segregation in female meiosis is especially error-prone (Warburton, 1997; Holubcová *et al.*, 2015; Namgoong, Kim and Christenson, 2018), and such flaws result in congenital disorders or abortions.

As in mitosis, a MT based spindle is assembled to equally separate the genetic material into two daughter cells (Figure 1.2A). Importantly, oocytes of many species do not have centrosomes (Müller-Reichert *et al.*, 2010; Dumont and Desai, 2012; Bennabi, Terret and Verlhac, 2016; Gruss, 2018), and rely on other pathways of MT nucleation (Chapter 3). One of the hallmarks of meiosis is the reductional nature of the first meiotic division (MI). There are several mechanisms in place that assure the halving of ploidy: 1) modified architecture of the sister-KTs and mono-orientation of chromosomes (Yokobayashi and Watanabe, 2005; Hauf *et al.*, 2007; Sakuno *et al.*, 2011); 2) specialized cohesin and hierarchical control of cohesin cleavage by separase (Watanabe and Nurse, 1999; Tomoya S. Kitajima *et al.*, 2003; Kitajima, Kawashima and Watanabe, 2004; Miyazaki *et al.*, 2017). These mechanisms ensure mono-orientation of homologous chromosomes in a bivalent, i.e. sister-KTs attach to the MTs emanating from the same spindle pole. When the SAC is satisfied, separase cleaves the cohesin between the chromosome arms, but not in the centromeric region, where cohesin is protected (Miyazaki *et al.*, 2017). Spindle elongation in anaphase B of MI ensures the homologous chromosomes from a bivalent are sufficiently separated, and MI ends with two daughter cells that carry half of the chromosome number compared to the mother cell.

In some species, MI is followed by a brief period of rest called interkinesis, a so far cryptic form of interphase, in which the two nuclei of daughter cells can be observed. Meiosis II (MII) is often compared to mitosis, for the chromosome architecture is similarly organized (Figure 1.2B). An MII spindle forms in each nucleus, and separates sister-chromatids into two new daughter cells. MII ends with four daughter cells containing half of the starting amount of genetic material.

In female oocytes, cell division is very asymmetric in MI, and the products of MI are a mature egg, with halved chromosome number, and a small first polar body that does not play a further role (Sanders and Jones, 2018). The mature egg undergoes MII, and, in most vertebrates, it pauses at metaphase of MII until it gets fertilized by a sperm. MII is also asymmetric, and upon fertilization and resumption of division, an embryo and a second polar body are formed.

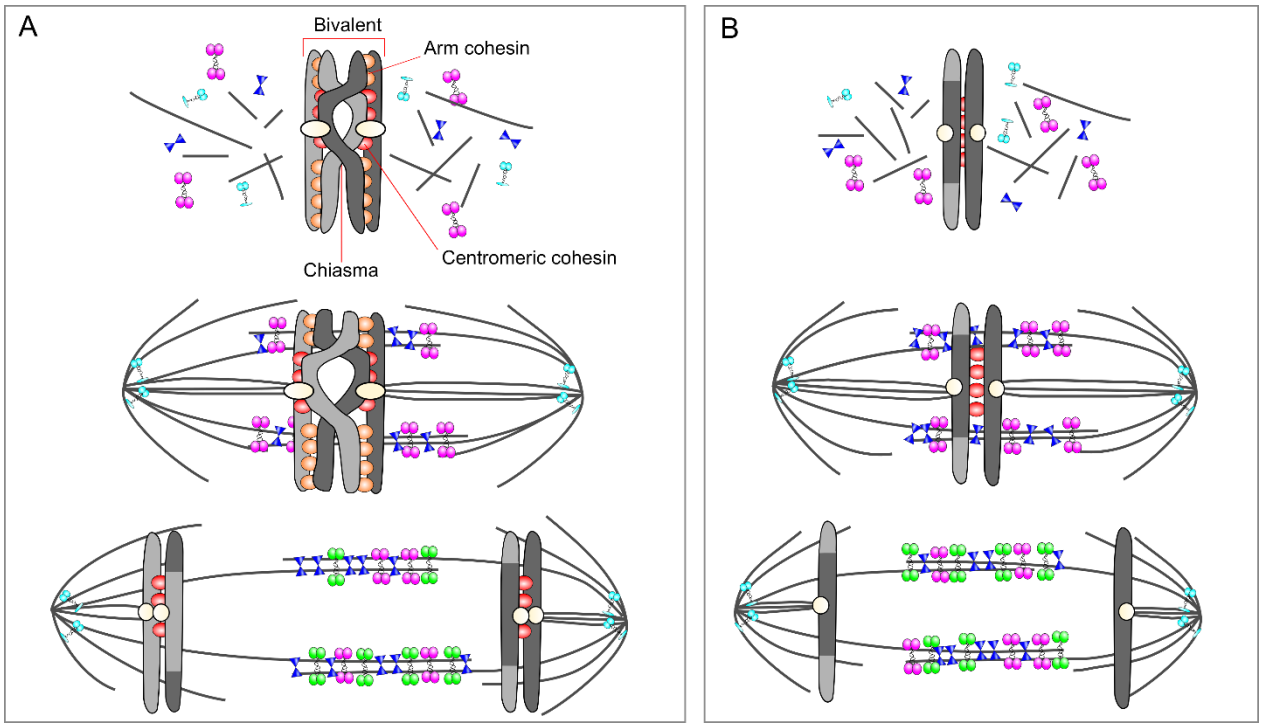

Figure 1.2. Meiosis consists of two divisions. (A) Two homologous chromosomes form a bivalent which is held together by cohesin and chiasma, site of recombination. Meiosis I is a reductional division in which the ploidy of the cells halves as recombinant homologous chromosomes are separated to the opposite spindle poles. The spindle is shown to nucleate in a centrosome independent way, as the majority of oocytes assemble a spindle without centrosomes. **(B)** In meiosis II, the spindle separates sister-chromatids to the opposite spindle poles.

2. Spindle components

The key structure in cell division is the spindle. It is an intricate cellular machine composed of hundreds of different proteins that fine-tune and regulate its function. Broadly speaking, spindle

components are shared between mitosis and meiosis, with some crucial differences that will be explored in the following chapter.

2.1. Microtubules (MTs)

As MTs are the building blocks of the spindle, so is tubulin the building block of the MT (Figure 2.1A). There are two types of tubulin that make an MT: α-tubulin and β-tubulin (Bryan and Wilson, 1971). The α- and β-tubulin associate into dimers, which in turn assemble into a linear protofilament. Typically, 13 protofilaments are linked in a parallel fashion to form a rigid, hollow cylinder that is the MT, but other number of protofilaments have also been observed (Desai and Mitchison, 1997; Chaaban and Brouhard, 2017).

The protofilament is polar in nature and the assembly of the protofilaments occurs on what is termed the plus-end, where β-tubulin resides (Mitchison, 1993; Nogales, 1999). The α-tubulin is found on the slow-polymerizing minus-end. Both tubulin units can bind one molecule of guanosine triphosphate (GTP): non-hydrolysable on the α-tubulin and hydrolysable on the β-tubulin. During the growth of the MT, GTP hydrolysis rate is slower than the rate of tubulin heterodimers incorporation rate, and MT plus-ends are decorated with a GTP cap. When GTP hydrolysis is faster than incorporation rate, the GTP cap is lost, which makes the MT unstable. This triggers a catastrophe, an event where MTs switch from the period of growth to a period of shrinkage (Figure 2.1B, C) (Mitchison and Kirschner, 1984). During shrinkage, an MT may reverse back to growth, an event termed rescue. This highly dynamic attribute of the MT where it shifts between periods of growth and shrinkage is named dynamic instability (Mitchison and Kirschner, 1984). It was proposed by Kirschner and Mitchison that the dynamic instability may facilitate KT capture during

the initial stages of spindle assembly, also known as the ‘‘search and capture’’ model (Kirschner and Mitchison, 1986).

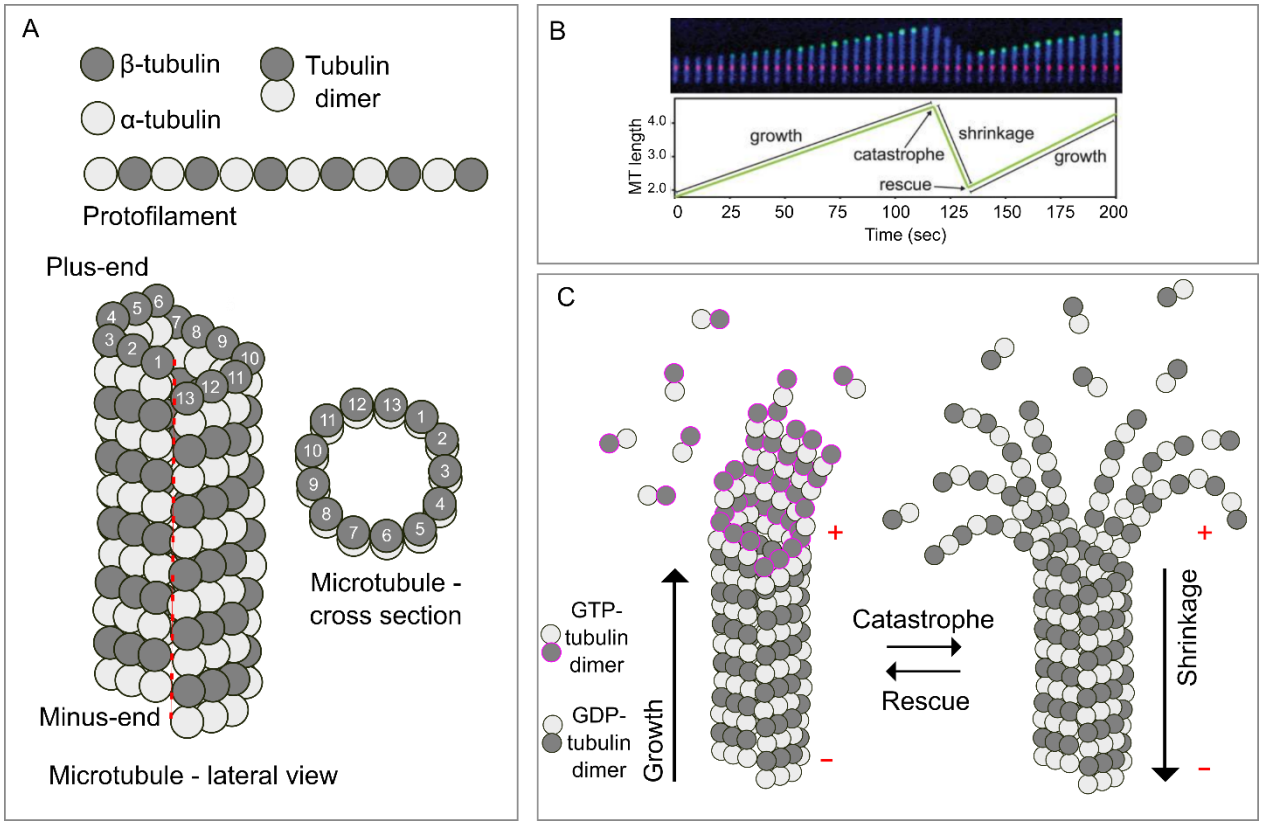

Figure 2.1. MTs are characterized by dynamic instability. (A) αβ-tubulin dimers associate into a protofilament, and 13 protofilaments assemble into a cylindrical MT. Red dashed line indicates a ‘‘seam’’, a lattice discontinuity caused by lateral dimer association. Adapted from (Akhmanova and Steinmetz, 2008). **(B)** Kymograph shows a MT (blue) growing from a seed (red), labelled with plus-end tracking protein (green). The corresponding plot of MT length through time depicts MT dynamic instability. Phases of MT growth and shrinkage, as well as the transitions between the two (catastrophe and rescue) are indicated on the plot. (Zwetsloot, Tut and Straube, 2018) **(C)** Schematic view of MT dynamic instability. During MT growth, a GTP-tubulin dimer rich region is present at the plus-end of the MT. During shrinkage, GTP-tubulin dimers are disassociated from the plus-end, and the lateral bonds in the protofilament break. MT polarity is indicated in red. Adapted from (Roostalu and Surrey, 2017)**.**

The MT cytoskeleton has many essential roles, ranging from cellular trafficking to cell shape maintenance. Notably, MTs assemble a spindle during cell division, and thereby orchestrate chromosome segregation. Dynamic instability of MTs, i.e. growth and shrinkage of MTs in

spindles can produce pushing or pulling forces respectively (Dogterom *et al.*, 2005). In early stages of spindle assembly, MT polymerization can produce pushing forces that together with dynein and kinesin-14 organize the bipolar spindle (Cytrynbaum, Scholey and Mogilner, 2003).

Polarity is another key characteristic of MTs in spindle morphogenesis and function. Motor proteins use polarity to move directionally along the MTs and transport cargo. Also, MTs in the spindle can be classified into three distinct subcategories – MTs that 1) attach KTs, 2) interdigitate between the spindle poles, or 3) grow outwards toward the cell cortex forming astral MTs (Figure 2.2).

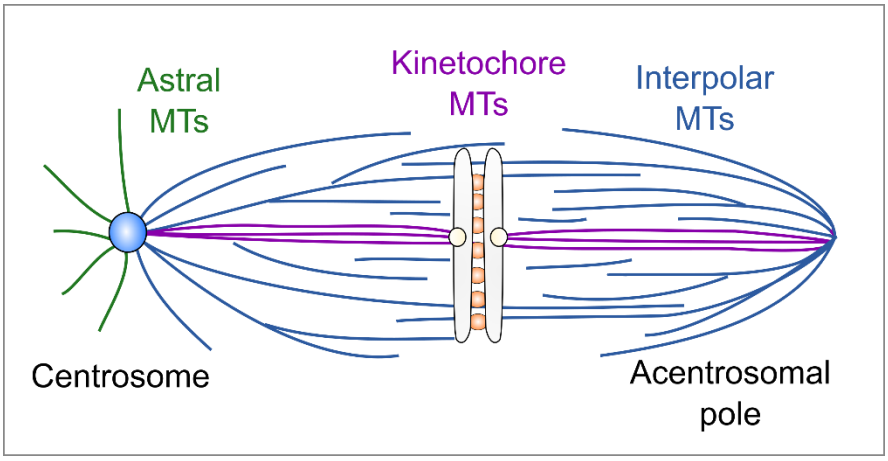

Figure 2.2. Categories of spindle MTs. A schematic representation of a spindle with different subsets of MTs color-coded. Adapted from (Petry, 2016).

2.1.1. Kinetochore microtubules (KT-MTs)

In the spindle, MTs that are attached to the KT are called the kinetochore-MTs (KT-MTs). They are usually considered to span from or near the spindle poles to the KTs, where they are attached via their plus-end (Figure 2.2) (McDonald *et al.*, 1992), although recent work in *C. elegans* mitotic spindles showed only very few KT-MTs are anchored directly to the spindle poles (Redemann *et al.*, 2017). In mammalian cells, 10–30 MTs are attached to a KT (McEwen, Ding and Heagle, 1998), while in budding yeast a single MT attaches to a KT (Winey and O'Toole, 2001).

KT-MTs can be formed by the search and capture model (Kirschner and Mitchison, 1986), but the stochastic character of this model is not consistent with the observed kinetics of K-fibre formation in animal cells, as it would require hours to capture all of the KTs (Wollman *et al.*, 2005). Studies with *Xenopus* egg extracts have demonstrated that MTs can self-assemble around chromatin in the absence of centrosomes via a Ran-GTP pathway (Heald *et al.*, 1996, 1997; Gruss *et al.*, 2001). The same pathway of MT nucleation was also shown to operate in centrosome containing mammalian cells and *Drosophila* S2 cell line, thus displaying the co-existence of these two pathways and their simultaneous activity (Khodjakov *et al.*, 2003; Maiato, Rieder and Khodjakov, 2004).

Besides silencing the SAC to prevent an early transition to anaphase (Musacchio and Salmon, 2007), the role of KT-MTs is to pull on the chromosomes at KTs, and maintain their position at the cell equator, thus contributing to the net force of the spindle (Dogterom *et al.*, 2005; Goshima *et al.*, 2005; Dumont and Mitchison, 2009; Pavin and Tolić, 2016). These pulling forces originate from MT depolymerization, and can be modulated by motors and MAPs present at the KTs (McIntosh, Grishchuk and West, 2002; Maddox *et al.*, 2003; DeLuca *et al.*, 2006).

2.1.2. Interpolar MTs (iMTs)

The set of MTs between spindle poles that is not attached to KTs is termed interpolar-MTs (iMTs) (Figure 2.2). Minus-ends of iMTs can be found throughout meiotic spindles (Burbank *et al.*, 2006; Petry *et al.*, 2013), and their nucleation was shown to occur throughout the mitotic spindles of *Drosophila* S2 cell lines and human cell lines (Mahoney *et al.*, 2006; Lawo *et al.*, 2009; Uehara *et al.*, 2009).

It was proposed that iMTs ensure spindle bipolarity, and it was demonstrated in *Xenopus* that bipolar meiotic spindles can assemble exclusively from non-KT-MTs (Heald *et al.*, 1996). Some

iMTs are cross-linked in an antiparallel manner in the spindle midzone by MT cross-linker proteins of the MAP65/Ase1/PRC1 family (Walczak and Shaw, 2010), and could provide structural stability to the spindle.

In metaphase spindles, forces produced by antagonistic motor proteins maintain the spindle length constant (Saunders and Hoyt, 1992; Goshima *et al.*, 2005; Syrovaktina, Fu and Tran, 2013). Most notably, kinesin-5 motors, that typically move toward the plus-end of the MT, separate the spindle poles by cross-linking and sliding apart the antiparallel MTs (Hagan and Yanagida, 1990; Sharp *et al.*, 1999; Kapitein *et al.*, 2005; Ferenz, Gable and Wadsworth, 2010). Kinesin-5 produced outward pushing force is opposed by motors that move toward the minus-end of the MTs, such as kinesin-14 motors or dynein, and that produce pulling forces (Chalovich and Eisenberg, 2013; Olmsted *et al.*, 2014). Hence, iMTs are a region of the spindle where motors operate and contribute pushing or pulling forces in the spindle.

2.1.3. Astral MTs (aMTs)

A subgroup of MTs that are anchored via their minus-end at the spindle pole, and grow with their plus end toward the cortex is called astral MTs (aMTs) (Figure 2.2). They can be found in centrosomal mitotic and meiotic spindles (Miyazaki, Kato and Nemoto, 2005; Burdyniuk *et al.*, 2018), while their occurrence varies in different types of acentrosomal meiotic spindles. When activated *in vitro*, acentrosomal spindles of *Drosophila* oocytes contained aMTs, but not in laid eggs (Wilson and Borisy, 1998; Endow and Hallen, 2011), and aMTs are not reported in *Xenopus* oocytes (Burbank *et al.*, 2006). In other acentrosomal MI spindles, like those of mouse oocytes and *C. elegans*, aMTs have been observed (Schuh and Ellenberg, 2007; Vargas *et al.*, 2019). However, these observations were not subsequently reported, and are currently considered to be the result of

poor experimental procedures, and not a reflection of reality. Importantly, human oocyte spindles lack aMTs (Holubcová *et al.*, 2015), but further research on healthy oocytes is necessary.

Studies showed aMTs are capable of producing pulling or pushing forces through dynamic instability, that can also be promoted by action of motor proteins and MAPs, and the principal function of aMTs appears to be spindle positioning (Pearson and Bloom, 2004). Additionally, aMTs are involved in the production of so called polar ejection force (PEF), that is suggested to contribute to chromosome congression and oscillation by pushing the arms of chromosomes away from the spindle poles (Rieder *et al.*, 1986). Drug induced destabilization/depolymerization of aMTs caused chromosomes to no longer be repelled from the pole (Ault *et al.*, 1991). Conversely, stabilization/polymerization of aMTs resulted in an increase of PEFs, as chromosomes were pushed further away from the pole than in non-treated cells. Moreover, in *C. elegans*, dynein-mediated astral pulling forces prompt anaphase B spindle elongation (Hara and Kimura, 2009).

2.2. Centrosome and spindle pole body (SPB)

In the majority of dividing mitotic cells, MT nucleation is assigned to the centrosomes. The centrosome is extensively studied, and much of its structure and proteome is mapped and described (Andersen *et al.*, 2003; Müller *et al.*, 2010; Lawo *et al.*, 2012; Ito and Bettencourt-Dias, 2018).

The centrosome is composed of two orthogonally oriented centrioles, one mother and one daughter centriole, and of pericentriolar material (PCM), that nucleates and stabilizes the MTs, and anchors MT minus-ends to the centrosomes (Figure 2.3A) (Wu and Akhmanova, 2017). Phosphorylation of PCM components by PLK1 and Aurora A is pivotal to promote PCM assembly (Lee and Rhee, 2011; Joukov, Walter and De Nicolo, 2014; Conduit, Wainman and Raff, 2015). Two molecules of γ-tubulin, a member of the tubulin superfamily indispensable for MT nucleation, GCP2 and

GCP3, form a γ-tubulin small complex (γ-TuSC) (Figure 2.3B). PCM proteins, like CDK5RAP2 and Pericentrin, promote the assembly of multiple γ-TuSCs into an active γ-tubulin ring complex (γ-TuRC), which acts as a template for MT nucleation (Figure 2.3B) (Zimmerman *et al.*, 2004; Fong *et al.*, 2008).

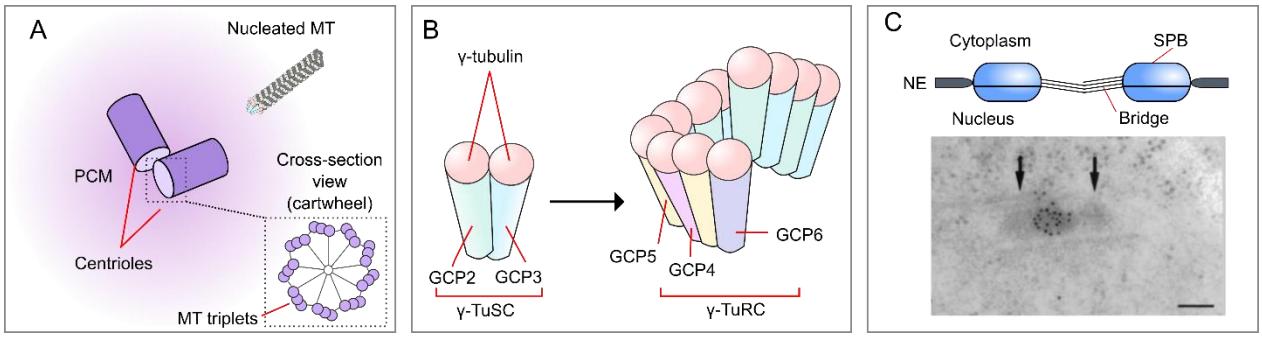

Figure 2.3. Centrosome is the major MT organizing center in the cell. (A) A schematic representation of the centrosome. The orthogonally positioned centrioles are surrounded by the protein-packed pericentriolar material (PCM). The centriole is a hollow cylinder made of nine MT triplets, which are organized in a cartwheel-like formation. Adapted from (Rale, Kadzik and Petry, 2018) **(B)** Schematic view of the γ-tubulin small complex (γ-TuSC). γ-TuSCs oligomerize into a more complex structure named the γ-tubulin ring complex (γ-TuRC). Adapted from (Tovey and Conduit, 2018; Liu *et al.*, 2020) **(C)** Schematic view of the duplicated fission yeast spindle pole body (SPB) connected by a bridge (upper) and an immunoelectron microscopy image of a duplicated SPB. The SPBs are embedded in the nuclear envelope (NE) through a process named polar fenestration. Scale bar, 100 nm. Adapted from (Cavanaugh and Jaspersen, 2017), (Paoletti *et al.*, 2003)..

The centrosomes of fission yeast, the spindle pole bodies (SPBs), are ellipsoid, laminar structures that do not contain centrioles (Figure 2.3C) (Cavanaugh and Jaspersen, 2017). As mitosis is closed in fission yeast, i.e. no nuclear envelope breakdown (NEBD) occurs, the cytoplasmic interphase SPBs have to be incorporated into the nuclear membrane to nucleate the spindle (Ding, McDonald and McIntosh, 1993). As in mammalian cells, γ-TuC complex serves as a template for MT nucleation in *S. pombe* and is composed of (human/fission yeast): γ-tubulin/Gtb1 and five GCP proteins (GCP2/Alp4, GCP3/Alp6, GCP4/Gfh1, GCP5/Mod21 and GCP6/Alp16). Fission yeast

also contains Pericentrin, Pcp1, that recruits both γ-TuRC and PLK1/Plo1 to the nuclear side of the SPB and is vital for mitotic entry and spindle formation (Fong, Sato and Toda, 2010). Unlike centrosomes in most systems, in fission yeast meiosis SPBs are retained and function as spindle MT nucleators.

Acentrosomal spindles, like the ones in most female oocyte meiosis, lack centrosomes, and therefore rely on non-centrosomal MTOCs (ncMTOC) to nucleate MTs. Research so far has showed that ncMTOCs during spindle assembly can be formed around chromatin, KTs or along pre-existing MTs (Heald *et al.*, 1996; Maiato, Rieder and Khodjakov, 2004; Petry *et al.*, 2013). Unlike the centrosome, little is known of the ncMTOC structure, but it is presumed they would have to include MT minus-end proteins and adapter-proteins that could attach MTs to specific locations (Sanchez and Feldman, 2015). Interestingly, it was shown that laser ablation of centrosomes in mammalian cells does not abolish spindle assembly (Khodjakov et al., 2000), demonstrating that ncMTOCs are operating during centrosomal spindle assembly as well.

2.3. Chromosomes and KTs

While regarded as passive elements during cell division in the past (Mazia, 1961), with the observation of Ran-GTP-dependent pathway of MT nucleation during spindle assembly, as well as the force contribution of the chromosomal region to force-balance in the metaphase spindle (Civelekoglu-Scholey and Scholey, 2010), chromosomes are now recognized as important contributors to spindle assembly and function.

In mitosis and MII, the chromosomes are individual entities, and consist of two sister-chromatids connected by the cohesin complex (Figure 2.4A). Mitotic cohesin complex is made up of four subunits (in human/fission yeast): Smc1α/Psm1, Smc3/Psm3, Rad21 and SA1/2/Psc3 (Tomonaga

et al., 2000; Mehta, Rizvi and Ghosh, 2012). Smc1 and Smc3 make a V-shaped dimer, which is enclosed by Rad21 associated with SA1/2 (Figure 2.4B). This ring structure encompasses sister-chromatids and keeps them together from S-phase until anaphase.

Each sister-chromatid contains a KT, where spindle MTs are attached during mitosis. To achieve bi-orientation, sister-KTs on mitotic chromosomes are positioned back-to-back (Figure 2.4A), and thus arranged to face the opposite spindle poles, which facilitates bipolar attachment (Östergren, 1951; Nicklas, 1997; Sakuno, Tada and Watanabe, 2009; Zaytsev and Grishchuk, 2015). In prometaphase, Aurora-B kinase concentrates at centromeres, and is likely activated by the lack of tension between the sister-chromatids. If Aurora B is close enough to the KTs (insufficient tension), it will phosphorylate its substrates at the KT, and destabilize the attachment of MTs to KTs, thereby promoting correct bi-orientation (Liu *et al.*, 2009). KT attachment and bi-orientation of chromosomes distances sister-KTs, and causes tension in the centromeric area that inactivates SAC (Musacchio and Salmon, 2007). Anaphase promoting complex (APC) is activated by Cdc20, and the ensuing cascade activates separase, that cleaves the cohesin between the sister-chromatids, and triggers their disjunction.

In MI, the homologous chromosomes are synapsed into a bivalent through the action of the synaptonemal complex, and consist of four sister-chromatids connected by cohesin and chiasmata – regions where crossing-over occurred and where the homologous chromosomes are physically linked (Figure 2.4A) (Miyazaki & Orr-Weaver, 1994; Page & Hawley, 2003). The meiotic cohesin ring is modified, such that the Rad21 subunit is exchanged with the meiosis specific Rec8 subunit (Figure 2.4B). Additionally, in mammals Smc1β replaces Smc1α (Revenkova *et al.*, 2004), and STAG3 replaces SA1/SA2 in meiosis (Prieto *et al.*, 2001). In fission yeast, cohesin complexes are

organized in distinct domains – chromosome arm cohesin, where Rec11 is in complex with Rec8, and centromeric cohesin, where Psc3 is in complex with Rec8 (Tomoya S Kitajima *et al.*, 2003).

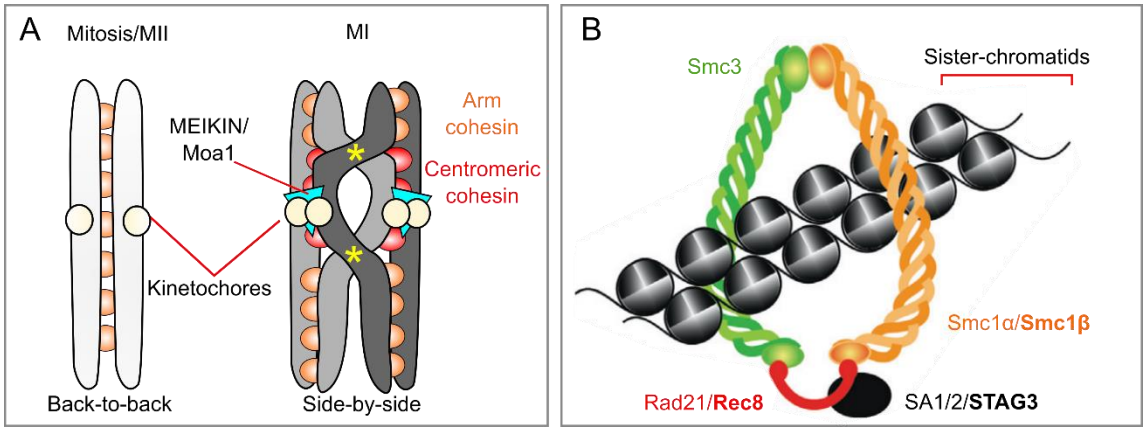

Figure 2.4. Architecture of an independent and paired chromosome. (A) An illustration of chromosomal architecture in mitosis and meiosis. In mitosis, the chromosome is a singular entity where sister-chromatids are connected by the mitotic cohesin complex. The KTs are oriented back-to-back, which facilitates bi-orientation of the chromosome, and separation of sister-chromatids to the opposite spindle poles upon the destruction of cohesin complex. In meiosis I (MI), the homologous chromosomes are paired into a bivalent, which facilitates recombination. The yellow asterisks on the illustration indicate sites where recombination occurred, and where the chromosomes are intertwined. Meiosis-specific cohesin and MEIKIN/Moa1 ''clamp'' the KTs on sister chromatids together, and bring them into a side-by-side arrangement. This facilitates the mono-orientation of each homologous chromosome in a bivalent. Adapted from (Sakuno, Tada and Watanabe, 2009)**. (B)** Schematic representation of the cohesin complex in mitosis (regular font) and meiosis (bold font) (Mehta *et al.*, 2013)**.**

Because of this meiosis-specific cohesin configuration, cohesin can be removed from the chromosome in a step-wise manner (Miyazaki *et al.*, 2017). First, once all the bivalents are bi-oriented, cohesin is removed from the chromosome arms, but not from the centromeric region (Figure 1.2A). This allows halving of ploidy, as the homologous chromosomes are separated from the bivalent configuration to the opposite spindle poles in MI. In MII, the remaining centromeric cohesin is removed (Figure 1.2B), and sister-chromatids are separated in anaphase II. Studies in fission yeast have identified an evolutionary conserved protein Sgo1 as the key molecule protecting

the cohesin in the centromeric region from destruction (Kitajima, Kawashima and Watanabe, 2004; Watanabe *et al.*, 2005; Kitajima *et al.*, 2006). Sgo1 is recruited through activity of Bub1 (Kawashima *et al.*, 2010), and in turn recruits PP2A that ensures dephosphorylation of centromeric cohesin during MI, thereby preventing its destruction (Riedel *et al.*, 2006; Miyazaki *et al.*, 2017).

KT architecture in MI is also altered to ensure mono-orientation of bivalents – unlike the mitotic/MII back-to-back arrangement, the KTs in MI adopt a side-by-side arrangement (Figure 2.4A) (Östergren, 1951). The central molecule in establishment of cohesin-mediated monopolar attachment of KTs is the mammalian MEIKIN/fission yeast Moa1 (Yokobayashi and Watanabe, 2005; Kim *et al.*, 2015), that localizes in the centromeric region up to anaphase I onset. This molecule is also implicated in the MI cohesin protection through recruitment of Plo1/PLK1 (Kim *et al.*, 2015; Miyazaki *et al.*, 2017), whose activity at the KTs ensures Bub1 accumulation (Miyazaki *et al.*, 2017).

2.3.1. Chromokinesins & polar ejection force (PEF)

At chromosomes, PEF is generated through the activity of chromokinesins, kinesin-like motor proteins that associate with chromatin during cell division. Chromokinesins identified so far belong to the kinesin-4 and kinesin-10 families, as well as kinesin-12 family (Vanneste, Ferreira and Vernos, 2011). They are low- or non-processive plus-end directed motors (Yajima *et al.*, 2003; Bringmann *et al.*, 2004) which are thought to advance chromosome congression by actively pushing the chromosome arms toward the spindle equator (Figure 2.5A). (Maiato *et al.*, 2017).

The first characterized chromokinesin was from the kinesin-4 family and was isolated from embryonic chick retina (Wang and Adler, 1995), and homologous proteins in *Xenopus*, *Drosophila*, mouse and human cells are identified (Sekine *et al.*, 1994; Vernos *et al.*, 1995; Williams *et al.*, 1995; Ha *et al.*, 2000). Depletion of both human and *Drosophila* kinesin-4 chromokinesin

(KLP4A/Klp3A) shows, to some extent, chromosome misalignment at metaphase plate (Goshima and Vale, 2003; Wandke *et al.*, 2012). Kif4 knockdown in mouse oocytes induced, among others, chromosome misalignment in MII (Camlin, McLaughlin and Holt, 2017).

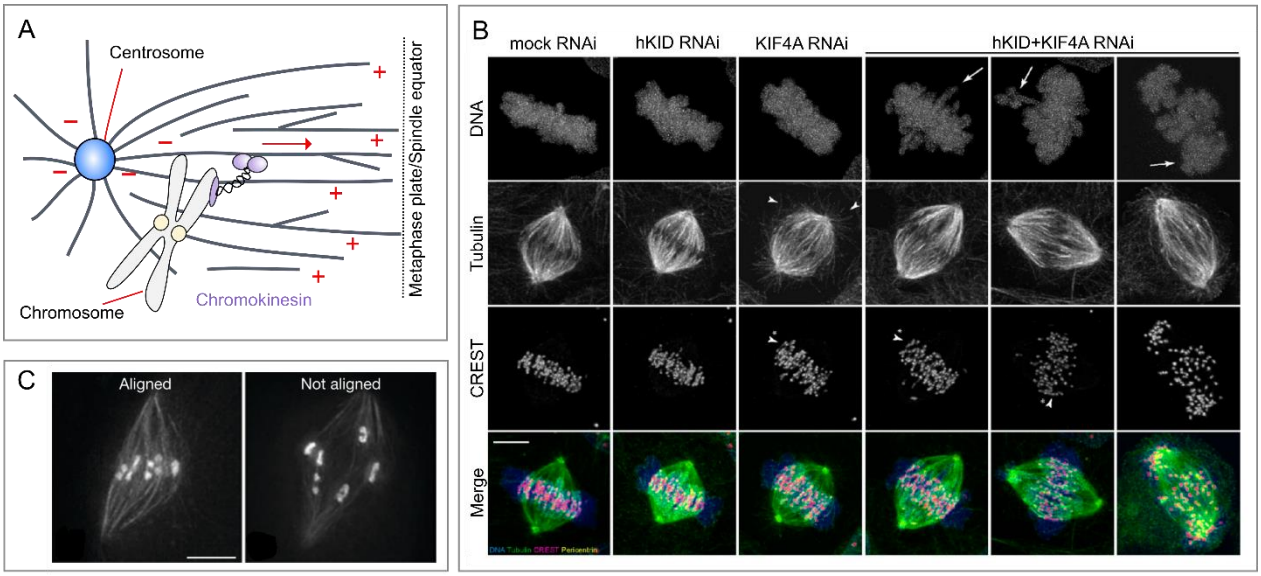

Figure 2.5. Chromokinesins exert forces on chromosomes to facilitate chromosome congression. (A) A schematic view of a chromokinesin bound to the chromosome arm, transporting the chromosome away from the centrosome and towards the metaphase plate. The arrow indicates the direction of chromokinesin motility. The polarity of MTs is indicated in red. Adapted from (David J. Sharp, Rogers and Scholey, 2000) **(B)** Images showing mitotic spindles in HeLa cells with MTs labeled in green, DNA in blue, KTs (CREST) in pink. Cells were either non-depleted (mock RNAi) or depleted for kinesin-10 (hKID RNAi), kinesin-4 (KIF4A RNAi), or both kinesins (hKID+KIF4A RNAi). Arrows point to misoriented chromosome arms and non-congressed chromosomes in hKID/KIF4A RNAi. Scale bar, 5 µm. (Wandke *et al.*, 2012) **(C)** Depletion of kinesin-4 KLP-19 in oocytes at MI provokes chromosomal misalignment at the spindle equator. Images show metaphase I spindles in *C. elegans* oocyte with fluorescently labelled tubulin and histones. Left image is a spindle in control oocytes (mock RNAi), and the right in oocyte depleted for KLP-19 (KLP-19 RNAi). Scale bar, 5 µm (Wignall and Villeneuve, 2009)**.**

Another kinesin-4 family chromokinesin, KLP-19, produces PEFs, and its deletion leads to aberrant chromosome poleward movements in *C.elegans* mitosis (Powers *et al.*, 2004). Proof that chromokinesins produce PEFs comes from observations of perturbed chromosome oscillations and metaphase alignment upon the disruption of kinesin-10 Kid in human cell lines and Xkid in

Xenopus egg extracts (Antonio *et al.*, 2000; Funabiki and Murray, 2000; Levesque and Compton, 2001). In human cells, kinesin-10 contributed the majority of PEFs while kinesin-4 stabilized MTs and reduced MT dynamics (Figure 2.5B) (Brouhard and Hunt, 2005; Wandke *et al.*, 2012).

Similarly to the effect observed in mitosis, absence of PEFs due to KLP-19 knockdown leads to chromosome alignment defects in oocyte MI (Figure 2.5C) (Wignall and Villeneuve, 2009), although a subsequent study did not confirm this role (Dumont, Oegema and Desai, 2010). In female *Drosophila* meiosis, orphan kinesin Nod was proposed to produce PEFs required to align chromosomes IV, which seldom recombine, but not the other chromosomes in meiosis or in mitosis (Zhang *et al.*, 1990; Theurkauf and Hawley, 1992). Alternatively, it was also proposed that Nod could act through its cross-linking activity as a break, as a chromosome tension regulator, or as an intermediate in chromosomal cohesion (Matthies, Baskin and Hawley, 2001).

In mitosis, computational analysis in the *Drosophila* embryo spindle estimated PEFs to be approximately 1.1 pN per MT (Marshall *et al.*, 2001). This value corresponds to both MT polymerization driven mechanism of PEF generation (Chapter 2.1.3. Astral MTs) and motor-generated PEF production. Research on the scope of PEFs produced per MT on a bivalent has not been performed.

2.4. Motor proteins

Dyneins and kinesins are motor proteins that bind and hydrolyse adenosine triphosphate (ATP) and translate that energy into movement along the MT lattice. MT-dependent motors are involved in a myriad of cellular functions, ranging from organelle transport to cell division and motility. Motor proteins exploit the polar nature of the MT in order to move along the lattice in a directed fashion. In general, the majority of kinesins move towards the plus-end of the MT, while kinesin-14s and

dyneins move toward the minus-end. It is well established that motors can produce force through binding and sliding of MTs or through modifying MT dynamics, and therefore play an integral role in the spindle force network. In the following sections, I will focus on data available on motor proteins that have functional homologues in fission yeast, and which play a role in fission yeast cell division processes during prophase and metaphase.

2.4.1. Dynein

Dyneins can be classified in two distinct subgroups: the axonemal and cytoplasmic dynein. The axonemal dynein is involved in ciliary and flagellar movements, and is not involved in spindle function. The cytoplasmic dynein can be further divided into dynein-1 and dynein-2, of which the latter is concerned with intra-flagellar transport. In this chapter, I will focus on cytoplasmic dynein-1 and its roles in the spindle force production.

Dynein-1 is an approximately 1.4 MDa complex composed of multiple polypeptide chains, and associates with numerous additional proteins and protein complexes to accomplish its cellular activities (Cianfrocco *et al.*, 2015). The stem is required for dimerization and for binding of other dynein accessory subunits such as intermediate chains, light intermediate chains, and light chains (Figure 2.6A). Two dynein heavy chains carry 6 AAA+-ATPase modules that make the motor-domain (Neuwald *et al.*, 1999). The motor and the tail domain are joined by a linker, an element required for dynein motility (Burgess *et al.*, 2003; Reck-Peterson *et al.*, 2006). The MT binding site is located on the N-terminal domain of dynein, the stalk, that extends from the motor domain (Gee, Heuser and Vallee, 1997; Koonce, 1997). Moreover, the tail domain also mediates interactions with cargo adaptor and dynein regulator proteins, which decide the function of dynein in the cell.

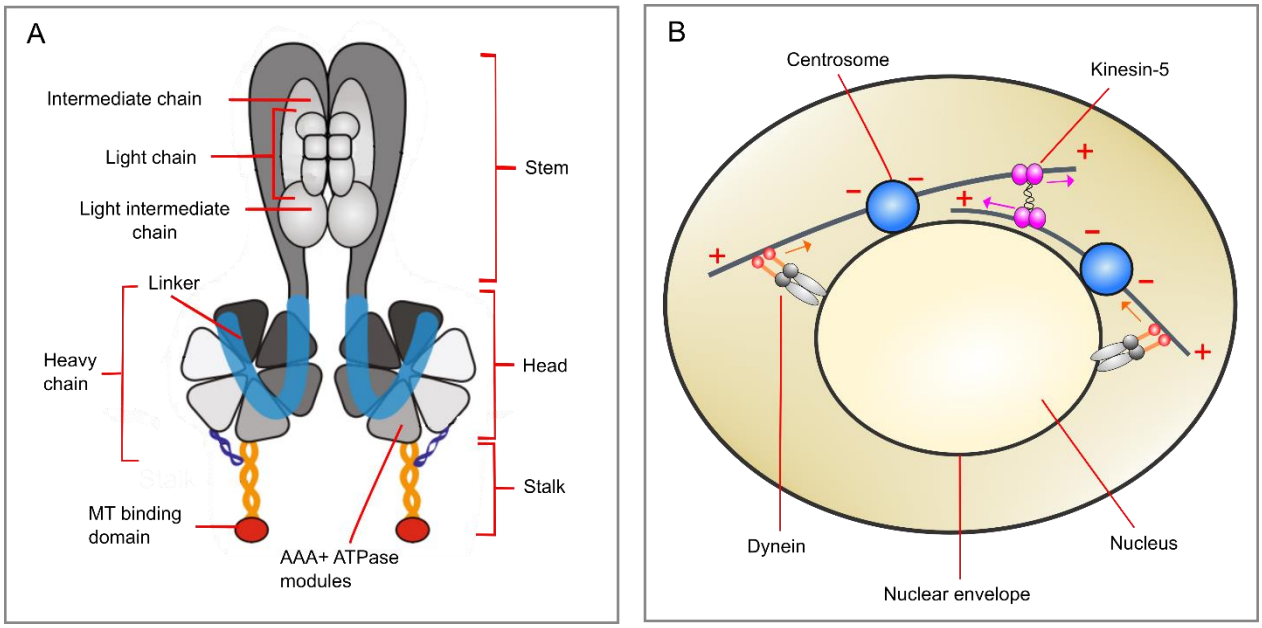

Figure 2.6. Dynein can induce centrosome separation. (A) A scheme of dynein-1 composition. Modified from (Raaijmakers and Medema, 2014) **(B)** An illustration of NE-bound dynein promoting centrosome separation. The arrows indicate the direction of kinesin-5 (pink) and dynein (orange) motility. MT polarity is indicated in red. Adapted from (Raaijmakers *et al.*, 2012).

As a result of interacting with a variety of adaptor proteins, dynein is found throughout the cell, from the nuclear envelope (NE) and cell cortex to MTs and KTs (Raaijmakers & Medema, 2014). In most systems, kinesin-5 is essential for spindle assembly and bipolarity establishment through centrosome separation. Some studies in mitotic and MII spindles have shown that dynein inhibition permits bipolar spindle assembly in kinesin-5 depleted systems, and that dynein might counteract kinesin-5 outward pushing forces (Mitchison *et al.*, 2005; Tanenbaum *et al.*, 2008; Chalovich and Eisenberg, 2013). Additionally, NE associated dynein was found to promote centrosome separation in *C. elegans* and *Drosophila* systems (Gönczy *et al.*, 1999; Robinson *et al.*, 1999). More recent studies, performed in a human cell line in which spindle assembly is independent of kinesin-5 activity, have further demonstrated the role of dynein-dependent pulling forces exerted on centrosomes in their separation (Figure 2.6B) (Raaijmakers *et al.*, 2012). Dynein mediated pulling

was also proposed to move chromosomes towards the spindle pole in prometaphase (Sharp et al., 2000; Yang et al., 2007).

2.4.2. Kinesin-5

Kinesin-5s are generally MT plus-end directed homotetramers, although there is experimental proof of kinesin-5 minus-end directed movement as well (Yajima *et al.*, 2003; Gerson-gurwitz *et al.*, 2011; Roostalu *et al.*, 2011; Winters *et al.*, 2019). Each monomer consists of the N-terminal head domain containing the motor, a coiled-coil stalk, and a C-terminal tail domain (Cole *et al.*, 1994; Kashina *et al.*, 1996). Such a structure allows kinesin-5 to crosslink and slide apart antiparallel MTs in the initial stages of spindle assembly, thereby producing outward pushing forces necessary to achieve spindle bipolarity (Figure 2.7A) (Kashina *et al.*, 1996; Kapitein *et al.*, 2005). Kinesin-5 localizes to the centrosomes and spindle MTs, which is consistent with its ability to bind both parallel and antiparallel MT bundles (Kapitein *et al.*, 2005; van den Wildenberg *et al.*, 2008).

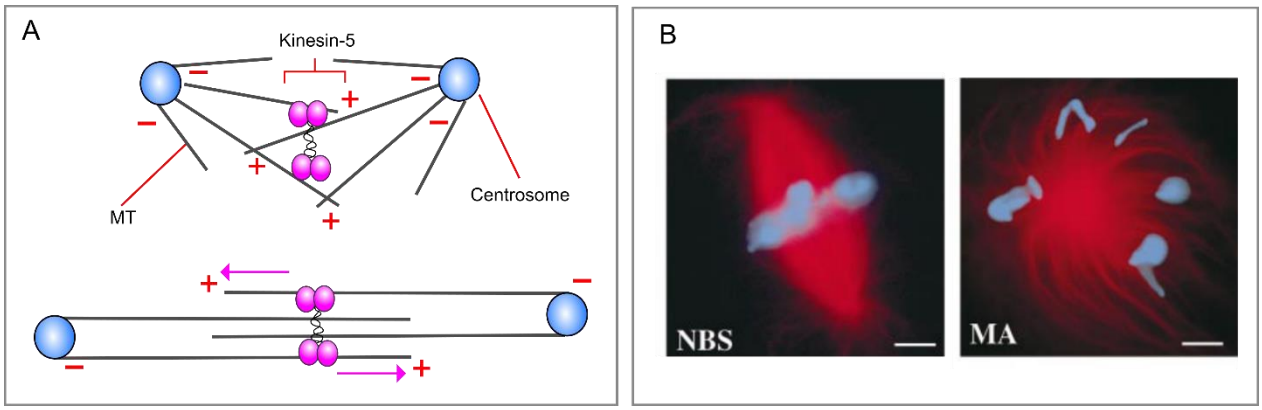

Figure 2.7. Kinesin-5 is essential for spindle bipolarity. (A) A simple model of kinesin-5 dependent spindle bipolarity establishment. In the initial stages of spindle assembly, kinesin-5 cross-link antiparallel MTs. Cross-linked anti-parallel MTs are subsequently pushed apart, thereby also distancing the spindle poles. **(B)** Chemical inhibition of kinesin-5 activity with monastrol in *Xenopus* egg extracts perturbs bipolar spindle assembly. Left image shows a normal bipolar spindle (NBS) and the right image shows a monoastral (monopolar) spindle (MA). Tubulin is shown in red and chromatin in blue. Scale bar, 5 µm (Kapoor *et al.*, 2000).

Kinesin-5s are essential in many systems, as their inactivity leads to formation of monopolar spindles and centrosome separation failure (Figure 2.7B) (Kashina, Rogers and Scholey, 1997; Kapoor *et al.*, 2000). Among the first kinesin-5s discovered were the one in fungus *A. nidulans* (Enos and Morris, 1990) and fission yeast (Hagan and Yanagida, 1990). In the following years kinesin-5s were identified in *Xenopus, Drosophila,* and human (Sawin *et al.*, 1992; Heck *et al.*, 1993; Blangy *et al.*, 1995). Interestingly, kinesin-5 BMK-1 mutants of *C. elegans*, or CaKip1 deficient *C. albicans,* do not show monopolar spindles, suggesting that in these organisms kinesin-5 is not essential for the establishment of spindle bipolarity (Bishop, Han and Schumacker, 2005; Shoukat, Frazer and Allingham, 2019).

Kinesin-5 plays a role in meiosis as well, implied by immunostaining of Eg5 on meiotic spindles in *Xenopus* (Houliston *et al.*, 1994). Similarly to mitosis, inhibition of Eg5 in mouse oocytes leads to an increase in frequency of monopolar spindles in MI, while also interfering with chromosome alignment at the metaphase plate and causing multipolar spindle assembly (Mailhes, Mastromatteo and Fuseler, 2004). Treatment of a human oocyte arrested in MII with monastrol, kinesin-5 inhibitor, switched the MII spindle from bipolar to monopolar state, (Duncan, Hornick and Woodruff, 2012), further indicating that kinesin-5 operates in meiotic spindles. Differently, knockdown of kinesin-5 in *Drosophila* oocytes did not result in monopolar, but in asymmetric spindles, e.g. one half-spindle showed a higher tubulin intensity (Radford, Go and McKim, 2017). The mechanism behind this result is not identified, but it is interesting to speculate that kinesin-5 may regulate MT nucleation as it was shown in fission yeast (Olmsted *et al.*, 2014).

2.4.3. Kinesin-8

Kinesin-8s are MT plus-end directed dimers implicated in MT dynamics regulation (Figure 2.8A) (Shrestha *et al.*, 2019). The motor domain is located at the N-terminal part of the protein, connected

to the C-terminal tail through a stalk domain that allows dimerization. The C-terminal tail binds cargo, and includes an additional MT binding site (Weaver *et al.*, 2011). Kinesin-8s were shown to localize to MT plus-ends *in vivo* (Mayr *et al.*, 2007; Tanenbaum *et al.*, 2011).

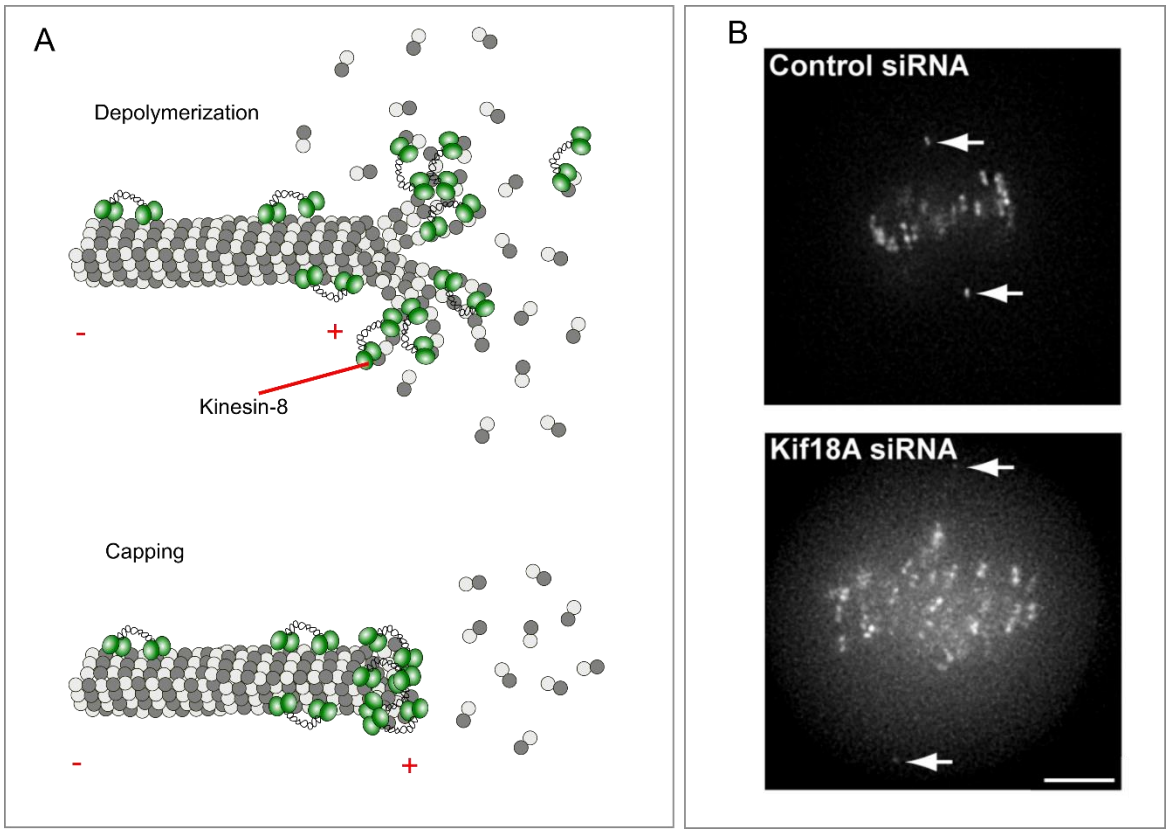

Figure 2.8. Kinesin-8s regulate MT dynamics. (A) An illustration of two possible models of kinesin-8-dependent MT depolymerization. In the upper illustration, kinesin-8 walks along the MT lattice towards the plus-end, where it actively depolymerizes MTs. In the lower illustration, kinesin-8 suppresses MT dynamics by capping the plus-end of the MT. The kinesin-8 cap would eventually trigger catastrophe and MT shrinkage. Adapted from (Shrestha *et al.*, 2019). **(B)** Images of control and Kif18A (kinesin-8) depleted HeLa cells (Kif18A siRNA) with fluorescently labelled spindle poles and centromeres. The pole-to-pole distance is increased upon Kif18A inhibition. Scale bar, 5 μm (Stumpff *et al.*, 2008).

Research done in mitotic systems has demonstrated that kinesin-8 proteins function as MT depolymerases (Figure 2.8A), and complete their function in a length-dependent manner (Varga *et al.*, 2009; Stout *et al.*, 2011; Tanenbaum *et al.*, 2011). Fission yeast kinesin-8 family members were proposed to rather destabilize MTs, as there is evidence for a Klp5/Klp6 dependent increase in

catastrophe frequency as a fuinction of MT length (Tischer, Brunner and Dogterom, 2009), and they did not show depolymerization activity *in vitro* (Erent, Drummond and Cross, 2012). Additionally, there is evidence that budding yeast kinesin-8 can cross-link and slide MTs (Su *et al.*, 2013; Rizk *et al.*, 2014). Likely due to its roles in MT dynamics regulation, inactivation of kinesin-8s disrupts chromosome congression in several systems (Figure 2.8B) (West, Malmstrom and McIntosh, 2002; Stumpff *et al.*, 2008; Savoian and Glover, 2010). Moreover, spindle length is typically affected upon kinesin-8 disruption, so that the spindles in mutants are longer (West, Malmstrom and McIntosh, 2002; Mayr *et al.*, 2007; Stumpff *et al.*, 2008; Weaver *et al.*, 2011). The observed phenotypes point to kinesin-8s as players in spindle length maintenance.

Kinesin-8 function is explored to a modest extent in meiotic systems. Studies performed in *Drosophila* spermatocytes recapitulate roles for kinesin-8 in meiotic spindle length control and chromosome congression (Gandhi *et al.*, 2004; Savoian *et al.*, 2004). In *Xenopus*, kinesin-8 was shown to accumulate in oocytes as they progress through meiosis, and its depletion results in longer MII spindles with unfocused spindle poles (Möckel *et al.*, 2017). Further, fission yeast kinesin-8 mutants fail to progress through typical meiosis, evident by aberrant spore production (West *et al.*, 2001). No studies were done on kinesin-8 functions in acentrosomal MI spindles, but considering the available data, it is plausible to assume kinesin-8 could be an important player in MI spindle assembly/function, and further studies may be beneficial.

2.4.4. Kinesin-14

Kinesin-14s are dimeric MT minus-end directed motors (McDonald, Stewart and Goldstein, 1990). Like other kinesins, they consist of a globular head with a motor domain, a central coiled-coil stalk region, and a tail domain, with the distinction of the motor domain being C-terminal and the tail

domain N-terminal (Figure 2.9A) (Karabay and Walker, 1999). They contain two MT binding sites – one ATP dependent in the motor domain, and one ATP independent in the tail domain.

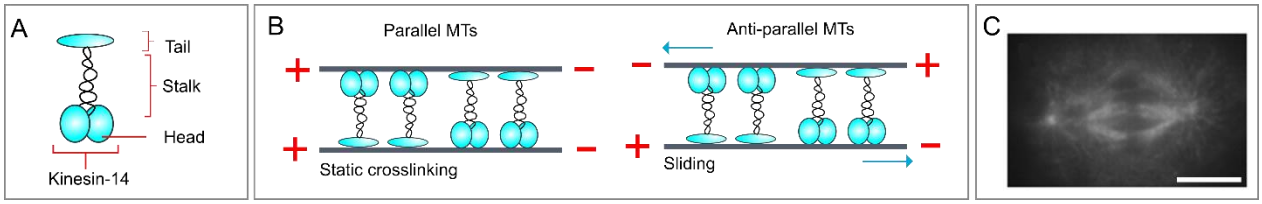

Figure 2.9. Kinesin-14 is a minus-end directed motor capable of bundling MTs. (A) Representation of kinesin-14 structure. **(B)** Kinesin-14 motors bind MTs by their motor- and tail-domains. Kinesin-14 can either statically cross-link parallel MTs (left), or slide anti-parallel MTs (right). The arrows indicate the direction of kinesin-14 motility. MT polarity is shown in red. Adapted from (She and Yang, 2017) **(C)** Fluorescently labelled *Drosophila* kinesin-14 Ncd localizes to the spindle MTs and spindle poles in S2 cells. Scale bar, 5 μm (Goshima, Nédélec and Vale, 2005).

Kinesin-14s are capable of bundling parallel and anti-parallel MT bundles, as well as sliding MTs (Figure 2.9B) (Oladipo, Cowan and Rodionov, 2007; Furuta and Toyoshima, 2008; Fink *et al.*, 2009). In mitotic cells, kinesin-14 localizes to the spindle poles and the spindle MTs (Figure 2.9C) (Walczak, Verma and Mitchison, 1997; Goshima, Nédélec and Vale, 2005). This is in line with its reported role in organizing and focusing the spindle poles (Walczak, Verma and Mitchison, 1997; Goshima, Nédélec and Vale, 2005; Syrovatkina and Tran, 2015). Upon inhibition of kinesin-14 activity, spindle length increases in some systems (Troxell *et al.*, 2001; Brust-Mascher and Scholey, 2002), but shortens in others (Cai *et al.*, 2009; Syrovaktina, Fu and Tran, 2013). These results portrait the specificity of each system, and may be a consequence of disruption of other kinesin-14 dependent functions, a distinct cellular response to kinesin-14 absence, or even existence of multiple kinesin-14s in a cell.

Data on kinesin-14 function in acentrosomal MI spindle assembly are limited to studies in *Drosophila* oocytes, where Ncd mutants had defective spindle structure with unfocused spindle

poles (Hatsumi and Endow, 1992). Further studies expanded on this findings, showing that MI spindles in which kinesin-14 activity is compromised form multipolar MI spindles, fail to elongate to wild-type (*wt*) MI spindle length, and either fail to achieve bipolarity, or are unable to maintain it (Matthies *et al.*, 1996; Endow and Komma, 1997). A model of acentrosomal MI spindle assembly relying on Ncd was also proposed (Sköld, Komma and Endow, 2005). A compelling study in mouse oocytes has shown that increasing the amount of kinesin-14 in MI results in a mitotic-like spindle assembly and erroneous chromosome segregation (Bennabi *et al.*, 2018). Available data indicates kinesin-14 may play a crucial role in MI spindle assembly in a dose-dependent way, while not being essential in mitotic spindle assembly.

2.5. Non-motor microtubule associated proteins (MAPs)

Spindle function and structure does not depend solely on motor proteins and MTs. The spindle and its components are coordinated by proteins that control cell-cycle progression, regulate motor function or promote MT nucleation (Manning and Compton, 2008). Among many regulatory and accessory proteins associated with the spindle and its components, non-motor MAPs are tasked with providing structural integrity to the spindle by crosslinking parallel or anti-parallel MT bundles. In this chapter, I will discuss non-motor proteins in the context of spindle structure and architecture.

Cross-linker proteins of the MAP65 family are arguably the most prominent spindle structure maintaining proteins besides kinesin-5. Mammalian member of the MAP65 family, PRC1, is a homodimer with four distinct domains: a dimerization domain, a rod domain, a spectrin domain, and a C-terminal domain (Figure 2.10A) (Subramanian *et al.*, 2010; Kellogg *et al.*, 2016). MAP65 members are crucial elements of the spindle midzone, for they crosslink iMTs, and support the

spindle structure (Figure 2.10B) (Mollinari *et al.*, 2002; Kurasawa *et al.*, 2004; Loïodice *et al.*, 2005). Mitotic spindles in cells deficient for MAP65 frequently break upon anaphase onset, producing two unorganized half-spindles.

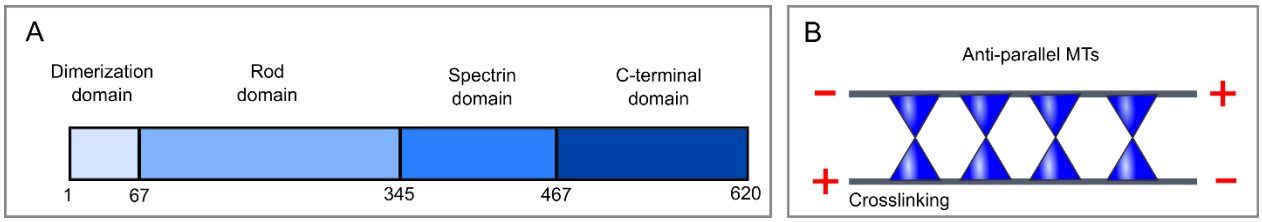

Figure 2.10. MAP65 members are cross-linker MAPs. (A) An illustration of domain structure of full-length PRC1. Numbers indicated below represent amino-acid residues. Adapted from (Kellogg *et al.*, 2016). **(B)** MAP65 members cross-link anti-parallel MTs at the spindle midzone. MT polarity is shown in red.

Depletion of SPD-1 in *C. elegans* female meiosis showed no defect in MT bundle formation, arguing for a secondary role of SPD-1 in MT bundling (Verbrugghe and White, 2004; Mullen and Wignall, 2017). Considering the spindle localization of MAP65 members during prometaphase in mammalian and fission yeast cells (Mollinari *et al.*, 2002; Fu *et al.*, 2010; Polak *et al.*, 2017), there is a possibility that MAP65 might be an essential player in meiotic spindle integrity maintenance in systems other than *C. elegans*. A recent study published by the Kitajima group has found that Prc1 accumulates at the kinetochore during MI, and is required for correct chromosome segregation. Additionally, such accumulation was not detected in human oocytes, where chromosome segregation is especially error-prone (Yoshida *et al.*, 2020).

3. Non-centrosomal pathways of spindle assembly

As mentioned earlier (Chapter 1.2. and Chapter 2.2.), many female oocytes lack centrosomes, yet manage to nucleate MTs and assemble a spindle. Moreover, mitotic cells in which centrosomes are ablated, or mutants that lack centrosomes, still assemble a mitotic spindle. Regardless of

differences, it appears that MT nucleation is mediated by γ-tubulin in all pathways. In the following chapter, I will address the most prominent centrosome-independent spindle assembly pathways.

3.1. Ran-GTP pathway.

The small GTPase Ran exists in a gradient around the chromosomes in mitosis and meiosis, and acts as an activator of spindle assembly factors (Figure 3.1). Ran is active in its GTP-bound form and inactive in its guanosine diphosphate (GDP)-bound form. Ran-GTP transition is promoted by chromatin associated RCC1, so the concentration of Ran-GTP is higher in the chromatin vicinity (Carazo-Salas *et al.*, 1999). Studies in *Xenopus* egg extract have shown that RCC1 coupled to a bead is capable of organizing MTs into a bipolar network around the bead (Halpin *et al.*, 2011). In contrast, Ran-GTP to Ran-GDP transition is promoted by the cytoplasmic RanGAP. Therefore, RCC1 and RanGAP form a gradient of Ran-GTP, with Ran-GTP enriched around the chromatin, and reducing in concentration away from it (Kaláb, Weis and Heald, 2002; Kaláb *et al.*, 2006).

Ran-GTP promotes spindle assembly by activating spindle assembly factors, most notably TPX2 (Figure 3.1) (Gruss *et al.*, 2001, 2002). TPX2 then activates Aurora A kinase which phosphorylates the γTuRC adaptor protein NEDD1 and initiates MT nucleation (Pinyol, Scrofani and Vernos, 2013; Scrofani *et al.*, 2015). Perturbations of Ran-GTP in *Xenopus* extracts result in loss of MT density in spindles, but do not show a great impact on spindle structure in centrosome containing mitotic cells (Kaláb *et al.*, 2006). This hints that Ran-GTP pathway might be more important in acentrosomal spindle assembly than the one relying on centrosomes.

In mouse oocytes, reduced or heightened levels of Ran-GTP did not prevent MI spindle assembly or functionality, likely because MI spindle assembly in mouse oocytes relies on acentriolar MTOCs (aMTOCs) (Dumont et al., 2007; Schuh & Ellenberg, 2007). Similarly, Ran-GTP is not required

for MI spindle assembly in *Xenopus* or *Drosophila* oocytes (Cesario and McKim, 2011). These data point to the existence of another essential spindle assembly mechanism, while Ran-GTP mostly contributes to the speed and efficiency of MI spindle assembly in oocytes. At the same time, MII spindle defects were much more pronounced upon Ran-GTP inactivation, and accompanied by chromosome segregation defects, suggesting a critical role of Ran-GTP pathway in MII spindle function.

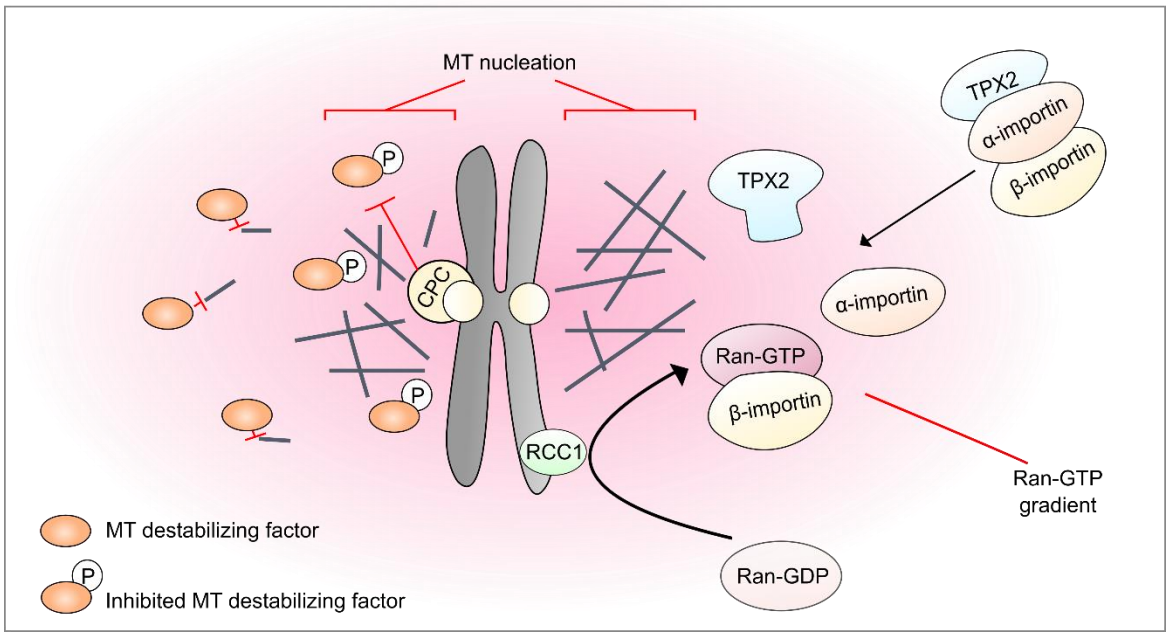

Figure 3.1. Chromosome associated pathways of spindle assembly. On the left side of the chromosome, the chromosomal passenger complex (CPC) dependent pathway of spindle assembly is illustrated. CPC is at the KT, where one of its components, Aurora B, phosphorylates and thereby inactivates MT-destabilizing factors. This results in an environment being formed around the KT that promotes MT stabilization. On the right side of the chromosome, the Ran-GTP pathway of spindle assembly is illustrated. Chromatin bound Rcc1 locally enriches the area around chromatin with Ran-GTP. In turn, Ran-GTP activates spindle assembly factors, like TPX2, by releasing them from importins. Adapted from (Gruss, 2018) and (Meunier and Vernos, 2016).

Blocking Ran-GTP dependent spindle assembly pathway delayed MT nucleation and hindered MI spindle assembly in human oocytes, suggesting its necessity for successful meiosis (Holubcová *et*

al., 2015). However, oocytes used in this study did not progress through meiosis upon hormonal cues, and may therefore behave differently than healthy human oocytes.

3.2. Chromosomal passenger complex (CPC) pathway

A second chromatin associated pathway of spindle assembly, which can operate separately of the Ran-GTP pathway, is called the CPC pathway (Maresca *et al.*, 2009). The CPC consists of (human/fission yeast) INCENP/Pic1, Aurora B/Ark1, Survivin/Bir1/Cut17 and Borealin/Nlb1 (Carmena *et al.*, 2012; van der Waal *et al.*, 2012). The mechanism of CPC-dependent assembly pathway is well explored in *Xenopus*, and involves Aurora B-dependent inhibition of MT-destabilizing agents, which in turn creates an environment where MT assembly is promoted (Figure 3.1) (Andrews *et al.*, 2004; Lan *et al.*, 2004; Sampath *et al.*, 2004; Gadea and Ruderman, 2006; Kelly *et al.*, 2007).

In *Drosophila* oocytes, CPC pathway is essential for acentrosomal spindle assembly, as its suppression prevents MI spindle assembly (Colombié *et al.*, 2008; Radford, Jang and McKim, 2012). In *C. elegans,* CPC was found to contribute to chromosome alignment in meiosis, the release of cohesion, and the proper assembly of the meiotic (Kaitna *et al.*, 2002; Rogers *et al.*, 2002; Wignall and Villeneuve, 2009; Dumont, Oegema and Desai, 2010). As Ran-GTP, inactivation of the CPC-pathway through INCENP knockdown, or chemically by inactivating Aurora B/C, did not abolish MI spindle assembly in mouse oocytes, but it did provoke chromosome misalignment at meiotic metaphase (Shuda *et al.*, 2009; Sharif *et al.*, 2010).

In other studies utilizing mouse oocytes, MI spindles were aberrant and disorganized in Aurora B and C mutants (Yang *et al.*, 2010; Balboula and Schindler, 2014). Discrepancies in obtained results may reflect the robustness of the methods or the targets used for the inhibition of the CPC-pathway.

Alternatively, they could be a consequence of redundancy, as multiple Auroras co-exist in mice. Indeed, Aurora A can take over Aurora B/Aurora C roles in mouse oocytes when both are inactivated (Nguyen *et al.*, 2018). In any case, further studies in model organisms and human oocytes are mandatory.

3.3. Acentriolar MTOCs (aMTOCs)

aMTOCs were discovered in mouse oocytes (Calarco, Donahue and Szollosi, 1972), and are of significant importance in MI spindle assembly (Namgoong, Kim and Christenson, 2018). Their structure and function is still under investigation, but it has been established that they associate with canonical PCM proteins such as γ-tubulin, pericentrin and NEDD1 (Gueth-Hallonet *et al.*, 1993; Carabatsos *et al.*, 2000; Ma, Baumann and Viveiros, 2010).

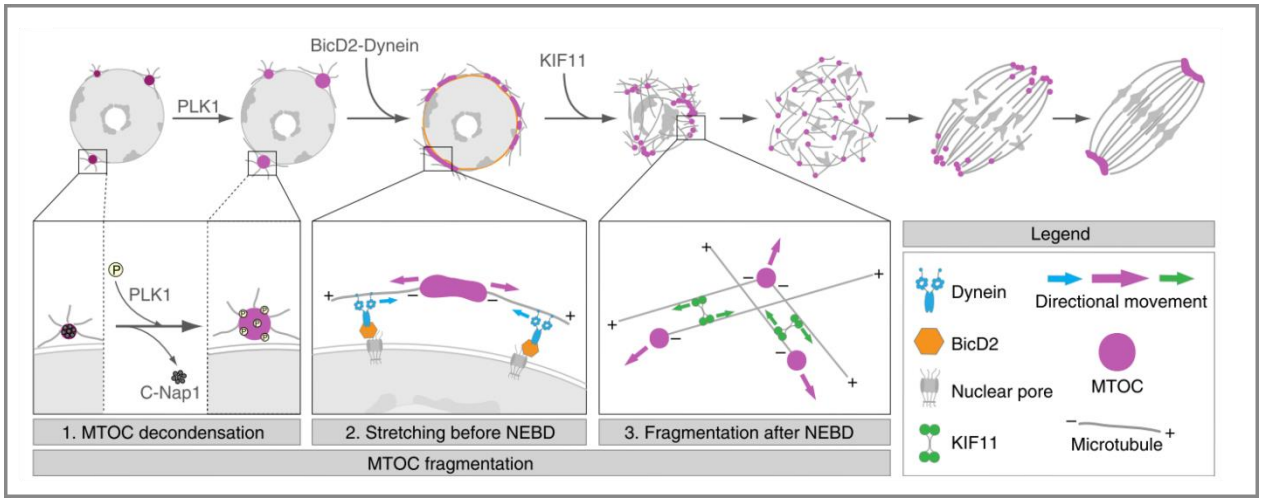

Figure 3.2. A model for bipolar spindle assembly in mouse oocytes. First, PLK1 triggers a decondensation of MTOC structure. Secondly, BicD2 recruits dynein to the NE, which stretches the MTOCs into ribbons and fragments them. Thirdly, kinesin-5 KIF11 further fragments the MTOCs so that they can be arranged between the spindle poles (Clift and Schuh, 2015).

aMTOC-dependent spindle assembly is well characterized in mouse oocytes where three crucial steps were observed (Figure 3.2) (Clift and Schuh, 2015). Mouse oocytes contain multiple aMTOCs

before NEBD (Clift and Schuh, 2015), which are first decondensed by PLK1. Next, dynein and MTs stretch aMTOCs and split them into pieces along the NE (Łuksza *et al.*, 2013). After NEBD, kinesin-5 further fragments aMTOCs into smaller foci. It appears that aMTOC fragmentation is required to achieve MI spindle bipolarity, as spindle assembly is defective when PLK1 decondensation of aMTOCs is inhibited (Clift and Schuh, 2015). Following NEBD, a MT ball is formed through Ran-GTP pathway, and then organized into a stable central array (Brunet *et al.*, 2008; Clift and Schuh, 2015), along which aMTOCs are sorted to the spindle poles (Breuer *et al.*, 2010).

It remains to be explored whether such a mechanism of spindle assembly functions in human oocytes. Studies conducted so far did not report aMTOCs in human atretic oocytes (Holubcová *et al.*, 2015). Whether they are present in healthy human oocytes is unknown.

3.4. Augmin pathway

MTs can be nucleated within the mitotic spindles from pre-existing MTs (Mahoney *et al.*, 2006), and the complex responsible for it is the Augmin complex (Goshima *et al.*, 2008). Augmin is not required for the initial spindle assembly, but rather to enrich spindles with MTs. Augmin promotes MT nucleation from pre-existing MTs by recruiting γ-tubulin in several mitotic systems, including human cell lines (Goshima *et al.*, 2007; Uehara *et al.*, 2009), and was also shown to recruit γ-TuRC to MTs *in vitro* (Song *et al.*, 2018).

Augmin deficiency lowers MT density within the spindle, compromises spindle bipolarity, and results in chromosome misalignment (Lawo *et al.*, 2009; Uehara and Goshima, 2010). In *Xenopus* MII arrested extracts, both Augmin and Ran-GTP target TPX2 to initiate nucleation of branching

MTs (Petry *et al.*, 2013). Because of that finding, and the reported co-immunoprecipitation of Augmin with TPX2 (Petry *et al.*, 2013), it was suggested that the two pathways are linked.

In *Drosophila* oocytes mutated for Augmin, chromosomes often misalign, but initial spindle assembly is not perturbed (Meireles *et al.*, 2009). Instead, Augmin is required for MT assembly near the acentrosomal MI spindle poles (Colombié *et al.*, 2013). Augmin pathway is functional in male meiotic spindle assembly, but Augmin localizes to the KTs instead on the spindle MTs (Savoian and Glover, 2014). The contribution of Augmin pathway obviously differs from system to system, so it would be most beneficial to expand studies on it in MI spindles of other species, like *Xenopus*, and ultimately, in human oocytes.

4. Fission yeast as model system for analysing mitotic and meiotic spindle dynamics

Fission yeast is a single cell eukaryotic rod-like organism that typically reaches 12-14 μm in length at mitotic division (Figure 4.1A). Because of the simplicity of its MT cytoskeleton, the evolutionary conservancy of major players in spindle assembly and function, and the fact that, as in higher eukaryotes, multiple MTs attach to a KT (Ding, McDonald and McIntosh, 1993), it makes for an exceptional model organism to study the spindle in mitosis and meiosis.

When in nutrient-rich conditions, fission yeast mostly lives as a haploid, and undergoes mitosis in order to proliferate. Like mammalian cells, it shows the typical progression of cell cycle stages: G1, S, G2 and M (Figure 4.1A) (Mitchison and Creanor, 1971; Nurse, Thuriaux and Nasmyth, 1976). Under starvation conditions, most notably nitrogen starvation, fission yeast exits the vegetative cell cycle and enters the meiosis pathway (Figure 4.1B) (Harigaya and Yamamoto, 2007). Two different mating types can be distinguished in fission yeast cells: h^+ and h^-. If the

neighbouring cells are of opposite mating type, and are starved of nitrogen, they can fuse and form a zygote. The diploid zygote may either proliferate as a diploid, if nutrient-rich medium becomes available, or continue with meiotic divisions. Upon meiosis completion, the zygote will have formed four haploid nuclei that are encapsulated by a forespore membrane into four haploid spores. Once conditions are favourable, the haploid spores can germinate and commence the vegetative cell cycle anew. Although nitrogen starvation induces conjugation of haploid cells into zygotes, haploid fission yeast cells can also continue to proliferate by mitosis. This allows studying both cell division types simultaneously, under the same conditions.

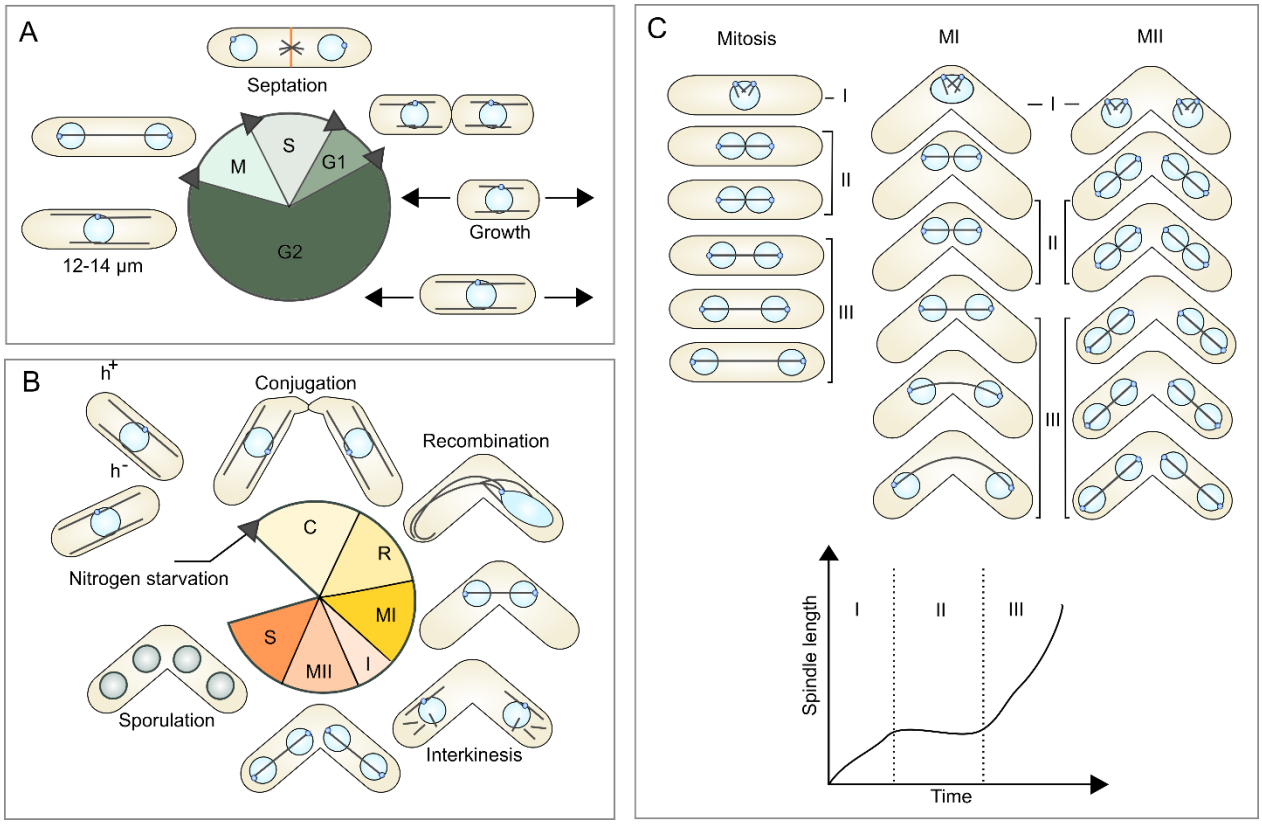

Figure 4.1. Fission yeast is a good tool to comparatively study mitotic and meiotic spindles. (A) Vegetative life cycle of haploid fission yeast cells. In an exponentially growing culture at 25°C it takes approximately 3 hours to complete one cycle. Fission yeast spends the majority of the cell-cycle in G2 phase. S phase, when DNA duplicates, takes places simultaneously with septation, and G1 phase is almost unnoticeable. **(B)** Meiotic life cycle of fission yeast. Two haploid fission yeast cells of opposite mating types can fuse into a zygote when starved for nitrogen. The DNA undergoes recombination and two successive

meiotic divisions. Meiosis ends with four haploid nuclei encapsulated into four haploid spores. **(C)** Spindle dynamics commonly show three phases of elongation. Upper illustration shows fission yeast cells in mitosis and meiosis, with phases of spindle elongation indicated on the right side (mitosis) and in between the cells (meiosis). The lower part depicts a spindle dynamics plot that shows spindle length through time. Dotted lines indicate transition between three phases of spindle elongation.

Spindle assembly and elongation are stereotypical in fission yeast, in both mitosis and meiosis, and consist of three distinct stages (Figure 4.1C) (Nabeshima, Nakagawa, Straight, Murray, *et al.*, 1998). Phase I of spindle assembly starts in prophase, and refers to the initial stages of spindle formation, e.g. bipolarity establishment. Phase II is marked by steady-state spindle length maintenance, accompanied by chromosome congression to the metaphase plate. At the end of phase II, sister-chromatids are separated to the opposite poles. Finally, in phase III, the spindle elongates rapidly and further separates the chromosomes.

4.1. Phase I – Initial stages of spindle nucleation

MT nucleation in fission yeast mitotic spindle assembly is mediated by the SPB, and dependent on γ-tubulin (Masuda and Shibata, 1996; Fong, Sato and Toda, 2010). Mitosis is closed in fission yeast, so to nucleate spindle MTs on the nuclear side, SPBs first have to be inserted into the NE in the process known as fenestration (Jaspersen and Ghosh, 2012; Cavanaugh and Jaspersen, 2017). Once inserted, SPBs can nucleate MTs through γ-TuC activity.

The γ-TuC in fission yeast is comparable in size and structure to the γ-TuRC of metazoans, and all of the homologous subunits have been identified in fission yeast (Vardy and Toda, 2000; Fujita *et al.*, 2002; Venkatram *et al.*, 2004; Anders, Lourenço and Sawin, 2006). γ-TuC components γ-tubulin/Gtb1, GCP2/Alp4 and GCP3/Alp6 are essential, and mutants defective for these proteins

assembled aberrant monopolar spindles (Horio *et al.*, 1991; Vardy and Toda, 2000). Interaction of MOZART1/Mzt1 with GCP3/Alp6 allows γ-TuC to be targeted to the SPBs (Figure 4.2) (Fong, Sato and Toda, 2010; Dhani *et al.*, 2013; Masuda *et al.*, 2013). Upon mitotic entry, CDK/Cdc2 phosphorylation of TACC/Alp7 triggers transport of the TACC-TOG/Alp7-Alp14 complex to the nucleus, where it interacts with γ-TuC to initiate MT nucleation and spindle assembly (Sato *et al.*, 2004; Okada *et al.*, 2014; Tang *et al.*, 2014; Flor-Parra, Iglesias-Romero and Chang, 2018; Yukawa, Kawakami, *et al.*, 2019).

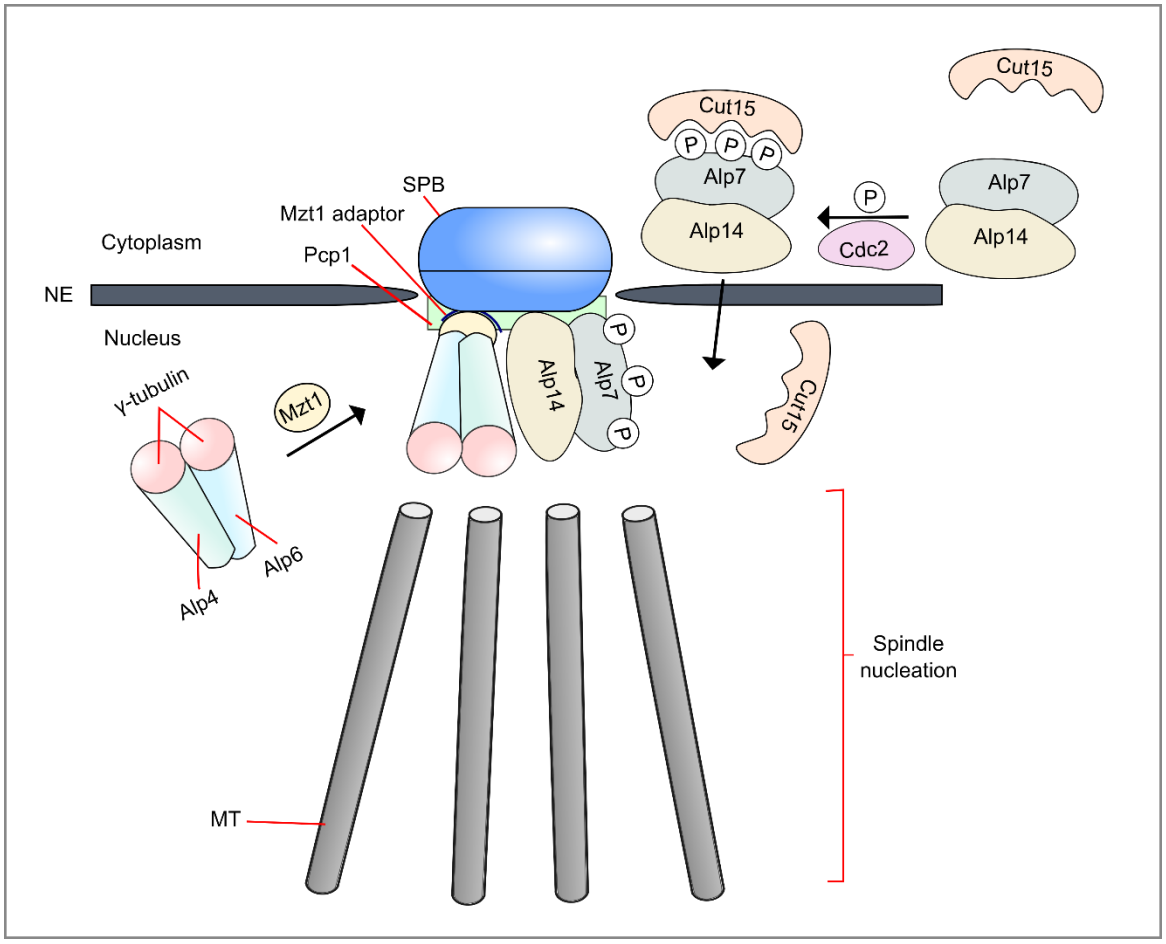

Figure 4.2. A simplified scheme showing spindle nucleation in fission yeast. Mzt1 forms a complex with γ-tubulin, Alp4, and Alp6 (that comprise the γ-TuC), and it is necessary for γ-TuC attachment to the SPBs. At mitosis, Alp7 in Alp7-Alp14 complex is phosphorylayed by Cdc2, which allows the interaction of Alp7-Alp14 complex with the importin Cut15. The complex is translocated to the nucleus where it is recruited to the SPB, among others, by Pcp1.

Ran-dependent spindle assembly pathway was also observed in fission yeast, albeit somewhat differently than described in the chapter 3.1. Firstly, as mitosis is closed in fission yeast, there is most likely no Ran-GTP gradient, and secondly, nucleation seems to be promoted at SPBs, and remains unproven around chromatin in mitosis (Okada & Sato, 2015). Fission yeast mutants for Ran-GTP/Sim1 and RCC1/Pim1 showed aberrant monopolar or star-shaped spindles, indicating impaired spindle assembly (Fleig et al., 2000; Hirose et al., 2006; Salus et al., 2002). TACC/Alp7 was shown to be the primary target of RCC1/Pim1 (Sato & Toda, 2007). Upon RCC1 inactivation, TACC/Alp7, and consequently TOG/Alp14, localization to the nucleus was prevented, and therefore nuclear mitotic spindle assembly was perturbed.

In meiosis of fission yeast, spindle assembles from the SPBs as well, although some of its components are modified from mitosis to meiosis (Ohta, Sato and Yamamoto, 2012). As research on MT nucleation in meiosis of fission yeast is lacking, presumably, a similar mechanism of γ-tubulin SPB-dependent MT nucleation operates in fission yeast meiosis as in mitosis. In support of this hypothesis, Gtb1 mutants were shown to assemble monopolar spindles in meiosis, accompanied by frequent chromosome mis-segregation (Tange *et al.*, 2004). Interestingly, a recent study has shown that a spindle can assemble in meiosis without SPB insertion into the NE (Pineda-Santaella and Fernández-Álvarez, 2019). Taken with the fact that Alp7/TACC, a target of RCC1/Pim1, localizes to the KTs at MI but not at mitotic entry (Kakui *et al.*, 2013), it is plausible to presume chromatin-dependent MT nucleation pathway may exist in MI of fission yeast.

4.2. Phase I – Establishment of a bipolar spindle

MT minus-ends are anchored at the SPBs (Toya *et al.*, 2007; Yukawa, Ikebe and Toda, 2015), while their plus-ends grow and explore the area around the SPBs. The growing MTs are bundled

together by fission yeast kinesin-5 Cut7, and it was proposed this is done by Cut7 rotating growing MTs and organizing them into antiparallel fashion (Hagan and Yanagida, 1990, 1992a; Winters *et al.*, 2019). Cut7 exerts forces on the antiparallel-bundle and slides the MTs apart, thereby pushing away the SPBs and establishing bipolarity. Cut7 is an essential protein, and mutants deficient for Cut7 assemble monopolar mitotic spindles (Figure 4.3A) (Hagan and Yanagida, 1990, 1992a). Cut7 localizes to the spindle poles and to the spindle MTs in the initial stages of spindle assembly in both mitosis and meiosis.

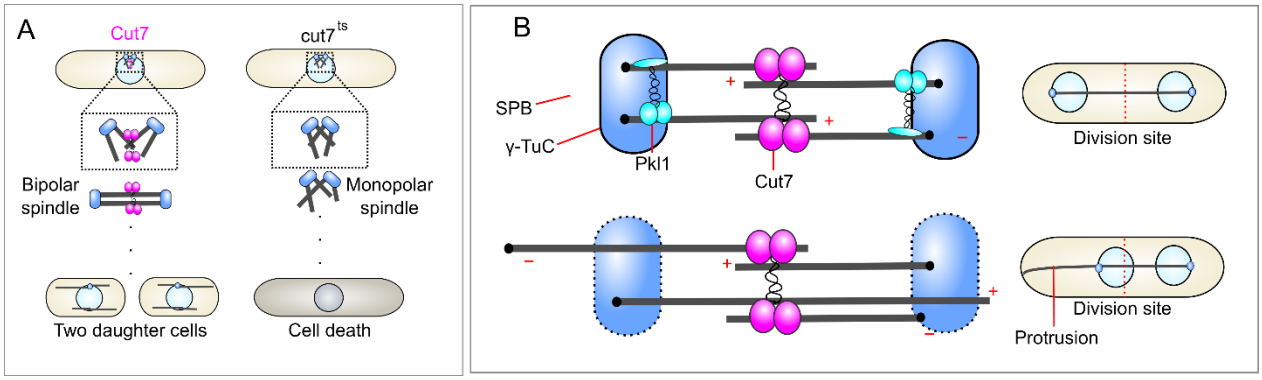

Figure 4.3. Cut7 establishes bipolarity, and Pkl1 organizes spindle poles. (A) Cut7 is essential for spindle bipolarity establishment in fission yeast. Cut7 mutants build a monopolar spindle, which ultimately leads to cell death. **(B)** An illustration of Pkl1 function at the spindle poles. Pkl1 localizes to the SPB, where it crosslinks parallel MTs. In cells lacking Pkl1, the spindle poles are unfocused. Cut7-dependent outward pushing forces can slide minus-end MTs outward from the unfocused poles, thus forming a long MT protrusion. The protrusion can push the chromosome to the cell division site, resulting in chromosome cut and aneuploidy. Adapted from (Syrovatkina and Tran, 2015).

Another player in the initial stages of spindle assembly in fission yeast is a minus-end directed motor protein, kinesin-14 Pkl1, which localizes to the SPBs and faintly to the spindle MTs at the initial stages of mitotic spindle assembly (Pidoux, LeDizet and Cande, 1996; Syrovatkina and Tran, 2015; Yukawa, Ikebe and Toda, 2015). Pkl1 forms a complex together with Msd1 and Wdr8, which is important in anchoring the spindle MTs to the SPBs (Toya *et al.*, 2007; Yukawa, Ikebe and Toda, 2015). Additionally, Pkl1 probably crosslinks parallel MT bundles to provide structural stability at

the spindle poles in view of outward force exerted by Cut7. In fission yeast cells lacking Pkl1 activity, spindle poles are unfocused, which is evident in Cut7-mediated MT protrusion formation, which may cause aneuploidy (Figure 4.3B) (Syrovatkina and Tran, 2015). After the report that immunostaining failed to show Pkl1 localization on spindle MTs in meiosis (Pidoux, LeDizet and Cande, 1996), its localization in meiosis was not further explored. However, it was reported that zygotes deleted for Pkl1 produce the typical four spores upon meiosis completion less frequently (Troxell *et al.*, 2001), arguing for a role of Pkl1 in meiosis.

Two other significant players in spindle bipolarity establishment are a MAP65 family cross-linker PRC1/Ase1 and CLASP/Peg1, a MT binding protein. Upon mitosis onset, Ase1 localizes around the SPBs, and once bipolarity is established, it localizes to the SPBs and to the spindle midzone, although its localization at the midzone is reduced compared to late mitosis (Loïodice *et al.*, 2005; Yamashita *et al.*, 2005; Fu *et al.*, 2010). Ase1 contributes to early spindle stability by recruiting Peg1, and is required for Cut7-independent spindle assembly (Bratman and Chang, 2007; Rincon *et al.*, 2017; Yukawa *et al.*, 2017). The role of Ase1 in the recruitment of Peg1 to early spindle midzone is somewhat controversial, as one study found that Peg1 localization to early spindle midzone was not dependent on Ase1 (Ebina, Ji and Sato, 2019), while another found that Peg1 localization required Ase1 (Rincon *et al.*, 2017). In any case, strains lacking Ase1 activity show defects in spindle integrity, but they are more pronounced in phases II and III than in phase I (Loïodice *et al.*, 2005; Yamashita *et al.*, 2005; Syrovaktina, Fu and Tran, 2013). Fission yeast Peg1 localizes to the mitotic spindle midzone, and mutants were shown to generate aberrant phase I mitotic spindles (Grallert *et al.*, 2006; Bratman and Chang, 2007). Interestingly, localization and function of these two molecules was not explored in fission yeast meiosis, except for a report that meiotic nuclear oscillations are perturbed in Ase1 deficient zygotes (Yamashita *et al.*, 2005).

4.3. PhaseI/phaseII – Chromosome attachment to the spindle and congression

In fission yeast mitosis, the chromosomes are grouped at the NE, and their KTs are associated with the SPBs (Figure 4.4A) (Hironori *et al.*, 1993). In meiosis, the telomeres are associated with the SPBs, and KTs are distal (Figure 4.4A) (Chikashige *et al.*, 1994; Fennell *et al.*, 2015). Once spindle MT nucleation begins, the growing plus-ends of MTs pivot, and explore the region around the SPBs searching for KTs in both mitosis and MI (Kalinina *et al.*, 2013; Cojoc *et al.*, 2016; Winters *et al.*, 2019).

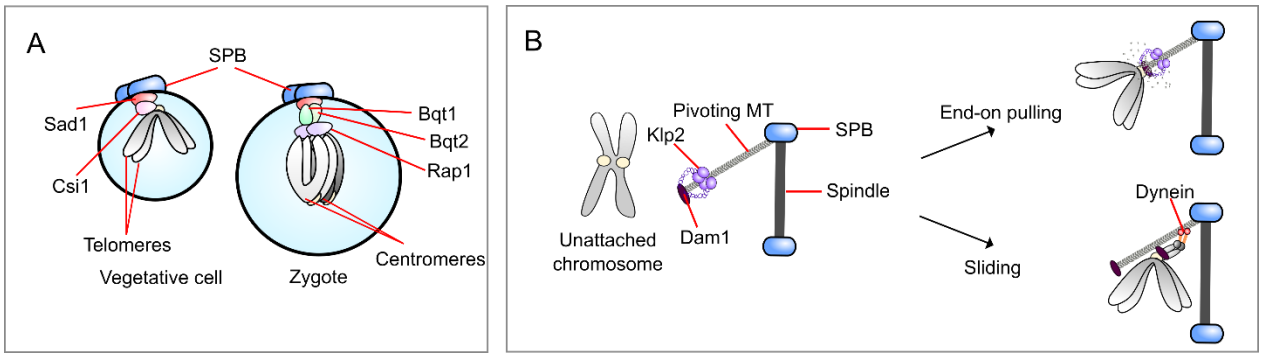

Figure 4.4. Chromosome localization at spindle assembly onset and chromosome recapture. (A) A depiction of chromosome orientation in mitosis and MI. In mitosis, the centrosomal region is localized below the SPBs, and the telomeres are distant, while in MI the orientation is opposite, resulting in a ‘’bouquet’’ organization. **(B)** A schematic representation of chromosome recapture in fission yeast by Dam1, Klp2 and dynein. End-on pulling was found to be the dominant mechanism, but lateral sliding also operates in the process of chromosome recapture. Adapted from (Gachet *et al.*, 2008).

In the process of mitotic chromosome bi-orientation, minus directed motors dynein/Dhc1 and Pkl1 play a role in promoting bi-orientation through their spindle pole organizing activities, but are not required for its establishment (Grishchuk, Spiridonov and McIntosh, 2007). Furthermore, another study found that dynein deficient cells show chromosome segregation defects, likely originating from faulty bi-orientation (Courtheoux *et al.*, 2007). Klp2, the second kinesin-14 in fission yeast, was found to associate with the KTs in mitosis (Troxell *et al.*, 2001), and to be important in KT

recapture and chromosome translocation to the SPBs through MT sliding and depolymerization together with Dam1 (Figure 4.4B) (Grishchuk and McIntosh, 2006; Franco, Meadows and Millar, 2007; Gachet *et al.*, 2008). That congression and bi-orientation of the chromosomes are not impeded in the absence of one or more fission yeast minus-end directed proteins, demonstrates this process is likely motor independent, and probably relies on MT dynamics (Grishchuk and McIntosh, 2006; Grishchuk, Spiridonov and McIntosh, 2007). Even though each homologous chromosome in a bivalent is attached to a single, opposite pole, the bivalents in MI are actually bi-oriented. However, the role of motors in this process in meiosis was not explored in fission yeast.

Chromokinesins have not been identified in fission yeast (Wood *et al.*, 2002), and astral MTs are absent until end of phase II/onset of phase III, when they are assembled on the cytoplasmic side of the nucleus (Hagan and Hyams, 1988). Therefore, PEFs probably do not operate in fission yeast spindles to drive chromosome congression. A hetero-complex of kinesin-8, Klp5/Klp6, was identified as a key complex in mitotic chromosome congression and bi-orientation, together with Dam1 (Garcia, Koonrugsa and Toda, 2002; West, Malmstrom and McIntosh, 2002; Sanchez-Perez *et al.*, 2005; Grissom *et al.*, 2009; Buttrick *et al.*, 2012; Mary *et al.*, 2015; Klemm *et al.*, 2018). Klp5/Klp6 localizes to KTs from phase I until the onset of phase III, when it localizes to the spindle midzone. It governs chromosome congression by controlling pulling forces in a length-dependent manner, i.e. accumulating at the plus-ends of long MTs, and promoting catastrophe (Figure 2.8A). Function and localization of Klp5/Klp6 in meiosis was not reported. It was reported that Klp5 and Klp6 appear to be essential for successful meiosis outcome, as zygotes defective for one or both molecules failed to produce the typical four spores (West *et al.*, 2001). As Klp5/Klp6 is not essential for vegetative growth and mitosis, it would be beneficial to explore what is the cause of their essentiality in meiosis.

4.4. Phase II – Spindle forces and force-balance maintenance in fission yeast

In phase II, the spindle maintains a constant length, while the KTs move back and forth along the spindle. The constant length of phase II spindle was observed in many other organisms and cell types, and was proposed to be the result of the balance of forces produced by spindle motors and MAPs (Saunders and Hoyt, 1992; Dumont and Mitchison, 2009; Goshima and Scholey, 2010; Syrovaktina, Fu and Tran, 2013).

In the fission yeast mitotic phase II spindle, kinesin-5 is recognized as a motor that produces outward pushing force. Its inactivation results in phase II spindle shortening and collapse (Syrovaktina, Fu and Tran, 2013). Regarding other motors and MAPs in fission yeast, spindle II length changes have been observed upon Pkl1 and Ase1 inactivation (shorter spindles), and Klp5/Klp6, Klp2, and Dam1 inactivation (longer spindles) (Troxell *et al.*, 2001; Garcia, Koonrugsa and Toda, 2002; Syrovaktina, Fu and Tran, 2013). Dam1 is a MAP conserved only in fungi, and is one of the subunits of the DASH complex that is associated with the KTs during mitosis (Liu *et al.*, 2005). It was suggested that its role in inward pulling force production in phase II mitotic spindles stems from its ability to convert MT depolymerization into chromosome movement (Gachet *et al.*, 2008; Grissom *et al.*, 2009).

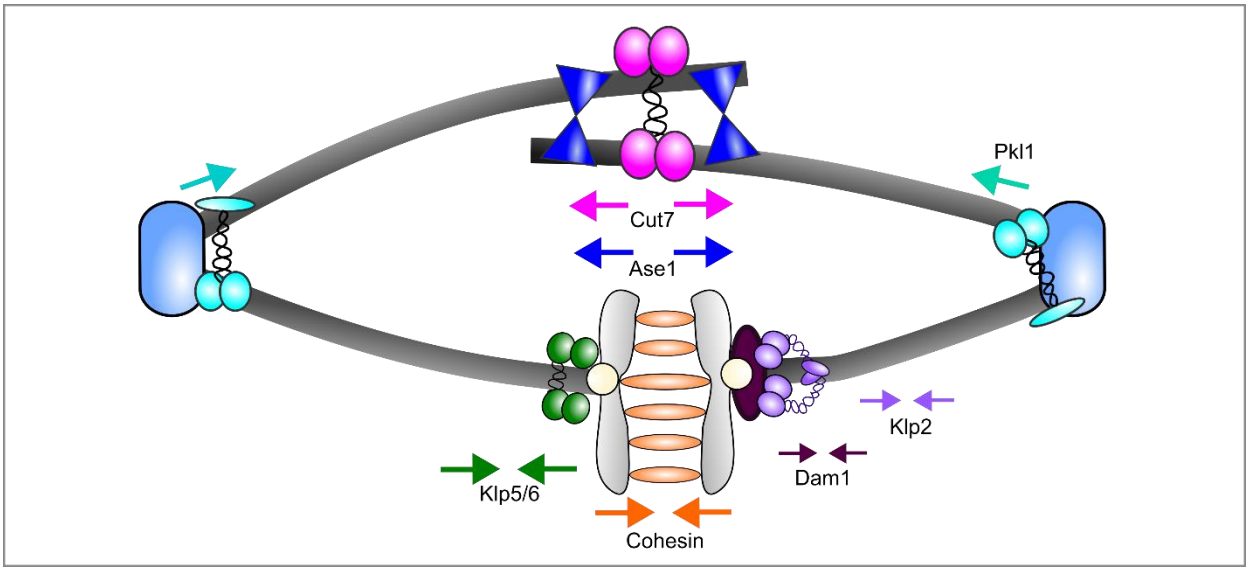

Figure 4.5. Simplified map of force producers in the fission yeast mitotic phase II spindle. In phase II, the spindle length is maintained constant. The activity of indicated motors and MAPs was experimentally shown to influence phase II spindle length *in vivo*. Additionally, simultaneous deletion of force generators producing opposite forces has been shown to restore phase II spindle length.

Interestingly, Pkl1 overexpression also results in shorter spindles, arguing for its role in production of inward pulling forces (Rincon *et al.*, 2017). Finding that Pkl1 inactivation results in shorter spindles could be the consequence of its role in spindle pole focusing and spindle assembly. Moreover, simultaneous deletion of Cut7 and Pkl1, or simultaneous overexpression, restores spindle bipolarity, thereby demonstrating these two proteins produce opposite forces and act antagonistically to maintain constant mitotic phase II spindle length (Olmsted *et al.*, 2014; Rincon *et al.*, 2017; Yukawa *et al.*, 2017). Similar antagonistic relationship was also observed for Klp5-Ase1 and Dam1-Ase1 (Syrovaktina, Fu and Tran, 2013).

KT-MT attachment and chromosome cohesion also influence mitotic phase II spindle length. Mutants for KT proteins Mis12, Mis6 and Mis4 all show longer metaphase spindles (Goshima, Saitoh and Yanagida, 1999). Chromosomes likely produce inward forces through tension production upon attachment of KTs to MTs. Constant metaphase spindle length and force-balance

maintenance are important in promoting correct KT-MT attachment and chromosome segregation (Choi and McCollum, 2012; Syrovaktina, Fu and Tran, 2013). Although necessary for precise chromosome segregation, force-balance, or the key molecules producing force in phase II meiotic spindles, have not been explored in fission yeast meiosis.

4.5. Phase III – Final spindle elongation

At the end of phase II, the genetic material is separated to the opposite sides of the cell as KT-MTs depolymerize (Nabeshima, Nakagawa, Straight, Murray, *et al.*, 1998; Ward *et al.*, 2014). In phase III, MTs resume growth and the spindle elongates rapidly.

The majority of anti-parallel MT sliding in phase III is carried out by the homo-tetrameric kinesin-6 Klp9, while Ase1 cross-links the anti-parallel MTs at the spindle midzone, and maintains the integrity of the spindle (Tolić-Nørrelykke *et al.*, 2004; Courtheoux *et al.*, 2009; Fu *et al.*, 2010; Krüger *et al.*, 2019; Yukawa, Okazaki, *et al.*, 2019). Cut7 was also shown to play a role in MT sliding, as its inactivity results in lower phase III elongation velocity (Rincon *et al.*, 2017; Krüger *et al.*, 2019; Yukawa, Okazaki, *et al.*, 2019). Additionally, minus-end directed Klp2 is important for final spindle elongation, as its persistence at the phase III spindle leads to lower spindle elongation velocity (Mana-Capelli *et al.*, 2012; Krüger *et al.*, 2019). Unfortunately, our understanding of meiosis spindle elongation is lacking, as no studies were performed to date.

4.6. Comparison of mitotic and meiotic spindle dynamics in fission yeast

Mitosis and meiosis have both been extensively studied, and many studies have investigated the roles of motors, MAPs and MT dynamics in the spindle. Despite the considerable amount of research, no studies to date have compared spindle dynamics simultaneously in mitosis and meiosis in the same system. This is most likely because such a task remains technically impossible in many

systems, or there are too many factors that would influence data interpretation. Additionally, such studies in humans are extremely complicated, because oocytes are regarded as precious material. Taking into account the fundamental nature of mitotic and meiotic processes, a comparison of mitotic and meiotic spindle dynamics would be central in exploring the differences and peculiarities in each cell division type respectively.

Fission yeast has emerged as a good model system for such a study. Its MT cytoskeleton is simple and well characterized, and the key molecular players in spindle assembly are conserved from fission yeast to human. Importantly, fission yeast can undergo mitosis and meiosis in the same environment, and under the same conditions, making comparative study of mitotic and meiotic spindle dynamics feasible.

Aim of this work

Force-balance maintenance in phase II spindle, and components producing forces in the spindle are very well explored in fission yeast mitosis. Additionally, spindle dynamics in *wt* and mutant vegetative cells have been measured and studied. However, the corresponding data for meiotic spindles is lacking. Moreover, no study was conducted to compare mitotic and meiotic spindle dynamics in the same organism, and to reveal how and why cells modify spindles from mitosis to meiosis.

The antagonistic relationship of Cut7 and Pkl1 is extensively investigated in fission yeast mitotic spindles. That Pkl1 can bypass Cut7 essentiality is explained by its ability to generate forces in the spindle that are opposite to Cut7-dependent forces (Rincon *et al.*, 2017; Yukawa *et al.*, 2017). Additionally, it was proposed that Pkl1 may inhibit MT nucleation, and that Cut7 counters this function (Olmsted *et al.*, 2014). More recent studies have further suggested that another fission yeast kinesin-14, Klp2 may collaborate with Pkl1 in antagonizing Cut7 (Yukawa *et al.*, 2018).

We discovered that while *cut7Δpkl1Δ* mutants complete mitosis, they fail to successfully complete meiosis, evident by their failure to produce typical four spores. This finding raised the possibility of the spindle not being functional in meiosis of *cut7Δpkl1Δ*, although it is functional in mitosis, and provided us with a tool to study differences in mitotic and meiotic spindles in fission yeast. Taking into consideration that function of motors and MAPs has not been thoroughly explored in fission yeast meiosis, I have set out to investigate why *cut7Δpkl1Δ* mutants fail to complete meiosis, and what is the difference between mitotic and meiotic spindle dynamics in fission yeast.

Results

The results obtained in this study are presented in the form of an article entitled: *Kinesin-14s and microtubule dynamics define fission yeast mitotic and meiotic spindle assembly and elongation.* This article is published in the Journal of Cell Science.

Kinesin-14s and microtubule dynamics define fission yeast mitotic and meiotic spindle assembly and elongation.

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Key words: Spindle, mitosis, meiosis, microtubule, kinesin, fission yeast

Running title: Mitotic and meiotic spindle analysis.

Summary

Chromosome segregation requires the microtubule-based spindle in both mitosis and meiosis. We report that kinesin-14s and microtubule dynamics function differently between mitosis and meiosis.

Abstract

To segregate the chromosomes faithfully during cell division, cells assemble a spindle that captures the kinetochores and pulls them towards opposite poles. Proper spindle function requires correct interplay between microtubule motors and non-motor proteins. Defects in spindle assembly or changes in spindle dynamics are associated with diseases like cancer or developmental disorders. Here we compared mitotic and meiotic spindles in fission yeast. We show that even though mitotic and meiotic spindles undergo the typical three phases of spindle elongation, they have distinct features. We found that the relative concentration of kinesin-14 Pkl1 is decreased in meiosis I compared to mitosis, while the concentration of kinesin-5 Cut7 remains constant. We identified the second kinesin-14 Klp2 and microtubule dynamics as factors necessary for proper meiotic spindle assembly. This work defines differences between mitotic and meiotic spindles in fission yeast, and provides prospect for future comparative studies.

Introduction

Cell division is an essential feature of all living organisms. Mitosis allows cell renewal and amplification by producing two daughter cells of the same ploidy as the mother cell. Meiosis promotes genetic mixing within species by producing four gametes of halved ploidy (Page and Hawley, 2003). In both cases, faithful chromosome segregation is crucial for a successful outcome of cell division. To accomplish this, cells build a spindle – a complex molecular machine that is comprised of microtubules (MTs), motors, non-motor MT-associated proteins (MAPs) and other regulatory proteins.

As it is well recognized that irregularities in spindle dynamics can result in chromosome segregation errors, potentially leading to cancer, congenital diseases and cell death (Holland and Cleveland, 2009; Thompson, Bakhoum and Compton, 2013), knowing the similarities and/or differences in spindle dynamics between mitosis and meiosis in the same organism may help our understanding of these processes (Ohkura, 2015). Fission yeast is a simple, genetically tractable organism with many of its genes conserved in humans. Importantly, fission yeast can complete mitosis and meiosis in the same environment. This allows robust simultaneous study of both processes and provides an opportunity to understand the intrinsic differences in their spindle dynamics.

In fission yeast, the initial assembly of the mitotic spindle occurs in phase I, during prophase/prometaphase, when nucleation of MTs from the spindle pole body (SPB, equivalent of centrosome) starts inside the nucleus (Fig. 1A). MTs are anchored to the SPBs via their minus-ends, which are bundled and focused by the kinesin-14 Pkl1 (Grishchuk, Spiridonov and McIntosh, 2007; Syrovatkina and Tran, 2015; Yukawa, Ikebe and Toda, 2015). Plus-ends of MTs originating

from the opposite SPBs grow and interact through motors and MAPs. Spindle bipolarity is achieved when interdigitating MTs are cross-linked and organized into antiparallel arrays by the kinesin-5 Cut7 (Hagan and Yanagida, 1992b; Sharp *et al.*, 1999; Kapitein *et al.*, 2005), inducing the separation of spindle poles. Spindle bipolarity then ensures the segregation of genetic material to the opposite sides of the cell. The bipolar spindle grows in length until metaphase/anaphase A (phase II), when a constant spindle length is reached and maintained by a "force-balance" mechanism (Saunders and Hoyt, 1992; Goshima *et al.*, 2005; Syrovaktina, Fu and Tran, 2013). The forces involved in maintaining the spindle length during chromosome congression originate from the spindle midzone (kinesin-5), the spindle poles (kinesin-14, dynein), the kinetochores (kinesin-8) and from the cohesion between the sister chromatids (cohesin) (Saunders and Hoyt, 1992; Pavin and Tolić, 2016). When all kinetochores are captured by MTs and the chromosomes are bi-oriented (sister-kinetochores are attached to the opposite poles), the spindle assembly checkpoint (SAC) is satisfied (Musacchio and Salmon, 2007). This activates an endopeptidase separase which abolishes the cohesion between the sister-chromatids and triggers chromatid disjunction (Uhlmann, Lottspeich and Nasmyth, 1999; Yanagida, 2000). In anaphase B (phase III), the spindle elongates, thus segregating the chromatids into two daughter cells which keep the same ploidy as the mother cell.

Haploid fission yeast cells can undergo mitosis, or, in nutrient-poor conditions, cells of opposite mating type can fuse and form a zygote (Fig. 1B). Zygote undergoes two meiotic divisions and produces four quiescent haploid spores. Meiotic spindle assembly is preceded by the so-called nuclear horsetail movement – long range oscillations led by the SPB which promote meiotic recombination (Chikashige *et al.*, 1994; Yamamoto *et al.*, 1999; Miki *et al.*, 2002). Differently than in mitosis, the homologous chromosomes are linked through the reciprocal recombination

(chiasmata) and form bivalents during the first meiotic division (MI). Due to specific meiotic cohesion and an altered kinetochore architecture (Watanabe and Nurse, 1999; Yokobayashi and Watanabe, 2005; Hauf *et al.*, 2007; Sakuno, Tada and Watanabe, 2009), the homologous chromosomes in the bivalent mono-orient (sister-kinetochores attach to the same pole). This, along with step-wise removal of cohesion by separase (Kitajima, Kawashima and Watanabe, 2004; Miyazaki *et al.*, 2017), results in segregation of homologues into daughter cells and halved ploidy in the two nuclei produced by the end of MI. In the second meiotic division (MII), the kinetochores have a mitotic-like architecture, resulting in the segregation of sister-chromatids into daughter cells, similarly to mitosis. The four haploid nuclei produced by the end of MII are finally encapsulated into spores by growth of the forespore membrane around them (Nakamura-Kubo et al., 2003).

In most organisms, including fission yeast, kinesin-5 deficiency leads to mitotic monopolar spindles and failure to segregate the chromosomes (Hagan and Yanagida, 1990; Kapoor *et al.*, 2000; Goshima *et al.*, 2005; Olmsted *et al.*, 2014). It was recently reported that force-balance and spindle bipolarity are restored in fission yeast cells defective simultaneously for kinesin-5 Cut7 and kinesin-14 Pkl1 (Olmsted *et al.*, 2014; Rincon *et al.*, 2017; Yukawa *et al.*, 2018). In this work, we show that, in contrast to what happens in mitosis, fission yeast zygotes deficient for Cut7 and Pkl1 fail to assemble a functional MI spindle, and instead persist in a monopolar state. While the total amounts of all spindle components tested in this work scale up from mitosis to MI, the total amount of kinesin-14 Pkl1 remains constant. The second fission yeast kinesin-14, Klp2, additionally antagonizes Cut7 in MI and its deletion rescues the Cut7-independent bipolar spindle assembly. Finally, we report that MTs are more dynamic in zygotes than in vegetative cells. This suggests altered MT dynamics regulation between mitosis and meiosis, which also impacts the ability to assemble a functional spindle in the absence of Cut7 and Pkl1. Our data point out precise

adaptations in meiotic spindle dynamics that fine tune chromosome segregation, despite similarities with mitotic spindles.

Results

Spindle dynamics differ in mitosis and meiosis in fission yeast

To assess spindle dynamics in mitosis and meiosis, we imaged wild-type (*wt*) fission yeast cells expressing α2-tubulin tagged with mCherry (mCherry-Atb2) and the SPB component Sid4 tagged with GFP (Sid4-GFP), cultured in malt-extract (ME) medium to induce mating. Fission yeast MTs reorganized at the end of vegetative interphase (IP) to build a mitotic spindle, with typical three-phase dynamics (Fig. 1C) (Nabeshima, Nakagawa, Straight, Chikashige, *et al.*, 1998). Mitotic spindle studies were traditionally performed in rich medium (YE5S). Therefore, we checked if mitotic spindle dynamics are comparable in YE5S and ME medium (Fig. S1). We found that they are similar, except that the spindle elongation velocity in phase III is slightly slower in cells undergoing mitosis in ME medium, which results in ~10% longer mitosis duration (YE5S: 27±2 min; ME: 30±3 min). In zygotes (Fig. 1D), MI and MII spindles exhibited a three-phase progression similar to mitosis. MI spindle disassembly was followed by interkinesis (IK), a phase of rest between two meiotic divisions where MTs are cytoplasmic and undergo dynamic instability, analogous to vegetative IP. Two MII spindles then formed synchronously in phase I of MII, and meiosis ended with four distinctly separated SPBs. Parameters defining spindle dynamics during mitosis, MI and MII are provided in Table 1.

The final spindle lengths at each of the three phases, as well as the duration of phases, differed between mitosis, MI and MII. We next measured spindle dynamics over time (Fig. 1E). MI final spindle length was increased by 25% on average compared to mitotic spindles, and MI spindle

elongation velocity in phase III was faster than in mitosis. In contrast to vegetative mitotic cells, zygotes spent the largest portion of spindle elongation time in phase II of MI and MII. Finally, zygotes spent a similar proportion of time in phase I of MI and MII, but the velocity of spindle assembly in zygotes was lower than in mitotic cells. These differences in spindle dynamics implied qualitative and/or quantitative changes in spindle components, such as motors and MAPs, or changes in MT dynamics between mitosis and meiosis.

Chromosome segregation is severely compromised in *cut7Δpkl1Δ* zygotes

Kinesin-14 Pkl1 and kinesin-5 Cut7 are antagonistic motors involved in mitotic spindle assembly. While Cut7 deletion leads to monopolar spindles and is non-viable (Hagan and Yanagida, 1990), the simultaneous deletion of Cut7 and Pkl1 resulted in the formation of a short bipolar spindle in mitosis, capable of segregating the chromosomes (Syrovatkina and Tran, 2015; Rincon *et al.*, 2017; Yukawa *et al.*, 2018). However, when these mutants were mated, their zygotes failed to produce the typical four spores (20±1% zygotes with four spores; Fig. 2A, Fig. S2A), consistent with a recent report (Shirasugi and Sato, 2019). This was suggestive of a problem in meiotic chromosome segregation. We imaged *wt* and *cut7Δpkl1Δ* cells expressing GFP-Atb2 and Hht1-mCherry (histone H3) to visualize chromosome segregation. While the mitotic spindle in *cut7Δpkl1Δ* cells was capable of segregating the DNA into two separate pools of equal size (Fig. 2B), meiotic chromosome segregation was perturbed, and three distinct mutant phenotypes in *cut7Δpkl1Δ* meiosis could be detected (Fig. 2C). The first subset of zygotes managed to segregate the DNA mass in MI, but one of the MII spindles failed to complete DNA separation, resulting in three DNA masses upon MII completion (22% of zygotes). In the second subset of zygotes, the spindle appeared to elongate, as if the zygotes entered phase III of MI, but without segregating the DNA mass. A spindle assembled again, this time separating the DNA into two separate masses (45% of

zygotes). Finally, in the third subset, the spindle completely failed to separate the DNA mass, resulting in one DNA mass at the end of meiosis (9% of zygotes). These observed errors in chromosome segregation could explain the abnormal number of spores at the end of meiotic divisions.

Spindle integrity is compromised specifically in MI of the *cut7Δpkl1Δ* zygotes

To study spindle dynamics in *cut7Δpkl1Δ* meiosis, we imaged *cut7Δpkl1Δ* zygotes expressing mCherry-Atb2 and Sid4-GFP. Contrary to mitosis, where all of the *cut7Δpkl1Δ* cells assembled a bipolar spindle, *cut7Δpkl1Δ* zygotes failed to do so in meiosis (Fig. S2B-D): 74±2% of *cut7Δpkl1Δ* zygotes failed to establish a bipolar spindle and separate the SPBs during MI, consistent with a recent report (Shirasugi and Sato, 2019). In addition, among the zygotes that had assembled bipolar spindles in MI, another 8±2% failed to assemble a spindle in MII. Unsuccessful SPB separation in MII could result from errors in the preceding MI, or from the intrinsic properties of *cut7Δpkl1Δ* MII spindles.

In *wt* zygotes, there are two SPBs at the end of MI, which duplicate, and MII ends with four SPBs (Fig. 1D). In the *cut7Δpkl1Δ* zygotes which have initially failed to assemble a spindle, we have observed a second spindle assembly onset accompanied by an increase in SPB number (Fig. S2C, far right panel). An increase in SPB number after MI spindle failure could indicate that the SPBs in *cut7Δpkl1Δ* zygotes were duplicated a second time, and that *cut7Δpkl1Δ* zygotes entered MII despite chromosome segregation failure in MI. We confirmed this result by imaging *wt* and *cut7Δpkl1Δ* zygotes expressing Cdc13-GFP (cyclin-B) and mCherry-Atb2. In *wt* zygotes, Cdc13-GFP localization was visible in the nucleus, on the spindle, and the SPBs (Izawa *et al.*, 2005; Fu *et al.*, 2010). Upon phase III onset in MI, Cdc13-GFP signal was partially degraded (Fig. S2E). The

pool of Cdc13-GFP was replenished upon MII phase I onset, and was degraded again upon phase III onset of MII. Similar dynamics of Cdc13-GFP were visible in *cut7Δpkl1Δ* zygotes that formed monopolar spindles, except that Cdc13-GFP degradation relative to spindle assembly onset was delayed 16 minutes on average in *cut7Δpkl1Δ* MI (Fig. S2F). This suggests that the spindle assembly checkpoint (SAC) is active, but leaky, in *cut7Δpkl1Δ* zygotes, ultimately allowing progression through the meiotic stages regardless of spindle or chromosome segregation defects.

We then compared *wt* and *cut7Δpkl1Δ* spindle dynamics. In mitosis, pre-phase III spindles were shorter and phase III onset was delayed in the *cut7Δpkl1Δ* mutant compared to *wt* (Fig. 2D), which is in agreement with previous studies (Rincon *et al.*, 2017; Yukawa *et al.*, 2018). Similar results were observed for the MII spindle dynamics (Fig. 2E). In contrast, most MI spindles remained monopolar. The 25% of MI *cut7Δpkl1Δ* spindles that managed to achieve bipolarity and separate the SPBs, did not show the usual three-phase progression of spindle elongation (Fig. 2F), as phase I and II of spindle elongation could not be distinguished. Combined, our data indicate that spindle assembly and elongation are specifically perturbed during MI of *cut7Δpkl1Δ* zygotes.

We next analyzed the polarity of MT protrusions that formed in MI monopolar spindles by checking the localization of the MT-plus end tracking protein Mal3-GFP (EB1) (Busch and Brunner 2004). The analysis revealed that *cut7Δpkl1Δ* zygotes formed monopolar protrusions with distal MT-plus ends (Fig. S2G). This phenotype is similar to the phenotype of mitotic cells lacking Cut7 (Hagan and Yanagida, 1990).

We conclude that Pkl1 deletion cannot fully compensate for the absence of Cut7 in MI. This suggests that Cut7 and Pkl1 are differently regulated between mitosis and MI.

The ratio of Cut7-to-Pkl1 is higher in MI than mitosis spindles

To assess if Cut7 and Pkl1 are differently regulated in mitosis and MI, we imaged Cut7 or Pkl1 tagged with GFP together with the MTs tagged with mCherry in *wt* mitosis and MI. We first checked the impact of the GFP tag on motor functionality. Cut7 deficiency is lethal in fission yeast (Hagan and Yanagida, 1992b), resulting in cell death when Cut7 is inactive or inhibited. Cells expressing Cut7-GFP were viable. Of 45 observed zygotes with Cut7-GFP, 11% showed a collapsed MI spindle, and only 1 vegetative cell of more than 100 observed showed a monopolar spindle in mitosis. Tagging Cut7 with resulted in less zygotes producing the typical 4 spores (Fig. S3A; 91% compared to the 97% for the strain with no GFP tag), and no difference in gro*wt*h was observed in serial dilution plate assay (Fig. S3B). Mitotic and MI spindle dynamics of the strain with the tagged Cut7 are comparable to the strain with no GFP tag (Fig. S3C,D). The results indicate that the GFP tagged version of Cut7 is mostly functional.

Pkl1 deficiency was reported to lead to protrusions in phases II and III in 85% of the mitotic cells grown in YE5S medium (Syrovatkina and Tran, 2015). We have observed no protrusions in mitotic cells with 3XGFP tagged Pkl1 grown in YE5S. In ME medium, we have observed 13% of mitotic cells with protrusions in phase III and all of the zygotes showed protrusions in phase III of MI. Despite protrusions in phase III of MI, labelling Pkl1 with 3XGFP had no significant effect on spore production (Fig. S3A; 94% compared to the 97% for the strain with no GFP tag) or any consequence on the growth in serial dilution plate assay (Fig. S3B). Mitotic spindle dynamics of the strain with the Pkl1-3XGFP were similar to the dynamics of the strain with no GFP tag (Fig. S3E), while MI spindle dynamics showed a longer phase II duration and a shorter phase II spindle in MI (Fig. S3F). The results indicate that the 3XGFP tagged version of Pkl1 is mostly functional.

Despite slight changes regarding spindle dynamics, as well as observed protrusions and lower percentage of zygotes with 4 spores, suggesting a partial but not complete loss of functionality, no

changes have been observed in phase I. Therefore, we conclude that Cut7-GFP and Pkl1-3XGFP can be used to investigate the localization and recruitment of Cut7 and Pkl1 during phase I in both mitosis and MI.

Similar to previous observation (Hagan and Yanagida, 1992b), Cut7-GFP localized to the spindle and the spindle poles from spindle assembly to spindle disassembly in both mitosis and MI (Fig. 3A). Similarly, the localization pattern of Pkl1-3XGFP appeared unchanged between MI and mitosis. Pkl1-3XGFP localized to the spindle poles shortly after spindle assembly started, and remained associated with the SPBs until spindle disassembly. It was also detected on the spindle in phase I to phase II transition (Fig. 3B).

We then measured Cut7-GFP and Pkl1-3XGFP signal distribution along the spindles during phase I to phase II transition by linescan analysis, 1 minute before the spindle reached a constant length that marks the beginning of phase II.

Both Pkl1-3XGFP and Cut7-GFP displayed maximum intensity peaks at the start and at the end of the linescan, which corresponds to the spindle poles. Cut7-GFP maximum intensity was considerably higher in MI (Fig. 3C), while Pkl1-3XGFP maximum intensity was similar in mitosis and MI (Fig. 3D). Moreover, total intensity of Cut7-GFP, calculated by integrating the intensity profiles from the linescan, was elevated in MI compared to mitosis, while total intensity of Pkl1-3XGFP remained similar in both types of spindles (Fig. S3G, H). Because total MT intensity is different in mitotic and MI spindles (Fig. S3I), we normalized the intensity values obtained for Cut7-GFP and Pkl1-3XGFP to the MT intensity values. In this way we found that the relative concentration of Cut7 remained unchanged (Fig. 3E), while the relative concentration of Pkl1-3XGFP per MT was lower in MI than in mitotic spindles (Fig. 3F). This may explain why *pkl1*

deletion cannot compensate for *cut7* deletion in MI, and suggests that an additional factor might counteract Cut7 activity during phase II of MI to maintain force-balance.

Kinesin-14 Klp2 function is distinctive in MI and mitosis spindles

To identify additional spindle components that could counteract Cut7-dependent forces in meiosis (Hagan and Yanagida, 1990; Syrovaktina, Fu and Tran, 2013), we screened other motors and MAPs reported to produce forces in the spindle (Syrovaktina, Fu and Tran, 2013). We reasoned that if a protein antagonizes Cut7, its deletion in the *cut7Δpkl1Δ* background should restore bipolar spindle assembly in MI. This in turn would result in proper chromosome segregation and increase the percentage of zygotes producing the typical four spores. We have systematically deleted candidate motors and MAPs in the *cut7Δpkl1Δ* background, mated the triple mutants, and scored for spore number (Fig. 4A). Of all the proteins tested, only the deletion of kinesin-14 Klp2 resulted in zygotes that produced the typical four spores more frequently (44±2% of *cut7Δpkl1Δklp2Δ* zygotes produced four spores compared to 20±1% in the *cut7Δpkl1Δ* background). Next, we turned to live cell imaging of *cut7Δpkl1Δklp2* zygotes to study how *klp2* deletion conferred an increase in the number of zygotes producing four spores.

Live cell imaging of *cut7Δpkl1Δklp2Δ* zygotes revealed that they succeeded to build a bipolar spindle in MI that was capable of separating the SPBs (Fig. 4B). While only 26±2% of *cut7Δpkl1Δ* zygotes managed to establish spindle bipolarity and separate SPBs, 49±2% of *cut7Δpkl1Δklp2Δ* zygotes assembled bipolar spindles in MI (Fig. S4A). We next compared mitotic and MI spindle dynamics in the triple mutant. MI spindle dynamics comparison revealed that not only was spindle bipolarity reestablished, but the usual three-phase spindle elongation dynamics were partially

rescued (Fig. 4C). This differed strongly from *cut7Δpkl1Δ* zygotes in which the three-phase spindle dynamics was not observed (Fig. 2F and 4C).

It was recently reported that *klp2* deletion partially rescues *cut7Δpkl1Δ* mitotic spindle dynamics, indicating that Klp2 produces inward forces alongside Pkl1, thereby counteracting the Cut7-dependent forces (Yukawa *et al.*, 2018). Our mitotic spindle analyses showed that *klp2* deletion partially rescued the time needed to reach phase III, but it did not rescue phase III spindle length in *cut7Δpkl1Δ* cells (Fig. S4B-E). This result was unlike the one noted in *cut7Δpkl1Δklp2Δ* MI spindles, where the pre-phase III spindle length was partially restored (Fig. 4C). Together, these results suggest that kinesin-14 Klp2 counteracts Cut7-dependent outward pushing forces and represents a new player in the force-balance maintenance in phase II of MI spindles.

Klp2 is a minus-end directed motor that can crosslink and slide MTs, affecting spindle length (Troxell *et al.*, 2001; Carazo-Salas, Antony and Nurse, 2005; Janson *et al.*, 2007; Syrovaktina, Fu and Tran, 2013). We tagged Klp2 with GFP to study its localization in mitotic and MI spindles. Tagging Klp2 had no effect on serial dilution assay results when compared to the non-tagged strain (Fig. S4F) and had a non-significant decrease in the percentage of zygotes producing 4 spores (Fig. S4G; 94% compared to the 97% for the strain with no GFP tag). The velocity of spindle elongation in phase III of mitotic spindle dynamics was faster and phase II duration was increased in the strain with tagged Klp2 (Fig. S4H). Tagging Klp2 resulted in a longer phase II spindle in MI (Fig S.4I) and 2 of the 17 observed zygotes required a longer time to reach spindle bipolarity. The results indicate that Klp2-GFP is mostly functional.

Imaging of Klp2-GFP showed that it localized along the spindle, starting from spindle assembly until early phase III in both mitosis and MI (Mana-Capelli *et al.*, 2012; Yukawa *et al.*, 2018) (Fig.

4D). Linescan analysis of Klp2-GFP showed higher maximum intensity peaks in MI, indicating an increase in the total amount of Klp2-GFP in MI compared to mitosis (Fig. 4E). However, the normalized concentration of Klp2-GFP relative to spindle MTs remained unchanged (Fig. 4F). We also noticed that Klp2-GFP localized more intensely at the spindle poles in MI compared to mitosis. This is likely not an artifact of mitosis and MI spindle length difference, as mitotic Pkl13XGFP signal was clearly observed as two distinct peaks in linescan analysis (Fig. 3D), regardless of the shorter length of the mitotic spindle.

Suppressing MT dynamics restored MI spindle bipolarity in *cut7Δpkl1Δ* zygotes

Considering that *klp2* deletion rescued spindle bipolarity only partially in the *cut7Δpkl1Δ* zygotes, we searched for additional factors that may participate in force-balance in MI phase II spindles. We considered perhaps MT dynamics may be differently regulated between mitosis and meiosis. Because current imaging limitations prevented us from measuring individual MT dynamics within the spindles, where there are many crosslinked MTs, we instead measured individual MT bundle dynamics before mitosis onset (IP) and in the pause period between MI and MII (IK). We compared the dynamics of individual MT bundles that grew straight and originated from the SPBs. Parameters defining MT dynamics during IP and IK are provided in Table 2.

Kymographs and plots of MT bundle dynamics in a vegetative cell and a zygote are shown in Fig. 5A. MT bundles begun their gro*wt*h at the SPB and grew continuously until they abruptly underwent catastrophe, switching to rapid shortening, and shrank back to the SPB. We found MT bundles shrank slower and grew faster in IK, but reached shorter maximum length than bundles in IP. Additionally, IK MT bundles underwent catastrophe two times more frequently than IP MT bundles. In general, meiotic IK MTs are more dynamic than mitotic IP MTs. Although not

measured within the spindles, these results on IP and IK MT bundles suggest that MT dynamics are differently regulated between mitotic cells and meiotic zygotes. We therefore tested if a modification in MT dynamics could influence MI spindle assembly and spore formation in *cut7Δpkl1Δ* zygotes.

We perturbed MT dynamics by utilizing low doses of methyl benzimidazole carbamate (MBC), a drug that inhibits MT polymerization and suppresses MT dynamics (Yenjerla *et al.*, 2009; Vela-Corcía *et al.*, 2018). Mating *wt* zygotes on ME plates with 5 µg ml^{-1} MBC resulted in a slightly lower frequency of zygotes with four spores (Fig. 5B). In contrast, *cut7Δpkl1Δ* zygotes produced four spores more frequently with the addition of MBC (44±1% with the addition of MBC compared to 20±1% when no MBC was added). Live-cell imaging showed that the addition of 5 µg ml^{-1} MBC allowed bipolar spindle assembly in MI in 80±4% of *cut7Δpkl1Δ* zygotes compared to 26±2% of *cut7Δpkl1Δ* zygotes when no MBC was added (Fig. 5C and Fig. S5A).

We also noticed that in *wt* zygotes treated with MBC, the MI phase II spindle frequently exhibited transient breakage (Fig. 5C, asterisk), a phenotype that was never observed in the *cut7Δpkl1Δ* zygotes. Accordingly, we saw an abrupt decrease of SPB distance in phase II of MI upon spindle breakage (Fig. 5D). However, spindle integrity was subsequently re-established and MI phase III could proceed. Nevertheless, this spindle instability due to the MBC treatment could account for the slightly lower number of *wt* zygotes producing four spores.

Cut7Δpkl1Δ spindle dynamics with the addition of MBC showed a delayed onset of MI phase III as well as a shorter MI phase I/phase II spindle, similar to what was observed for *cut7Δpkl1Δ* mitotic progression in the absence of MBC (Fig. 2D). Unlike *cut7Δpkl1Δklp2Δ* (Fig. 4C), *cut7Δpkl1Δ* MI spindles in the presence of 5 µg ml^{-1} MBC did not exhibit a three-phase progression

of spindle elongation (Fig. 5D). This suggests that the mechanism of MBC-dependent rescue is distinct from the one that relies on *klp2* deletion.

Finally, addition of low doses of MBC did not seem to impact mitotic progression (Fig. S5B). The relationship between *wt* and *cut7Δpkl1Δ* spindle dynamics with the addition of 5 µg ml^{-1} MBC appeared similar to their relationship without MBC addition (Fig. 2D). We conclude that in phase II of MI, MT dynamics counteract Cut7-dependent outward pushing forces together with Klp2 to achieve proper force-balance and allow the formation of functional bipolar spindles.

Discussion

In this work, we have analyzed the differences between mitotic and meiotic spindle dynamics in fission yeast. We found that the total amount of MTs, as well as the total amount of molecular motors studied (except kinesin-14 Pkl1), were higher in MI than in mitosis. In fission yeast, spindle length scales with cell size (Cortés et al., 2018; Krüger et al., 2019). A simple scaling mechanism could therefore explain the increase in the final MI spindle lengths in all phases, as well as the increased velocity of MI phase III (Table 1; Krüger et al., 2019). Because zygotes are diploid and larger (Table 1), they presumably have more MTs, motors and MAPs to organize a spindle. The average 25% increase in MI final spindle length compared to mitotic spindle could be readily explained by the increased cell volume and increased availability of spindle components.

We further found that in MI, unlike in mitosis, the largest proportion of time was spent in phase II (Table 1). In MI, the homologous chromosomes in the bivalent are held together by cohesin and chiasmata (Watanabe and Nurse, 1999; Molnar *et al.*, 2003; Tomoya S Kitajima *et al.*, 2003). The temporal requirement to complete the resolution of chiasmata could explain why phase II of MI is prolonged compared to mitosis.

Finally, zygotes spent a similar time in phase I of the two meiotic divisions (Table 1), but the velocity of spindle elongation in phase I of MI was lower than in mitosis, indicating that it may not scale up with cell size, unlike in phase III. This lower phase I velocity might result from a modification in MT dynamics (Table 2), an hypothesis that needs further testing, as we could not to date access MT dynamics within the spindle for technical reasons.

It was reported that failure to form a meiotic bouquet leads to aberrant spindles (Tomita and Cooper, 2007). However, imaging of the telomere component Taz1-GFP and the SPB component Sid4-mCherry did not reveal any defect in bouquet formation (Fig. S5C). We have also considered that the kinetochores may not be attached to the spindle MTs in *cut7Δpkl1Δ* MI. Because of the bouquet rearrangement, the kinetochores are far from the SPBs during MI, making it relatively easy to detect kinetochore attachment to MTs. Imaging the kinetochore component Mis12 tagged with GFP and the SPB component Sid4 tagged with mCherry did not reveal a defect in kinetochore attachment (Fig. S5D).

Further, MI spindles have a specific organization of chromosomes in bivalents, with cohesin between the homologous chromosomes, the existence of chiasmata, and changes in kinetochore architecture (Molnar et al., 2003; Yokobayashi and Watanabe, 2005; Watanabe and Nurse, 1999; Kitajima et al., 2004). We examined if this organization could influence spindle assembly in the *cut7Δpkl1Δ* background, but the individual deletion of Rec8 (meiosis specific cohesin), Moa1 (meiotic kinetochore protein), Rec11 (meiotic sister-chromatid cohesion) or Rec12 (meiotic recombination endonuclease) did not rescue the *cut7Δpkl1Δ* MI spindle phenotype nor the number of zygotes producing the typical four spores (Fig. S5E). This indicates that the difference observed between mitotic and MI spindles does not simply stem from the organization of chromosomes. However, a study published by Shirasugi and Sato during the revision of this paper has

demonstrated that a double deletion of Rec12 and Moa1 partially rescued spindle bipolarity in *cut7Δpkl1Δ* background (Shirasugi and Sato, 2019).

Unlike the other spindle components tested in this work, the concentration of kinesin-14 Pkl1 relative to spindle MTs is lower in MI than in mitosis (Fig. 3). The regulation behind this decrease in relative concentration remains unknown. As we have measured the concentrations of motors in mitosis and MI by analyzing the intensity of GFP attached to the motor, it is possible that it does not reflect the wild-type state, especially in light of the fact that Cut7-GFP, Pkl1-3XGFP, and Klp2-GFP were not fully functional (Fig. 3S and 4S). Nevertheless, by measuring the intensities in the same cell but in different types of cell division, we argue that the differences we observe are indeed intrinsic to the type of cell division, and are not artifacts from the fluorescent tagging. It was recently published that mild overexpression of kinesin-14 HSET in MI accelerated spindle bipolarization during phase I, and resulted in more focused spindle poles, essentially switching from MI to a more mitotic-like spindle (Bennabi *et al.*, 2018). This in turn resulted in aberrant chromosome alignment. It is tempting to think that a downregulation of kinesin-14 concentration from mitosis to MI could be evolutionary conserved.

We identified Klp2 as an additional Cut7 antagonist in MI. It was demonstrated that in mitosis Klp2 tethering to SPBs can, to some degree, replace the role of Pkl1 in spindle anchorage and force generation, indicating that in such a situation, Klp2 can effectively oppose Cut7 outward forces (Yukawa *et al.*, 2018). Since Klp2-GFP localized more to the spindle poles in MI than in mitosis (Fig. 4E), we propose that Klp2 function may differ in MI compared to mitosis, and that it plays an important role in spindle bipolarity establishment by counteracting Cut7 outward pushing forces to compensate for the increase in the Cut7-to-Pkl1 ratio. How Klp2 regulation is tuned and which factors influence its functions remain open questions.

Another significant finding of our study was the possibility of restoring MI spindle bipolarity in *cut7Δpkl1Δ* by altering MT dynamics with low doses of MBC (Yenjerla et al., 2009; Cortés et al., 2018; Carazo-Salas et al., 2005). While we could measure parameters of dynamic MT bundles in IK compared to IP, we could not access MT dynamics within spindles. Nevertheless, our results imply changes in MT dynamics between mitosis and meiosis. Our hypothesis is that MT dynamics may be enhanced in MI phase I/phase II compared to mitotic phase I/phase II. In any case, the fact that suppressing MT dynamics in *cut7Δpkl1Δ zygotes* restores MI spindle bipolarity, and also alters the outcome of *wt* zygote meiosis, but without perturbing mitosis outcome, indicates that MI spindle formation is extremely sensitive to alterations in MT dynamics and that fine tuning of MT dynamics is crucial for the success of meiosis. Which factors alter MT dynamics between mitosis and meiosis remains to be studied.

In vegetative cells, centromeres are grouped at the nuclear envelope, below the SPB (Hironori *et al.*, 1993). In zygotes, a telomere bouquet is formed prior to meiotic divisions, which places telomeres below the SPB and centromeres farther away (Chikashige *et al.*, 1994; Fennell *et al.*, 2015). One possibility is that because centromeres/kinetochores are less accessible for attachment to MTs being farther away from the SPBs at MI, an increase in MT dynamics would increase the chances of MTs finding and attaching to kinetochores (Kirschner and Mitchison, 1986; Heald and Khodjakov, 2015). Moreover, it was shown that MT pivoting movement around the spindle poles speeds up kinetochore capture in mitosis (Kalinina *et al.*, 2013). Taking into account the decreased concentration of Pkl1 at the spindle poles in MI, it is plausible to assume that MTs may be more loosely organized at the SPB and that more MTs are being nucleated during MI (Olmsted *et al.*, 2014). This could result in greater range of MT pivoting motions, which would increase the chance of encountering and attaching a kinetochore. Indeed, we have observed an abundance of MTs in

the initial stages of spindle assembly in MI compared to mitosis where such structures were rarely detected (Fig. S5F). It is possible these MTs pivot, but this hypothesis remains unconfirmed as there are several MTs per time frame in MI, which makes tracking their position from frame to frame challenging. The fact that suppressing MT dynamics by MBC rescued spindle bipolarity in *cut7Δpkl1Δ* zygotes, suggests that MT dynamics also contribute to the force-balance establishment within meiotic spindles, possibly by limiting the outward pushing forces generated by MT gro*wt*h.

Our results open new questions on how motors, MAPs and MT dynamics are modified between mitosis and meiosis. Given the high degree of evolutionary conservation of the molecular players involved in spindle assembly, it will be most interesting to expand this study further in other organisms to develop our global understanding of mitosis and meiosis.

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Author contributions

A.L. and P.T.T. conceived and designed the study upon initial work by S.A.R. A.L. has performed the experiments, analyzed the data, and wrote the paper. M.L.R. wrote the semi-automated Matlab program for spindle dynamics measurements. S.A.R., A.P., and P.T.T. edited the manuscript.

Declaration of interest

The authors declare no competing interests.

List of Symbols and Abbreviations used

MT – microtubule

MAP – microtubule associated protein

SPB – spindle pole body

MI – meiosis I

MII – meiosis II

GFP – green fluorescent protein

mCherry – monomeric red fluorescent protein

ME – malt extract

IP - interphase

YE5S – yeast extract medium supplemented with Leu, Ura, Ade, His, and Lys

IK – interkinesis

DNA – deoxyribonucleic acid

SAC – spindle assembly checkpoint

MBC – methyl benzimidazole carbamate

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Table 1. Spindle length dynamics in mitosis, MI and MII.

Spindle characteristics		**Mitosis (n=60)**	**MI (n=53)**	**MII (n=99)**
Phase I	Final spindle length (μm)	1.26 ± 0.05	2.94 ± 0.11	1.64 ± 0.04
	Duration (min)	4.27 ± 0.18	9.60 ± 0.45	7.17 ± 0.26
	Relative duration of phase I (%)	17 ± 1	24 ± 1	21 ± 1
	Velocity (μm min^{-1})	0.36 ± 0.02	0.30 ± 0.02	0.27 ± 0.01
Phase II	Final spindle length (μm)	2.70 ± 0.06	5.09 ± 0.08	2.28 ± 0.05
	Duration (min)	8.52 ± 0.23	19.85 ± 0.60	21.23 ± 0.44
	Relative duration of phase II (%)	33 ± 1	49 ± 1	64 ± 1
Phase III	Final spindle length (μm)	10.97 ± 0.13	14.23 ± 0.18	4.94 ± 0.15
	Duration (min)	12.62 ± 0.27	10.72 ± 0.27	4.89 ± 0.17
	Relative duration of phase III (%)	50 ± 1	27 ± 1	15 ± 1
	Velocity (μm min^{-1})	0.70 ± 0.02	0.99 ± 0.03	0.75 ± 0.03
Total duration (min)		25.40 ± 0.33	40.17 ± 0.79	33.34 ± 0.49
Cell length (μm)		13.27 ± 0.13	18.13 ± 0.26	
Meiotic IK duration (min)		NA	33.64 ± 1.03	

Values in table are given as mean±s.e.m.

Table 2. Individual MT bundle dynamic parameters during IP and IK.

	IP (n≥80)	**IK (n≥105)**	**p-value**
Growth velocity (μm min^{-1})	2.12 ± 0.05	2.53 ± 0.09	<0.001
Shrinkage velocity (μm min^{-1})	6.28 ± 0.25	3.26 ± 0.13	<0.0001
Catastrophe frequency (min^{-1})	0.54 ± 0.02	1.07 ± 0.04	<0.0001
Maximum length (μm)	4.47 ± 0.12	2.63 ± 0.07	<0.0001

p-value was calculated by the Mann-Whitney test. Values in table are given as mean±s.e.m.

Figure Legends

Fig. 1. Fission yeast mitotic and meiotic spindle have distinct dynamics. (A) Model of spindle assembly and elongation in mitosis, MI and MII. **(B)** DIC image of a *wt* haploid cell post mitosis (cell indicated by an asterisk on the septum) and *wt* diploid zygote post MI and MII (zygote indicated by asterisks on 4 round spores) in the same field of view. **(C)** Time-lapse images of *wt* cell expressing mCherry-Atb2 (α-tubulin) and Sid4-GFP (SPB component) from IP (30 min prior to spindle formation) to spindle breakdown. Phases of mitotic spindle assembly and elongation are indicated on the left side; I = phase I, II = phase II, III = phase III. Each frame represents a 5 min interval. Scale bars, 5 µm. **(D)** Time-lapse images of *wt* zygotes expressing mCherry-Atb2 and Sid4-GFP from spindle assembly in MI to spindle disassembly in MII. Phases of meiotic spindle assembly and elongation are indicated on the left side. Each frame represents a 5 min interval. Scale bar, 5 µm. **(E)** Comparative plot of *wt* spindle length dynamics in mitosis (n=20), MI (n=19) and MII (n=34). Bold curves represent the average spindle.

Fig. 2. The *cut7Δpkl1Δ* spindle fails to segregate chromosomes during meiosis. (A) Bar graph comparison of the number of *wt* (n=749) and *cut7Δpkl1Δ* (n=669) zygotes producing the typical four spores. p-value was calculated by χ^2 test. **(B)** Time-lapse images of *wt* and *cut7Δpkl1Δ* mitotic cells expressing GFP-Atb2 and Hht1-mCherry (histone H3) from spindle assembly to spindle breakdown. Each frame represents a 5 min interval. Scale bar, 5 µm. **(C)** Time-lapse images of *wt* and *cut7Δpkl1Δ* zygotes expressing GFP-Atb2 and Hht1-mCherry from spindle assembly in MI to spindle breakdown in MII. Percentages below the three *cut7Δpkl1Δ* phenotypes represent their frequency (n=54). Each frame represents a 10 min interval. Scale bar, 5 µm. **(D)** Comparative plot of *wt* (n=19) and *cut7Δpkl1Δ* (n=20) mitotic spindle length dynamics. Dotted grey lines indicate a shift between phases for *wt* spindles. **(E)** Comparative plot of *wt* (n=41) and *cut7Δpkl1Δ* (n=35)

MII spindle length dynamics. Dotted grey lines indicate a shift between phases for *wt* spindles. **(F)** Comparative plot *wt* (n=17) and *cut7Δpkl1Δ* (n=20) MI spindle length dynamics. Dotted grey lines indicate a shift between phases for *wt* spindles.

Fig. 3. Kinesin-14 Pkl1 relative concentration is lower in MI than in mitosis. (A) Time-lapse images of cells expressing mCherry-Atb2 and kinesin-5 Cut7-GFP. Asterisks indicate the spindle at phase I/phase II transition, 1 minute before the spindle reaches a constant length in phase II. Scale bar, 5 μm. **(B)** Time-lapse images of cells expressing mCherry-Atb2 and kinesin-14 Pkl1-3XGFP. Asterisks indicate the spindle at phase I/phase II transition, 1 minute before the spindle reaches a constant length in phase II. Scale bar, 5 μm. **(C)** Linescan analysis of Cut7-GFP signal distribution on the spindle at phase I/phase II transition in mitosis (n=60) and MI (n=60). **(D)** Linescan analysis of Pkl1-3XGFP signal distribution on the spindle at phase I/phase II transition in mitosis (n=57) and MI (n=58). **(E)** Box-and-whisker plot comparison of relative concentration of Cut7-GFP per spindle MTs at phase I/phase II transition in mitosis (n=62) and MI (n=60). n.s.=not significant. **(F)** Box-and-whisker plot comparison of relative concentration of Pkl1-3XGFP per spindle MTs at phase I/phase II transition in mitosis (n=57) and MI (n=58). p-values were calculated by Mann-Whitney test.

Fig. 4. Kinesin-14 Klp2 deletion partially rescues bipolar spindle defects of *cut7Δpkl1Δ* zygotes during MI. (A) Bar graph comparison of the number of *cut7Δpkl1Δ* (n=669), *cut7Δpkl1Δklp2Δ* (n=650), *cut7Δpkl1Δklp6Δ* (n=483), *cut7Δpkl1Δdhc1Δ* (n=694), *cut7Δpkl1Δdam1Δ* (n=581), and *cut7Δpkl1Δrec8Δ* (n=522) zygotes producing the typical four spores. The red bar represents the *cut7Δpkl1Δ* zygotes and the green bar represents *cut7Δpkl1Δklp2Δ* zygotes. Black bars represent triple mutants that did not show a rescue in the number of zygotes producing four spores. p-value was calculated by χ^2 test. **(B)** Time-lapse images

of *cut7Δpkl1Δ* and *cut7Δpkl1Δklp2Δ* zygotes expressing mCherry-Atb2 and Sid4-GFP from spindle assembly to breakdown in MI. Each frame represents a 5 min interval. Scale bar, 5 µm. **(C)** Comparative plot of *cut7Δpkl1Δ* (n=20) and *cut7Δpkl1Δklp2Δ* (n=20) MI spindle length dynamics. Horizontal dotted lines indicate the final length of pre-phase III spindle for *cut7Δpkl1Δ* (red) and *cut7Δpkl1Δklp2Δ* (green). The box-and-whisker graph on the right shows the comparison of the final length of pre-phase III spindles in *cut7Δpkl1Δ* (n=24) and *cut7Δpkl1Δklp2Δ* (n=29). p-value was calculated by Mann-Whitney test. **(D)** Time-lapse images of cells expressing mCherry-Atb2 and Klp2-GFP. Asterisks indicate the spindle at phase I/phase II transition, 1 minute before the spindle reaches a constant length in phase II. Scale bar, 5 µm. **(E)** Linescan analysis of Klp2-GFP signal distribution on the spindle at phase I/phase II transition in mitosis (n=58) and MI (n=59). **(F)** Box-and-whisker plot comparison of relative concentration of Klp2-GFP per spindle MTs at phase I/phase II transition in mitosis (n=58) and MI (n=59). n.s.=not significant.

Fig. 5. Inhibition of MT polymerization restores MI spindle bipolarity in *cut7Δpkl1Δ* zygotes. **(A)** Representative zygote and vegetative cell expressing mCherry-Atb2 and Sid4-GFP. Scale bar, 5 µm. Kymographs are taken from boxed regions containing the SPB and the MT bundle in zygotes in IK and in vegetative cells in IP. Kymographs on the right shows representative IK- and IP-MT bundle dynamics over time. Scale bar, 2 µm. Comparative plot shows differences between IP- and IK-MT bundle dynamics. **(B)** Comparison of the number of zygotes producing the typical 4 spores with the addition of 5 µg ml^{-1} MBC (*wt* n=572, *cut7Δpkl1Δ* n=1162) and without the addition of MBC (*wt* n=749, *cut7Δpkl1Δ* n=669). p-value was calculated by χ^2 test. **(C)** Time-lapse images of *wt* and *cut7Δpkl1Δ* zygotes expressing mCherry-Atb2 and Sid4-GFP from spindle assembly to breakdown in MI. Yellow asterisk indicates a collapsed metaphase spindle. Scale bar, 5 µm. **(D)**

Comparative plot of *wt* (n=20) and *cut7Δpkl1Δ* (n=20) MI spindle dynamics with the addition of 5 µg ml^{-1} MBC.

Figure 1. Loncar et al., J Cell Sci, 2020

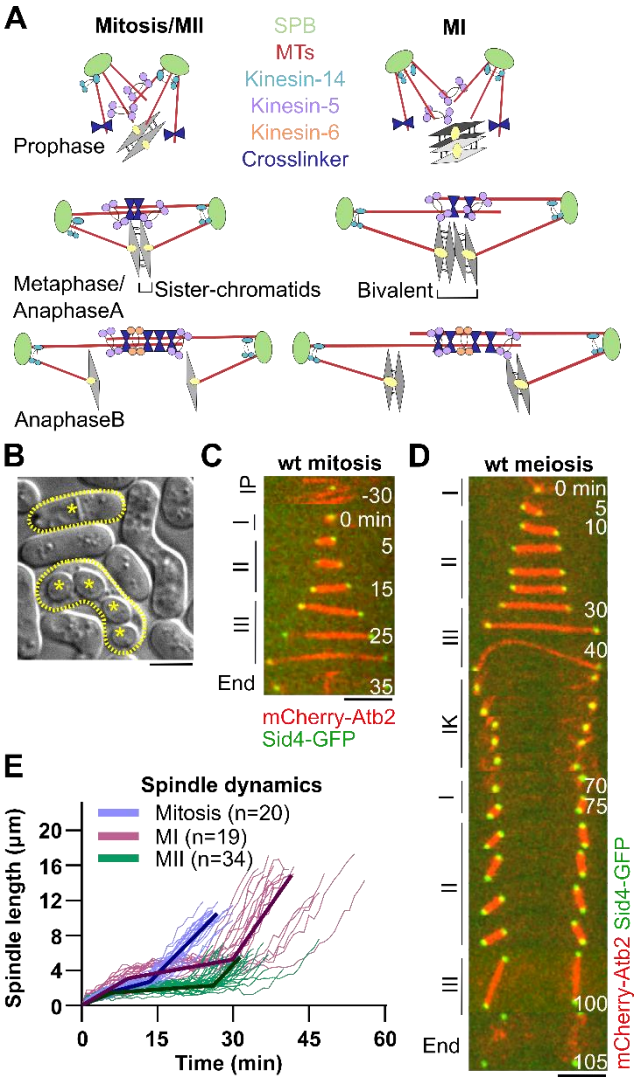
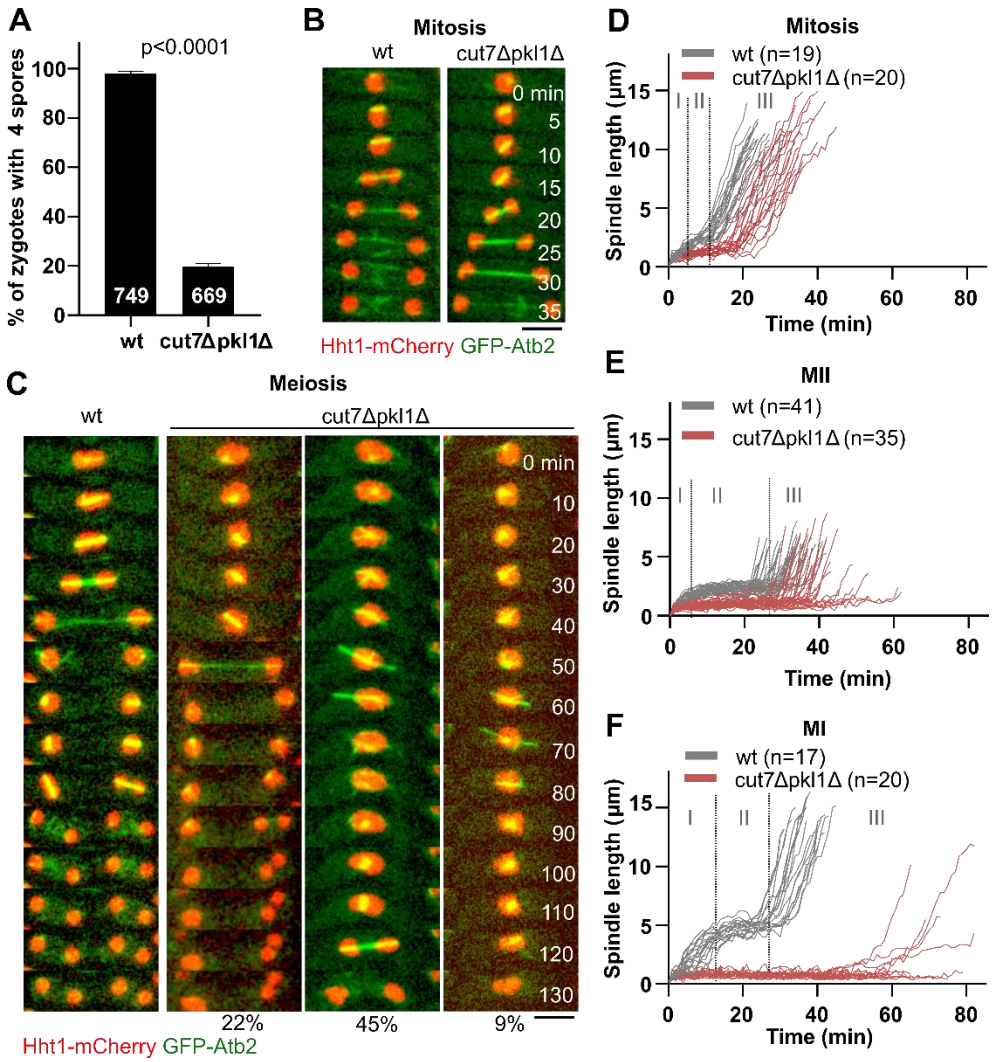


Figure 2. Loncar et al., J Cell Sci, 2020



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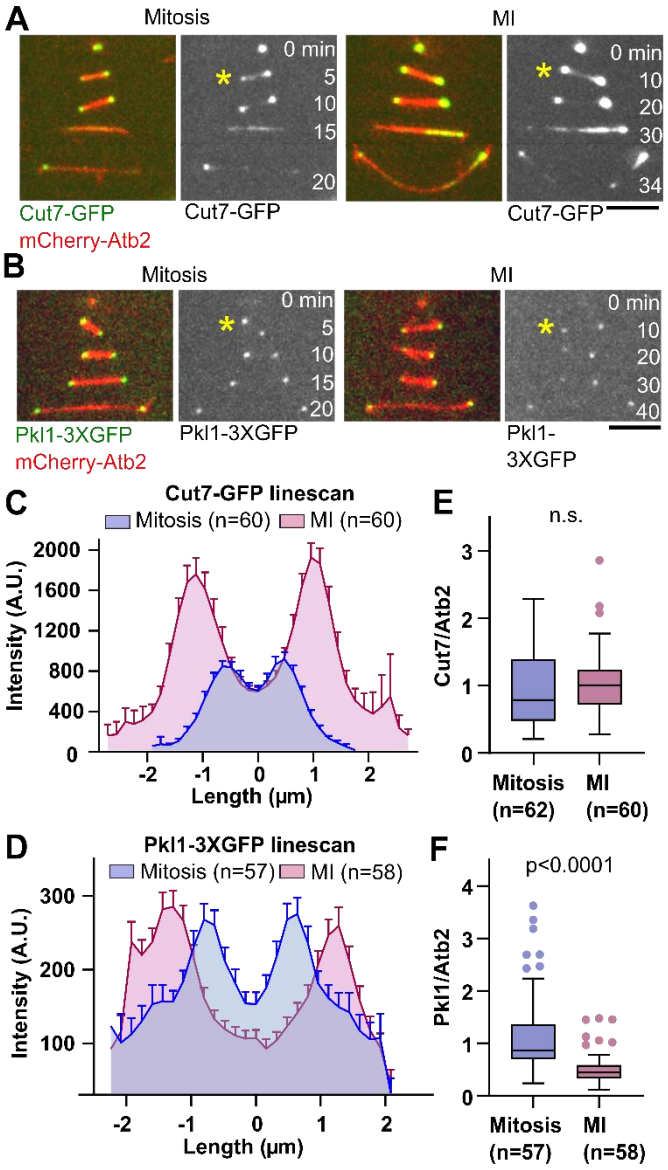

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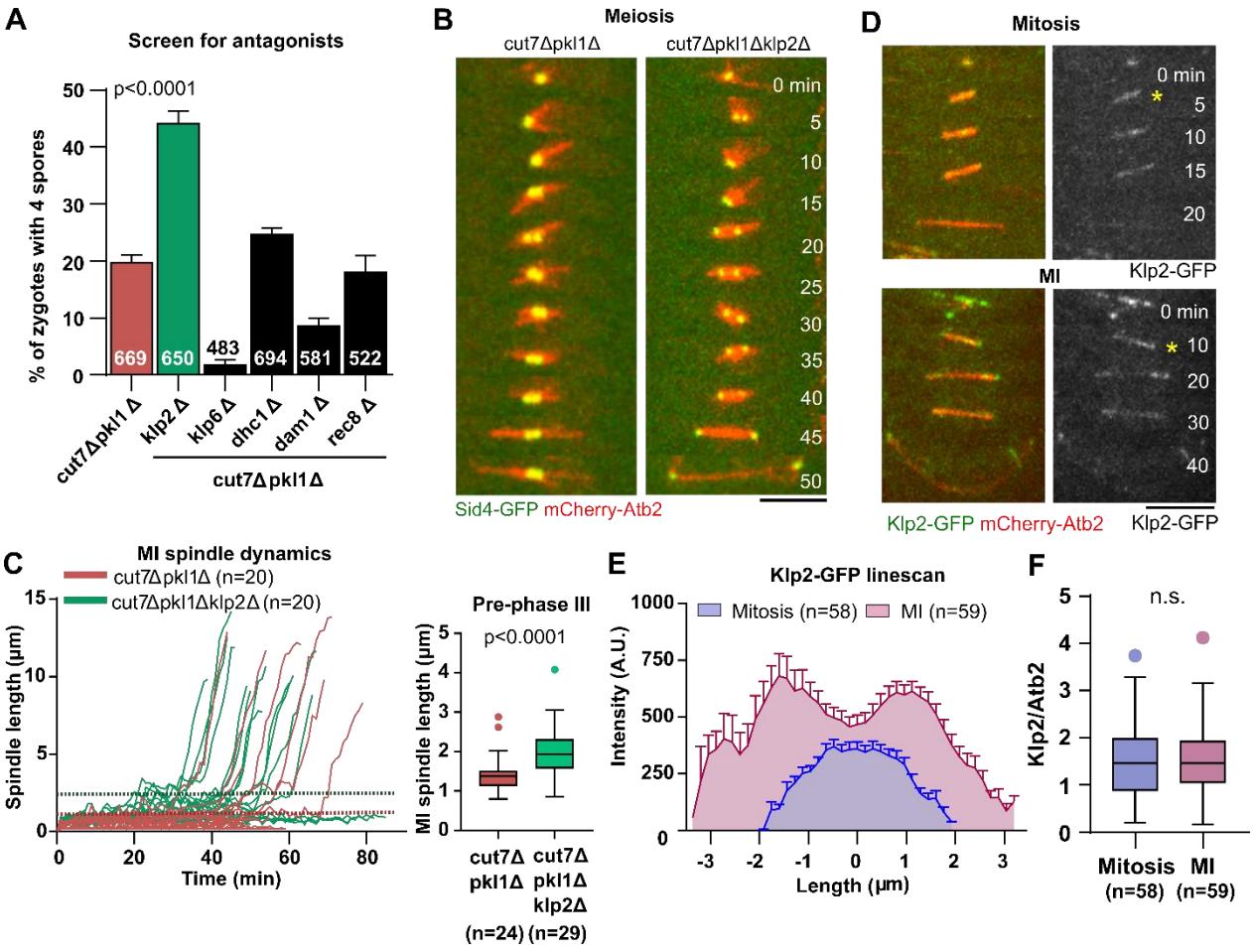
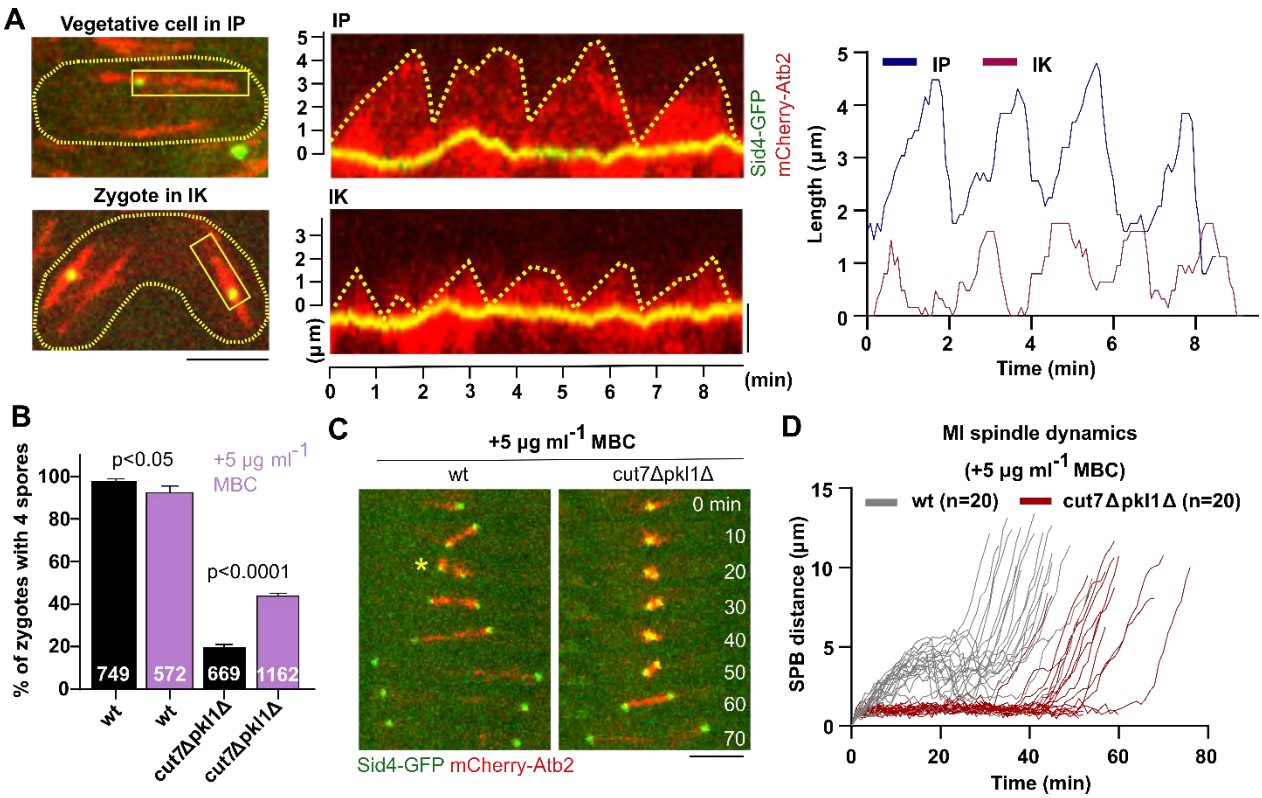


Figure 5. Loncar et al., J Cell Sci, 2020



Inventory of Supplemental information

Fig. S1. Mitotic dynamics are comparable in YE5S and ME media. Comparative plot of *wt* spindle dynamics in YE5S (n=20) and ME (n=20). Bold curves represent a mean spindle.

Fig. S2. *Cut7Δpkl1Δ* zygotes fail to establish a bipolar spindle in MI, but still enter MII. (A) DIC image of *cut7Δpkl1Δ* zygotes. Yellow lines indicate zygotes with abnormal spores (asterisks). **(B)** Time-lapse images of *wt* and *cut7Δpkl1Δ* vegetative cells expressing mCherry-Atb2 and Sid4-GFP from spindle assembly to spindle breakdown. Scale bar, 5 µm. **(C)** Time-lapse images of *wt* and *cut7Δpkl1Δ* zygotes expressing mCherry-Atb2 and Sid4-GFP from spindle assembly in MI to spindle breakdown in MII. Percentages below the two *cut7Δpkl1Δ* phenotypes represent their frequency (n=113). Scale bar, 5 µm. **(D)** Bar graph comparison of SPB separation in mitosis (*wt* n=201, *cut7Δpkl1Δ* n=219), MI (*wt* n=102, *cut7Δpkl1Δ* n=113), and MII (*wt* n=157, *cut7Δpkl1Δ* n=127). **(E)** Time-lapse images of *wt* and *cut7Δpkl1Δ* zygotes expressing mCherry-Atb2 and Cdc13-GFP (cyclin-B). Scale bar, 5 µm. **(F)** Box-and-whisker plot comparison of Cdc13-GFP degradation time after spindle assembly onset in MI of *wt* (n=37) and *cut7Δpkl1Δ* (n=22) zygotes. **(G)** Representative image of a *cut7Δpkl1Δ* zygote in phase I/phase II of MI expressing mCherry-Atb2 and Mal3-GFP. Scale bar, 5 µm. For insets, scale bar is 2 µm.

Fig. S3. Total amount of Pkl1 remains constant from mitosis to MI. (A) Bar-graph comparison of the number of zygotes producing the typical four spores in strains without GFP (n=494), in strains with Cut7-GFP (n=613), and in strains with Pkl1-3XGFP (n=490). p-values were calculated by χ^2 test. **(B)** Serial dilution (fourfold) assay showing gro*w*th of strains without GFP, and strains with Cut7-GFP or Pkl1-3XGFP. Plates were incubated 48 or 96 hours at the indicated temperatures. **(C)** Comparative plot of mitotic spindle length dynamics in the strain without GFP (n=18) and in the strain with Cut7-GFP (n=19). The green vertical dotted line represents a time point in phase I/phase II transition when the intensity of Cut7-GFP was measured. **(D)** Comparative plot of MI

spindle length dynamics in the strain without GFP (n=17) and in the strain with Cut7-GFP (n=20). The green vertical dotted line represents a time point in phase I/phase II transition when the intensity of Cut7-GFP was measured. **(E)** Comparative plot of mitotic spindle length dynamics in the strain without GFP (n=15) and in the strain with Pkl1-3XGFP (n=18). The green vertical dotted line represents a time point in phase I/phase II transition when the intensity of Pkl1-3XGFP was measured. **(F)** Comparative plot of MI spindle length dynamics in strains without GFP (n=24) and in strains with Pkl1-3XGFP (n=20). The green vertical dotted line represents a time point in phase I/phase II transition when the intensity of Pkl1-3XGFP was measured. **(G)** Box-and-whisker plot comparison of Cut7-GFP total intensity in mitosis (n=62) and MI (n=60). **(H)** Box-and-whisker plot comparison of Pkl1-3XGFP total intensity in mitosis (n=57) and MI (n=58). **(I)** Box-and-whisker plot comparison of MT total intensity per spindle length in mitosis (n=57) and MI (n=58). p-values were calculated by Mann-Whitney test.

Fig. S4. *Klp2* deletion rescues metaphase duration, but not final metaphase spindle length in *cut7Δpkl1Δ* mitosis. (A) Bar graph comparison of SPB separation in *cut7Δpkl1Δ* (n=113) and *cut7Δpkl1Δklp2Δ* (n=83) MI. **(B)** Time-lapse images of *cut7Δpkl1Δ* and *cut7Δpkl1Δklp2Δ* mitotic cells expressing mCherry-Atb2 and Sid4-GFP from spindle assembly to spindle disassembly. Scale bar, 5 µm. **(C)** Comparative plot of *cut7Δpkl1Δ* (n=20) and *cut7Δpkl1Δklp2Δ* (n=17) mitotic spindle length dynamics. **(D)** Box-and-whisker plot comparison of mitotic phase I/phase II duration in *cut7Δpkl1Δ* (n=46) and *cut7Δpkl1Δklp2Δ* (n=46). **(E)** Box plot comparison of pre-phase III mitotic spindle length of *cut7Δpkl1Δ* (n=46) and *cut7Δpkl1Δklp2Δ* (n=46). p-values were calculated by Mann-Whitney test. **(F)** Serial dilution (fourfold) assay showing gro*wt*h of strain without GFP, and strain with Klp2-GFP. Plates were incubated 48 or 96 hours at the indicated temperatures. **(G)** Bar-graph comparison of the number of zygotes producing the typical four spores in strains without

GFP (n=494) and with Klp2-GFP (n=445). p-values were calculated by χ^2 test. **(H)** Comparative plot of mitotic spindle length dynamics in the strain without GFP (n=26) and in the strain with Klp2-GFP (n=16). The green vertical dotted line represents an average time point in phase I/phase II transition when the intensity of Klp2-GFP was measured. **(F)** Comparative plot of MI spindle length dynamics in the strain without GFP (n=15) and in strains with Klp2-GFP (n=17). The green vertical dotted line represents a time point in phase I/phase II transition when the intensity of Klp2-GFP was measured.

Fig. S5. Chromosome organization and architecture are not the cause of failed *cut7Δpkl1Δ* meiosis. (A) Bar graph comparison of SPB separation in *cut7Δpkl1Δ* zygotes with (n=110) and without addition of 5 µg ml^{-1} MBC (n=124). **(B)** Comparative plot of *wt* (n=19) and *cut7Δpkl1Δ* (n=20) mitotic spindle dynamics with the addition of 5 µg ml^{-1} MBC. **(C)** Kymographs of *wt* and *cut7Δpkl1Δ* zygotes expressing Sid4-mCherry and a telomere component Taz1-GFP. Yellow asterisks indicate a time point in which telomeres start to dissociate from the SPB. Scale bar, 2 µm. **(D)** Kymographs of *wt* and *cut7Δpkl1Δ* zygotes expressing Sid4-mCherry and a kinetochore component Mis12-GFP. Yellow asterisks indicate a time point in which all the kinetochores are pulled to the SPB. Scale bar, 2 µm. **(E)** Bar graph comparison of the number of *cut7Δpkl1Δ* (n=122), *cut7Δpkl1Δrec11Δ* (n=168), *cut7Δpkl1Δrec12Δ* (n=235), *cut7Δpkl1Δsgo1Δ* (n=264), and *cut7Δpkl1Δmoa1Δ* zygotes (n=321) producing the typical four spores. **(F)** MTs in mitotic and MI from spindle assembly onset in phase I to phase II onset. Yellow arrows in vegetative cell indicate pivoting MTs. Yellow arrows in the zygote indicate a possible pivoting MT. Time indicated on the images is in seconds. Scale bar, 5 µm. For insets, scale bar is 2 µm.

Figure S1. Loncar et al., J Cell Sci, 2020

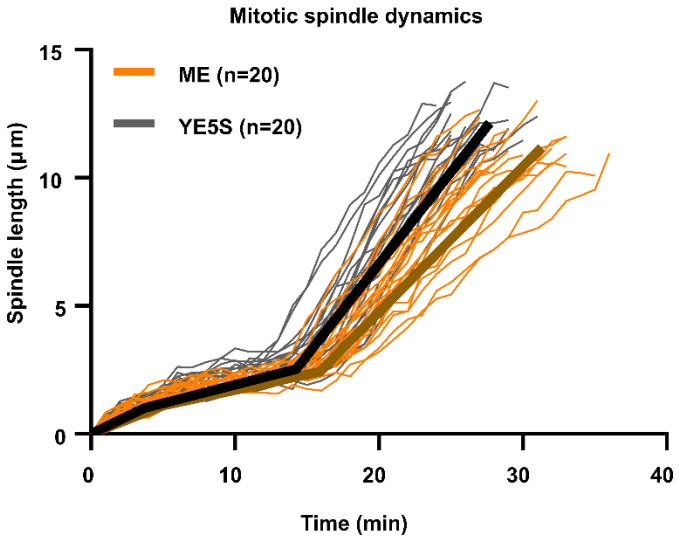


Figure S2. Loncar et al., J Cell Sci, 2020

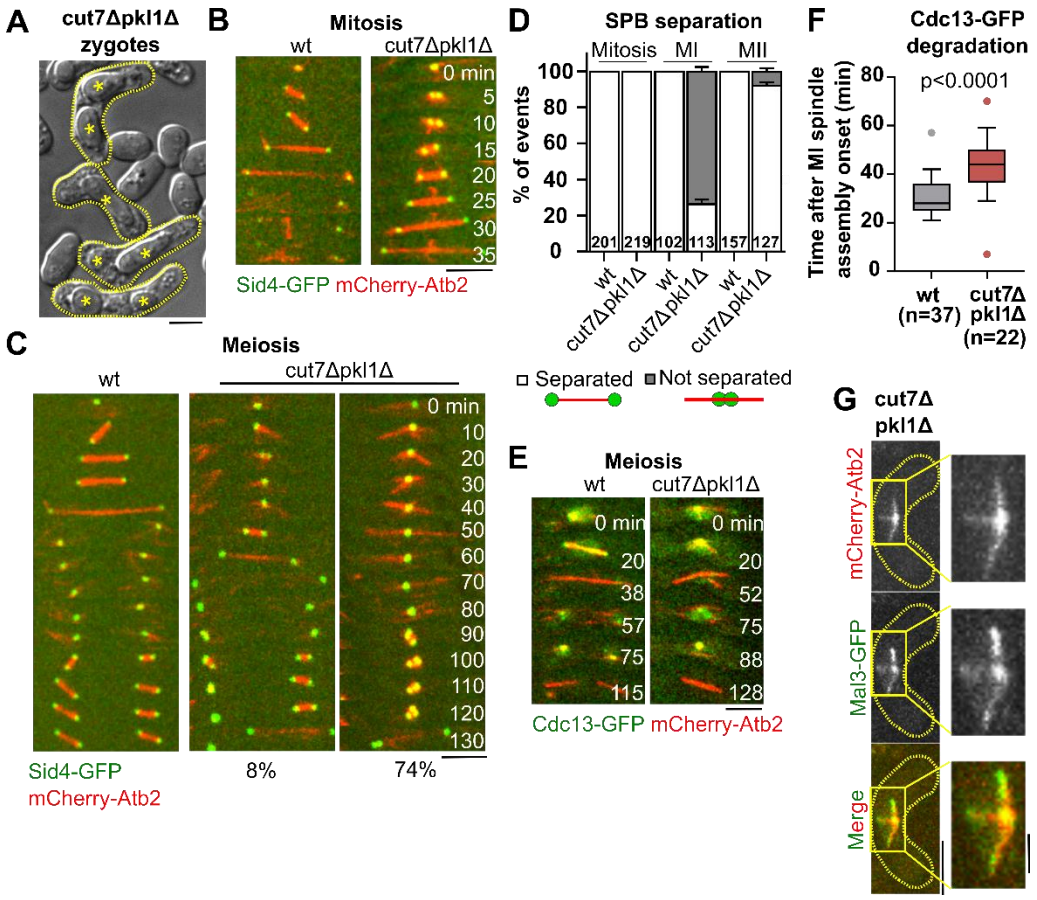


Figure S3. Loncar et al., J Cell Sci, 2020

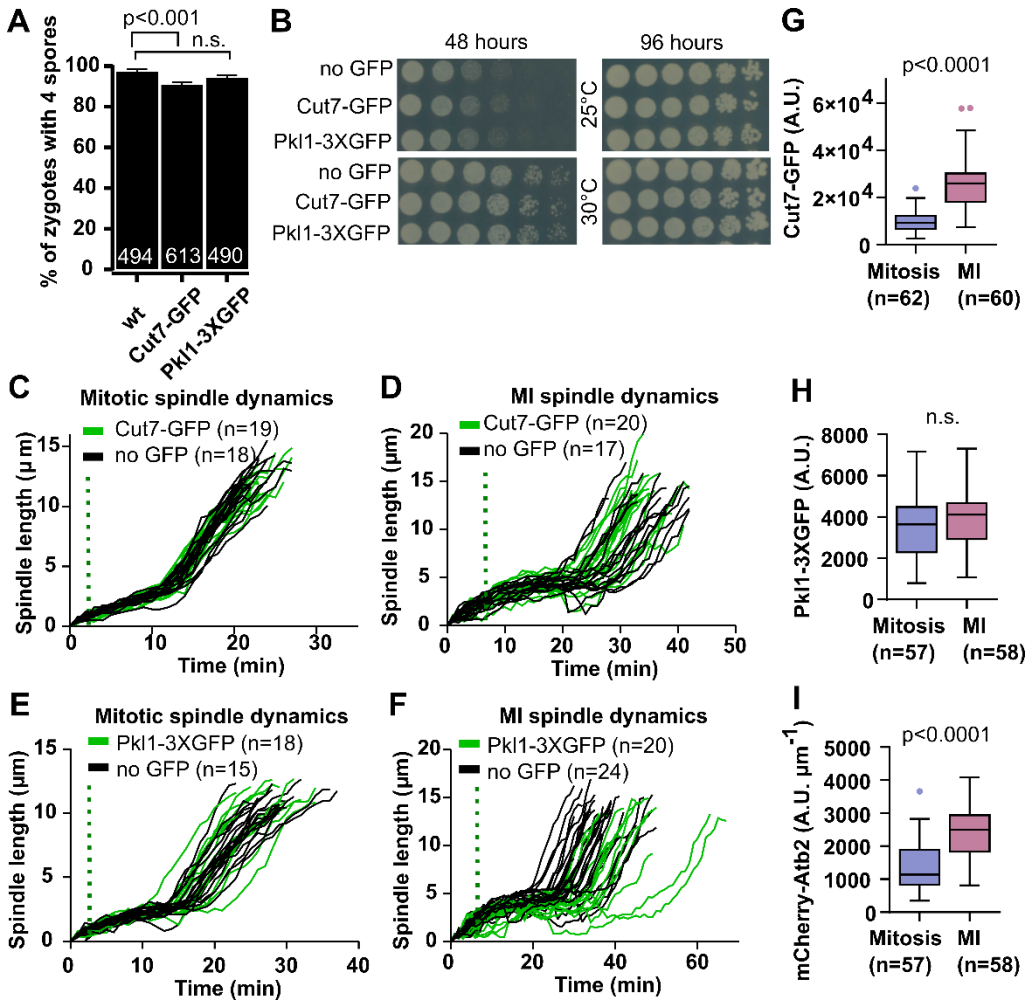


Figure S4. Loncar et al., J Cell Sci, 2020

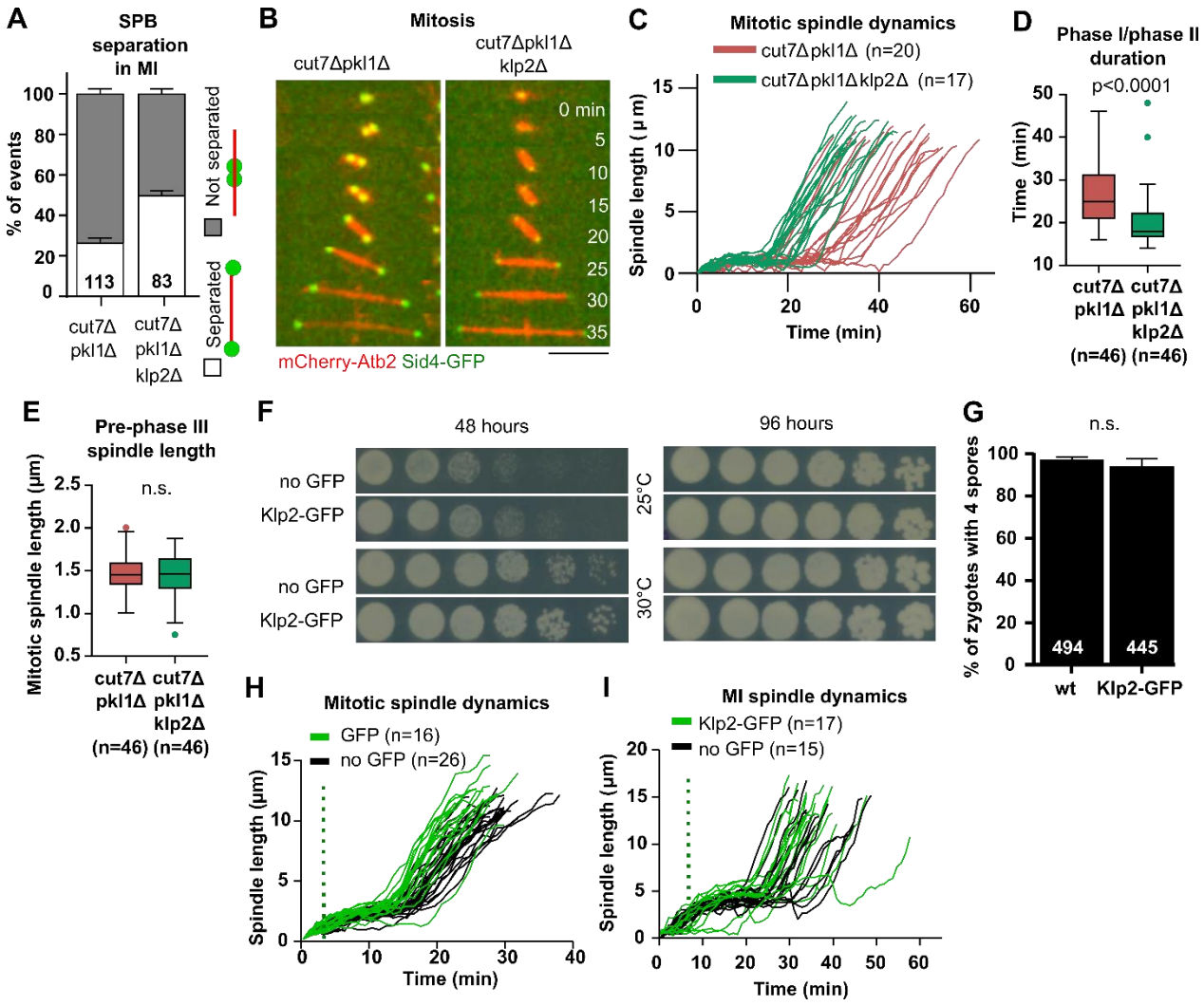
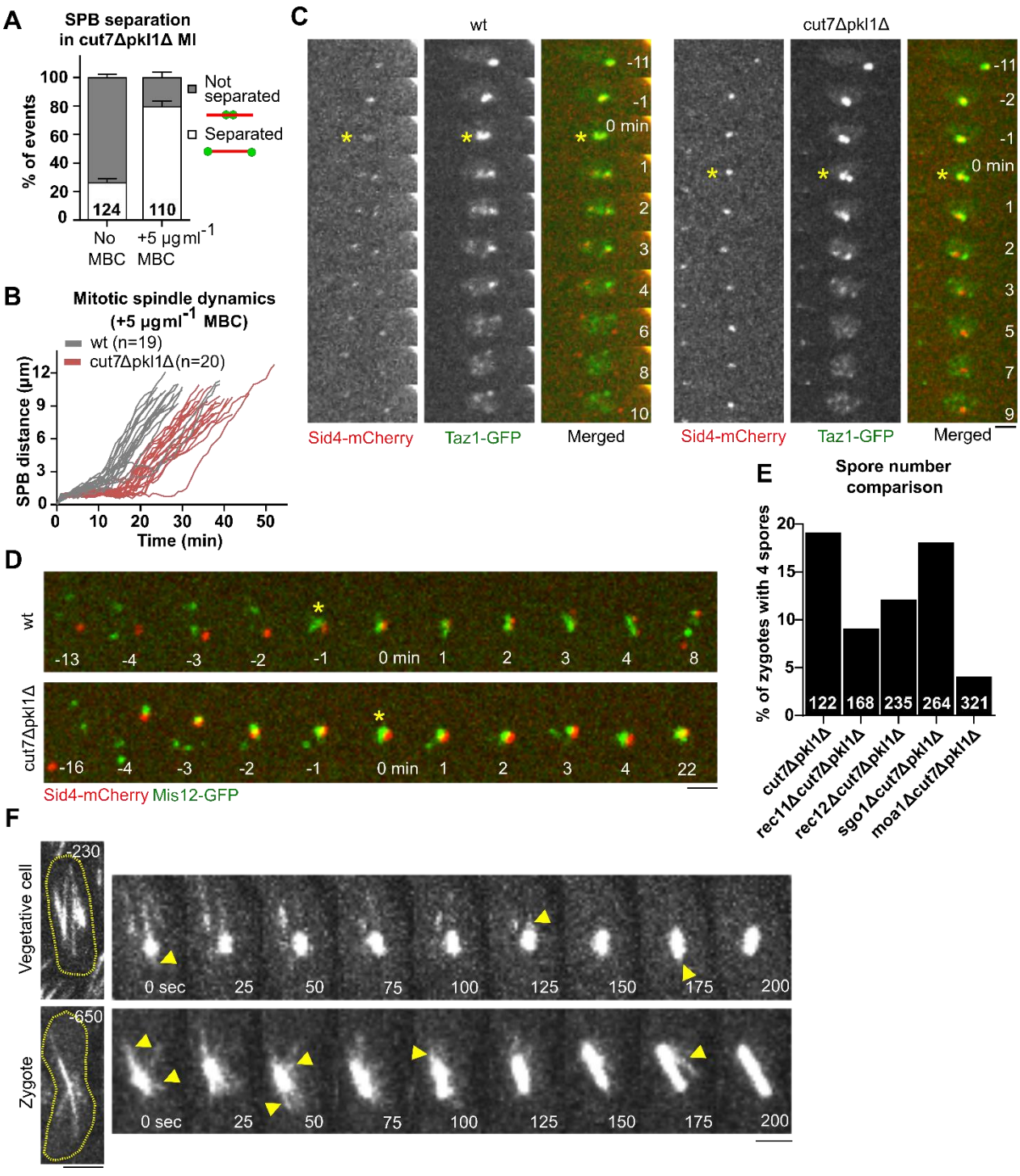


Figure S5. Loncar et al., J Cell Sci, 2020



Materials and methods

Production of *S. pombe* mutant strains

All strains used in this work are isogenic to wild-type 972 and were obtained from genetic crosses, selected by random spore germination and replicated on plates with appropriate drugs or supplements. All strains are listed in the Supplementary File 1. Gene deletion and tagging was performed in the endogenous locus, except for Sid4-GFP and Mis12-GFP which were integrated in the *leu1-32* locus, as described previously (Keeney and Boeke, 1994; Bähler *et al.*, 1998). Transformations were performed by using the lithium-DTT method. 25 mL of exponentially growing cells (OD_{600} 0.5-0.8) were harvested by centrifugation and washed with 10 mM Tris HCl pH 7.4. They were centrifuged again and re-suspended in 100 mM lithium acetate with 10 mM DTT. Suspension was incubated on an orbital wheel at room temperature for 40 minutes. 100 µL of suspension was mixed with 80 µL of 100 mM lithium acetate, 10 µL of single stranded DNA from salmon testes (D9156-5ML, Sigma) and 1 µg of the purified DNA. After 10 minutes of incubation on an orbital wheel, 300 µL of PEG 4000, dissolved in 100 mM lithium acetate was added. After incubation of 10 minutes on the wheel, 15 µL of DMSO was added. Mixture was heat shocked at 42°C for 20 minutes. Cells were washed with water and plated onto YE5S plates. After two days of incubation at 25°C, the plates were replicated on plates containing the corresponding selective media.

Fission yeast culture

All *S. pombe* strains were maintained at 25˚C and cultured in standard media. Generally, two days before imaging, cells were refreshed on a YE5S plate. The following day, cells of opposite mating types were mixed on an ME plate, or an ME plate supplemented with 5 µg ml^{-1} MBC, to induce conjugation. The plate with mating cells was incubated at 25˚C for 16-20 hours before imaging. For spore quantification, the mixture was incubated on an ME plate, or an ME plate supplemented with 5 µg ml^{-1} MBC, at 25˚C for two days and subsequently imaged.

Serial dilutions were performed by growing tested strains on a YE5S plate at 25˚C for 24 hours, and then resuspending some of the patch in water so the OD_{600} was 1.5. This suspension was serially fourfold diluted, 5 µl of the prepared dilutions was inoculated on YE5S plates and then incubated at 25˚C or 30 ˚C for 48 or 96 hours.

Live microscopy

For live-cell imaging, cells were put on ME agarose pads, containing 4% agarose (Tran et al., 2004). In experiments where MT dynamics were perturbed, MBC was added to the patch at a final concentration of 5 µg ml^{-1} MBC. Imaging was performed at 25˚C. Images were acquired on an inverted Spinning Disk Confocal (Roper/Nikon), equipped with Plan Apochromat 100×/1.4 NA objective lens (Nikon), a PIFOC (perfect image focus) objective stepper, and a charge-coupled device camera (EMCCD 512x512 QuantEM; Photometrics).

For spindle dynamics, stacks of 7 planes spaced by 1 µm were acquired for each channel with 200 msec exposure for 561 nm wave length and 100 msec exposure for 491 nm wavelength, binning one, and an electronic gain of 3 for both wavelengths. For each time-lapse movie, each stack was taken every minute for a duration of 180–240 min.

For intensity measurements, stacks of 11 planes spaced by 0.5 µm were acquired for each channel with 100 msec exposure, binning one, and electronic gain of 1 or 3. For each time-lapse movie, each stack was taken every minute for a duration of 90 min.

For MT bundle dynamics, stacks of 7 planes spaced by 1 µm were acquired for each channel with 100 msec exposure for 561 nm wave length and 100 msec exposure for 491 nm wavelength, binning one, and an electronic gain of 3 for both wavelengths. For each time-lapse movie, each stack was taken every 5 seconds for a duration of 30 min.

To quantify spores, bright-field images of zygotes were taken at random, and the spores inside the zygotes counted.

Image analysis

Analyses were performed by Metamorph 7.8 and ImageJ 1.52i. Maximum projections of each stack were performed for analysis of spindle dynamics and for presentation, unless stated otherwise, and sum projections for intensity measurements.

Spindle dynamics were determined by measuring the length of the mCherry-Atb2 signal between Sid4-GFP signals over time, or, when 5 µg ml^{-1} MBC was added, by measuring the distance between the SPBs over time, by a semi-automated Matlab program.

Intensity measurements were performed by linescan analysis, reading out the average intensity per pixel, subtracting the background and adding all positive values to obtain a total amount of intensity.

MT bundle dynamics were approximated by tracing the shape of mCherry-Atb2 signal on a kymograph, and calculating the slopes of the gathered curves to obtain the velocity of MT bundle gro*wt*h and shrinkage. The origins and the endings of the curves were approximated by extending the curves to the edges of the SPB signal. The movements of the SPB were accounted and corrected for in our measurements. The catastrophe frequency was approximated as a reciprocal value of time the MT bundle spent in elongation.

Image analysis: Feature detection

Microscopy images were analyzed with a semi-automated Matlab program that can find features in microscopy images. After the computer finds the features, they are presented to the user, and the user can manually correct them. In this work, the program was used to detect SPBs and spindles in maximal projections of confocal microscopy stacks. Features are represented as the coordinates of two points (SPBs) or a polygonal chain (spindle). The scripts for the analysis are integrated in a Graphical User Interface, and the complete version of the program is available upon request. We provide the Matlab scripts for feature extraction, and describe the pipeline of the analysis:

Cell segmentation: Initially, the user segments a cell manually in the relevant frames of the movie. Only the selected frames, and the area inside the segmented region of interest is considered for feature detection for a particular cell. Features are detected for each of the selected frames.

Spindle detection (1): Short spindles (less than 4 μm) are found by applying a 2 pixel gaussian blur to the image, and subsequently thresholding applying Otsu's method as provided in Matlab's *multithresh* (https://mathworks.com/help/images/ref/multithresh.htmL). Serial thresholding steps are applied until the area above the threshold contains less than 10% of the pixels of the region defined by the user as the cell. After that, regions above the threshold are merged using a convex hull algorithm as provided in Matlab's *bwconvhull* (https://mathworks.com/help/images/ref/bwconvhull.htmL) and the coordinates of the pixels above the threshold are fit to a first degree polynomial by regression. The resulting line is uniformly sampled such that points are distributed along its length at 1 pixel distance, and constitutes the

spindle feature. Once a spindle is found to be longer than 4 μm, spindles are detected using the method explained below.

Spindle detection (2): This method relies on the fact that long spindles are often aligned with the major axis of the cell. Therefore, the distance with respect to the major axis of the cell is very similar in neighboring pixels that belong to the spindle. A maximal projection of the image is made along the major axis of the region defined by the user as the cell; for each projected pixel, the position along the major axis ([X]), the intensity ([I]), and distance with respect to the major axis ([Y]) are stored in 1 dimensional vectors, noted in square brackets. To keep only the pixels belonging to the spindle, subsequent discarding steps are applied: A moving standard deviation filter of window size 3 pixels is applied to [Y], and positions with a standard deviation value above 3 pixels, are discarded. Next, groups ("group" denotes a series of consecutive non-discarded pixels) containing less than 4 pixels are discarded. Pixels for which the intensity in [I] is smaller than 20% of the maximum pixel in the cell are discarded. For the remaining groups, if the distance along the major axis [X] between the closest points of 2 consecutive groups divided by the difference in distance from the major axis [Y] between those two points is bigger than 1.5, the group that is furthest from the center of the cell is discarded. The curve defined by [Y] is smoothened applying a moving median filter of window size 10, resulting in [Y2]. Then, ([X,Y2]) coordinates are fit to a first order polynomial if the cumulative distance along the curve [X,Y2] is smaller than 8 μm, and to a third order polynomial otherwise. The resulting curve is sampled as described previously, and constitutes the spindle feature.

SPB detection: The 10 most intense pixels inside a cell are considered for the analysis. If 2 or more distinct regions are present in the image, the coordinates of the centroids of the two biggest regions are returned. If there is only one region (often when Spindle Pole Bodies are too close), the pixel with the weakest signal is removed from the analysis recursively until two distinct regions form, and the coordinates of the centroids of the two regions are returned. If this method does not succeed, the coordinates of the two most intense pixels in the cell are returned.

Image analysis: Measurements

Spindle length is defined from the N coordinates of the spindle polygonal chain $[x_1, x_2, \dots, x_N]$ and $[y_1, y_2, \dots, y_N]$, as the sum of Eucledian distances between consecutive points:

$$\sum_{i=1}^{N-1} \sqrt{(x_{i+1} - x_i)^2 + (y_{i+1} - y_i)^2}$$

SPB separation is defined as the Eucledian distance between the two points defining the spindle pole bodies.

Quantification and statistical tests

All graphs and box plots were generated using GraphPad 8.0 (Prism). All bar graph and line graph data are showed as mean values with error bars representing s.e.m. Data in box and whisker graphs are plotted by the Tukey method; the black line shows the median value. Analysis of statistical significance was performed by using Mann-Whitney or χ^2 test. Obtained p-values are shown within plots and data sets were defined as significantly different if $p<0.05$. n values, representing the number of cells, are shown inside the plots. All shown experiments were performed at least 3 times.

Supplementary table 1. List of strains used in this study.

Identifier	Genotype	Source
AP240	*h- ade6-M210 ura4-D18 leu1-32*	Lab collection
AP241	*h+ ade6-M210 ura4-D18 leu1-32*	Lab collection
TP2872	*h- cut7::Nat pkl1::ura4+ ade6-M210 ura4-D18 leu1-32*	Lab collection
TP2873	*h+ cut7::Nat pkl1::ura4+ ade6-M210 ura4-D18 leu1-32*	Lab collection
TP2927	*h- mCherry-Atb2:Hph leu1:Sid4-GFP leu1-32 ura4-D18*	Lab collection
TP2944	*h+ mCherry-Atb2:Hph leu1:Sid4-GFP leu1-32 ura4-D18*	Lab collection
TP3793	*h- GFP-Atb2:Hph Hht1-mCherry:Kan ade6-M210 ura4-D18 leu1-32*	This study
TP3794	*h+ GFP-Atb2:Hph Hht1-mCherry:Kan ade6-M210 ura4-D18 leu1-32*	This study
TP3653	*h- Hht1-mCherry:Kan GFP-Atb2:Hph cut7::Nat pkl1::ura4+ ade6-M210 ura4-D18 leu1-32*	This study
TP3654	*h+ Hht1-mCherry:Kan GFP-Atb2:Hph cut7::Nat pkl1::ura4+ ade6-M210 ura4-D18 leu1-32*	This study
TP2491	*h- cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph Sid4-GFP:Kan leu1-32 ura4-D18*	Lab collection
TP2492	*h+ cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph Sid4-GFP:Kan leu1-32 ura4-D18*	Lab collection
TP3453	*h+ Cdc13-GFP:Kan mCherry-Atb2:Hph ade6-M210 ura4-D18 leu1-32*	Lab collection
TP3454	*h- Cdc13-GFP:Kan mCherry-Atb2:Hph ade6-M210 ura4-D18 leu1-32*	Lab collection
TP4090	*h- Cdc13-GFP:Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph ade6-M210 ura4-D18 leu1-32*	This study
TP4091	*h+ Cdc13-GFP:Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph ade6-M210 ura4-D18 leu1-32*	This study
TP4224	*h- cut7::Nat pkl1::ura4+ Mal3-linker-GFP:Kan mCherry-Atb2::Hph ura4-D18 leu1-32*	This study
TP4225	*h+ cut7::Nat pkl1::ura4+ Mal3-linker-GFP:Kan mCherry-Atb2::Hph ura4-D18 leu1-32*	This study
TP116	*h- mCherry-Atb2::Hph ura4-D18 leu1-32*	Lab collection
TP319	*h+ mCherry-Atb2::Hph ura4-D18 leu1-32*	Lab collection

TP2508	*h- Pkl1-3xGFP:Nat mCherry-Atb2:Hph leu1-32 ura4-D18*	Lab collection
TP3834	*h+ Pkl1-3xGFP:Nat mCherry-Atb2:Hph leu1-32 ura4-D18*	Lab collection
TP2945	*h- Cut7-GFP:Kan mCherry-Atb2:Hph ade6-M210 ura4-D18 leu1-32*	Lab collection
TP2946	*h+ Cut7-GFP:Kan mCherry-Atb2:Hph ade6-M210 ura4-D18 leu1-32*	Lab collection
TP3166	*h- klp2::Kan cut7::Nat pkl1::ura4+ leu1-Sid4-GFP mCherry-Atb2:Hph leu1-32 ura4-D18*	Lab collection
TP3167	*h+ klp2::Kan cut7::Nat pkl1::ura4+ leu1-Sid4-GFP mCherry-Atb2:Hph leu1-32 ura4-D18*	Lab collection
TP3187	*h- dhc1::Kan cut7::Nat pkl1::ura4+ leu1:Sid4-GFP mCherry-Atb2:Hph ade6-M210 ura4-D18 leu1-32*	Lab collection
TP4542	*h+ dhc1::Kan cut7::Nat pkl1::ura4+ leu1:Sid4-GFP mCherry-Atb2:Hph ade6-M210 ura4-D18 leu1-32*	This study
TP3175	*h- dam1::Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph leu1:Sid4-GFP ade6-M210 ura4-D18 leu1-32*	Lab collection
TP4531	*h- dam1::Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph leu1:Sid4-GFP ura4-D18 leu1-32*	This study
TP3710	*h+ cut7::Nat pkl1::ura4+ rec8::Kan mCherry-Atb2:Hph leu1:Sid4-GFP ura4-D18 leu1-32*	This study
TP3891	*h- cut7::Nat pkl1::ura4+ rec8::Kan mCherry-Atb2:Hph leu1:Sid4-GFP ade6-M210 ura4-D18 leu1-32*	Lab collection
TP3905	*h- Klp2-GFP:ura4+ mCherry-Atb2:Hph ura4-D18 leu1-32*	Lab collection
TP4537	*h+ Klp2-GFP:ura4+ mCherry-Atb2:Hph ura4-D18 leu1-32*	This study
TP4445	*h+ Taz1-GFP:Kan Sid4-mCherry:Hph*	This study
TP4446	*h- Taz1-GFP:Kan Sid4-mCherry:Hph*	This study
TP4298	*h+ cut7::Nat pkl1::Kan Taz1-GFP:Kan Sid4-mCherry:Hph*	This study
TP4299	*h+ cut7::Nat pkl1::Kan Taz1-GFP:Kan Sid4-mCherry:Hph*	This study
TP4218	*h+ rec11::Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph leu1:Sid4-GFP*	This study
TP4219	*h- rec11::Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph leu1:Sid4-GFP*	This study
TP4221	*h+ rec12::Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph leu1:Sid4-GFP*	This study

TP4222	*h- rec12::Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph leu1:Sid4-GFP*	This study
TP4229	*h- sgo1::Kan cut7::Nat pkl1::ura4+ leu1:Sid4-GFP mCherry-Atb2:Hph*	This study
TP4230	*h+ sgo1::Kan cut7::Nat pkl1::ura4+ leu1:Sid4-GFP mCherry-Atb2:Hph*	This study
TP4539	*h+ moa1::Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph leu1:Sid4-GFP*	This study
TP4538	*h- moa1::Kan cut7::Nat pkl1::ura4+ mCherry-Atb2:Hph leu1:Sid4-GFP*	This study
TP1511	*h+ Mis12-GFP:leu2+ Sid4-mCherry:Hph ura4-D18 leu1-32*	Lab collection
TP2943	*h- Sid4-mCherry:Hph Mis12-GFP:leu2+ ura4-D18 leu1-32*	Lab collection
TP3223	*h- cut7::Nat pkl1::ura4+ Sid4-mCherry:Hph Mis12-GFP:leu2+ ade6-ura4-D18 leu1-32*	Lab collection
TP3062	*h+ cut7::Nat pkl1::ura4+ Sid4-mCherry:Hph Mis12-GFP:leu2+ ade6- ura4-D18 leu1-32*	Lab collection

Discussion

Spindle dynamics are distinguishing mitotic and meiotic spindles

In this work, spindle dynamics were explored and compared simultaneously in mitosis and meiosis, in the same organism, for the first time. This work has shown mitotic, MI and MII spindles exhibit distinct spindle dynamics patterns (Figure 1E in Results section), and that the characterized attributes of spindles are intrinsically different for each spindle type (Table 1 in Results section). Moreover, MT bundle dynamics measured in the time interval between two mitotic divisions and between two meiotic divisions showed that MT bundles are more dynamic in zygotes than in vegetative cells, as they undergo catastrophe more often (Table 2 in Results section). These data clearly show that mitotic and meiotic spindles are differently regulated, although they perform the same function, i.e. chromosome segregation, and that MT dynamics may be modified to fine tune spindle function in different cell-division types.

Zygotes are approximately 27% larger than vegetative cells, and assemble an MI spindle that is longer in all three spindle elongation phases. The phenomenon of spindle length scaling with cell size was observed in fission yeast previously (Krüger *et al.*, 2019), but also in other organisms (Wühr *et al.*, 2008; Hara and Kimura, 2009; Yang *et al.*, 2016), and it can be simply explained by a limited components model, i.e. limiting cytoplasmic components (Good *et al.*, 2013).

In mitosis of fission yeast, the spindle spends roughly half of its life in phase III, which is when spindle elongates and further separates sister chromatids, unlike MI and MII spindles, which spend nearly half or 2/3 of their life in phase II, respectively. As mitosis occurs in smaller cells, which additionally septate, i.e. produce a wall at the cell equator to separate daughter cells into two separate entities at the end of mitosis (Wolfe and Gould, 2005; Sipiczki, 2007), the principal role of the mitotic spindle may be to remove the sister-chromatids as far away from the site of cytokinesis to prevent the chromosome cut phenotype. In fission yeast meiosis there is no

cytokinesis to cause cut chromosomes, so there may be no necessity for the spindle to remain in phase III. In line with that, MII final spindle length is 45% shorter than mitotic spindle length, and only 15% of spindle life is spent in phase III, yet the DNA is successfully separated.

In MI, homologous chromosomes are associated into a bivalent, and physically linked at chiasmata (Molnar *et al.*, 2003; Hirose *et al.*, 2011). Possibly the MI spindle spends the majority of its life in phase II to resolve the chiasmata intermediates before homologous chromosomes are pulled to the opposite spindle poles at the end of phase II. That eggs are arrested at metaphase I or metaphase II, which coincides with phase II of meiosis spindle elongation in fission yeast, has been observed in many systems, including humans (Sagata, 1996; Tunquist and Maller, 2003; Sanders and Jones, 2018). Possibly, these phenomena are somehow correlated and may prove an interesting direction for future research.

Klp2 co-operates with Pkl1 to antagonize Cut7 in MI spindle

An important observation in this study was the failure of *cut7Δpkl1Δ* mutant to successfully complete meiosis, although mitosis outcome is successful (Olmsted *et al.*, 2014; Rincon *et al.*, 2017; Yukawa *et al.*, 2017; Shirasugi and Sato, 2019). Our study revealed that spindle assembly and elongation are specifically perturbed in MI, but not mitosis or MII. This confirms the hypothesis that meiotic and mitotic spindles are intrinsically different.

We confirmed that Cut7 and Pkl1 localize similarly in mitosis and MI spindle, but their relative amounts are changed, such that the ratio of Cut7 to Pkl1 is ~2 times higher in MI (Figure 3 in the Results section). Consequently, there are more Cut7 molecules to produce outward pushing force in MI spindle compared to the mitotic spindle. This has led us to speculate, that another molecule may antagonize Cut7. We have found that Klp2 partially counteracts Cut7-dependent forces in MI

and restores pre-phase III spindle length (Figure 4C in the Results section). Moreover, Klp2 localized more to the spindle poles in MI spindles, contrary to its dispersed localization along the spindle in mitosis (Figure 4D and E in in the Results section). It was shown previously that, in mitotic spindles, tethering Klp2 to the spindle poles in Pkl1 deficient cells allows it to produce inward forces to counteract Cut7-dependent forces (Yukawa *et al.*, 2018). We conclude that although Klp2 concentration is comparable in mitotic and MI spindles (Figure 4F in the Results section), its MI-specific localization to the spindle poles permits it to antagonize Cut7. The mechanism behind this regulation remains an open question. Considering that other organisms like mice and humans possess multiple kinesin-14s, it would be interesting to see if such kinesin-14 specialization in MI is evolutionary conserved.

MT dynamics in zygotes and vegetative cells may fine tune spindle function

In this study, we have observed that MI and MII spindles took longer time to assemble and establish a steady-state length, and have accomplished it at comparable velocities (Table 1 in the Results section). This has prompted us to investigate if spindle dynamics may differ from mitosis to meiosis. As technical limitations prevented us from studying MT dynamics inside the prometaphase spindles because their size precludes us from monitoring MT dynamics, we have opted to test MT bundle dynamics in between two mitotic divisions and the meiotic divisions.

We found MT bundles in zygotes grew faster and underwent catastrophe more often than in vegetative cells (Table 2 in the Results section). Under the assumption that MTs are more dynamic in MI, we have inhibited MT dynamics in *cut7Δpkl1Δ* zygotes by treatment with the MT drug MBC (Vela-Corcía *et al.*, 2018). Indeed, approximately 80% of treated *cut7Δpkl1Δ* zygotes assembled bipolar spindles compared to 26% of non-treated *cut7Δpkl1Δ* zygotes (Figure S5A in the Results section). Moreover, mitotic spindle dynamics were not sensitive to treatment with MBC, unlike MI

spindle dynamics, in both *wt* and *cut7Δpkl1Δ* zygotes (Figure 5D and FigureS5B in the Results section).

In zygotes, KTs are located further away from the SPBs, while in mitosis they are located underneath the SPBs (Figure 4.4A in the Introduction section) (Hironori *et al.*, 1993). This configuration may favour more dynamic MTs, as more frequent catastrophe would increase the chance of MT growing in the direction of the KT, and subsequent MT-KT attachment (Mitchison and Kirschner, 1984; Heald and Khodjakov, 2015).

In line with this, lower concentration of kinesin-14 Pkl1 in MI may further aid successful KT-MT attachment. Pkl1 was shown to negatively influence MT nucleation in fission yeast, and Cut7 was shown to inhibit this effect (Olmsted *et al.*, 2014). It may be that lower Pkl1 concentration in MI spindles favours more frequent MT nucleation. Moreover, as Pkl1 cross-links parallel MTs and organizes the spindle poles (Syrovatkina and Tran, 2015; Yukawa, Ikebe and Toda, 2015), its scarcity in in MI spindles could lead to MTs that are able to more freely explore the area around them (Figure S5F in the Results section) (Kalinina *et al.*, 2013; Cojoc *et al.*, 2016; Winters *et al.*, 2019). Further investigations on role of Pkl1 and Klp2 in MI spindles are needed, and measuring MT dynamics inside spindles is a necessary next step to confirm current findings.

Chromosome architecture is not a decisive factor in successful MI spindle assembly in *cut7Δpkl1Δ* zygotes

Arguably the most prominent difference between mitosis and MI is the chromosomal organization (Figure 2.4A in the Introduction section) (Ohkura, 2015). It was shown prior that cohesin influences the length of the phase II spindle (Goshima, Saitoh and Yanagida, 1999; Goshima *et al.*, 2005), and

proper tension between sister-chromatids is a feature used by the cell to monitor and amend proper MT-KT attachments (Musacchio and Salmon, 2007; Liu *et al.*, 2009).

We could not detect the influence of MI-specific chromosomal organization on spindle assembly in the *cut7Δpkl1Δ* background, but a recent study that studied homothallic strains in meiosis, has demonstrated that a double deletion of Rec12 and Moa1 partially rescued spindle bipolarity in *cut7Δpkl1Δ* background (Shirasugi and Sato, 2019). However, a quadruple mutant still showed only a modest, ~50% success in SPB separation, indicating that chromosomal architecture is not a pivotal factor in successful bipolarity establishment in *cut7Δpkl1Δ* MI.

Future perspectives for mitotic and meiotic spindle comparison

The work presented in this dissertation is but a beginning in comparative spindle studies. While Cut7, Pkl1, and Klp2 localization and intensities were investigated and compared, other motors and MAPs remain to be studied. Moreover, while localization and amounts of these three molecules are described, the regulation behind their respective peculiarities in MI compared to mitosis remains to be examined.

MII spindles remain somewhat ambiguous. While MI and mitotic spindle analyses are relatively straightforward, MII spindles are strongly impacted by the successfulness of preceding MI division. In order to probe MII spindles, new methodologies concerning protein inhibition and over-expression must be developed to specifically perturb a function of a molecule in MII but not in MI. Further, it is possible to study the role of many essential genes in mitosis because thermo-sensitive alleles of these genes exist, and as such, these proteins can be inactivated at 36°C. Such an approach is somewhat feasible in meiotic spindle studies, under the condition that conjugation and recombination are allowed to occur at permissive temperature (meiotic life cycle triggering is

severely inhibited at temperatures above 31°C). Generally speaking, methods to manipulate protein expression in meiosis are lacking, but are essential to perform meiotic spindle studies. Future work will surely have to be oriented towards new technology development, especially control of protein levels in meiosis.

This study has identified that MT dynamics and kinesin-14s are differently regulated in mitotic and MI spindles to coordinate chromosome segregation. In studies to follow, a more in depth characterization of the spindle must be achieved, that will ultimately allow us to manipulate the spindle in an efficient and targeted manner for therapeutic purposes.

Résumé

1. Introduction

1.1. Cell division

La cellule est une entité constituée de plusieurs organites communs aux organismes connus. Au cours de sa vie, la cellule effectue plusieurs cycles successifs composés de plusieurs phases. Chaque cycle cellulaire comprend l'interphase - comprenant les phases G1, S et G2 - suivie de la division cellulaire appelée mitose. Généralement, en phase G1 la cellule croît et se prépare à la réplication de son ADN (acide désoxyribonucléique) génomique qui aura lieu en phase S. Au cours de la phase G2, la cellule continue sa croissance et se prépare à la division cellulaire. Chez les eucaryotes, on distingue deux types de division cellulaire : la mitose et la méiose. La mitose permet la prolifération cellulaire qui consiste à générer deux cellules filles identiques tandis que la méiose permet de produire des cellules destinées à la reproduction sexuée. La mitose et la méiose se succèdent après un unique cycle de duplication de l'ADN – les cellules de la mitose se divisent une fois alors que lors de la méiose les cellules se divisent deux fois. La tâche impérative de la division cellulaire est de séparer précisément le matériel génomique dupliqué en deux cellules filles identiques.

1.1.1. La mitose

La mitose est un type de division cellulaire dans laquelle deux cellules filles identiques sont générées à partir d'une cellule parent unique, conservant la ploïdie parentale. C'est un processus essentiel dont le but est la prolifération cellulaire - qu'il s'agisse d'augmenter le nombre de cellules, pour permettre par exemple la croissance et le maintien d'un organisme multicellulaire, sa reproduction, comme par exemple dans le cas de la reproduction végétative des plantes. Par conséquent, il est essentiel que la ségrégation des chromosomes ne comporte aucune erreur car les inexactitudes peuvent conduire à une aneuploïdie qui pourrait entrainer des conséquences

catastrophiques telles que le cancer, des défauts au cours du développement ou la mort cellulaire (Holland and Cleveland, 2009; Thompson, Bakhoum and Compton, 2010).

Pour obtenir une séparation parfaite des chromosomes nécessaire au maintien de la ploïdie, les cellules forment un fuseau mitotique (Gatlin and Bloom, 2010; Heald and Khodjakov, 2015). Le fuseau mitotique est une structure macromoléculaire composée de microtubules (MTs), de protéines motrices et de protéines non motrices (David J. Sharp, Rogers and Scholey, 2000; Manning and Compton, 2008). Le fuseau mitotique est formé lors de la prophase lorsque les centrosomes, centres organisateurs des microtubules (MTOC) majeurs, procèdent à la nucléation des MTs (Rale, Kadzik and Petry, 2018). Les MTs en croissance sont attachées au centrosome par leur extrémité “moins” et leur extrémité “plus” *distale* leur permet d’explorer l’espace qui les entourent à la recherche d’autres MTs ou kinétochores (KTs), structures protéiques spécialisées situées dans la région centromérique de l'ADN qui servent de plate-forme pour la fixation du chromosome au fuseau médiée par les MTs.

La bipolarité du fuseau est établie lorsque l'interdigitation des MT des centrosomes opposés, sont réticulés via des moteurs et des protéines associées aux MT (MAPs) en faisceaux antiparallèles *qui glissent dans deux directions opposés* (Tanenbaum and Medema, 2010). Les chromosomes, qui sont attachés aux MTs du fuseau via les KT, congressent vers sur la plaque métaphasique et s'alignent par une interaction complexe de forces qui maintiennent une longueur de fuseau à l'état d’équilibre (Goshima *et al.,* 2005; Dumont and Mitchison, 2009). Une fois que tous les KT sœurs sont attachés aux MTs émanant du pôle opposé du fuseau (les chromosomes sont bi-orientés), le point de contrôle de l'assemblage du fuseau (‘spindle assembly checkpoint’ - SAC) est satisfait (Musacchio and Salmon, 2007). Cela active alors la séparase (Peters, 2006), qui clive ensuite la

cohésine, un complexe protéique qui relie les chromatides sœurs, permettant ainsi aux chromosomes d'être tirés vers les pôles opposés du fuseau en anaphase A.

On distingue deux mécanismes gouvernant les mouvements des chromosomes au cours de l' anaphase A: les flux nommés Pac-Man et "poleward", "vers le pôle". Dans le flux Pac-Man, les MTs attachés aux KTs (fibre- KT) dépolymérisent à l'extrémité plus et rétrécissent vers le pôle du fuseau, transportant les chromosomes aux deux pôles de façon simultanée (Gorbsky, Sammak and Borisy, 1987). Dans le flux 'poleward', le chromosome est translocalisé vers le pôle du fuseau par dépolymérisation de la fibre-KT près du pole du fuseau. (Mitchison, 1989).

Puis, un allongement rapide du fuseau a lieu en anaphase B qui permet la séparation du matériel génétique du site de la cytokinèse. La mitose se termine par l'obtention deux cellules filles qui ont le même nombre de chromosomes que la cellule mère.

1.1.2. La Méiose

La Méiose est un type de division cellulaire produisant des cellules porteuses de la moitié du matériel génétique de la cellule initiale. Ces cellules sont utilisées dans la reproduction sexuée lorsqu'elles fusionnent et produisent un zygote diploïde. La méiose est composée de deux divisions consécutives après un cycle de duplication de l'ADN. Contrairement à la mitose, les chromosomes homologues s'apparient en prophase I de méiose et subissent une recombinaison homologue (Page and Hawley, 2003). Ce processus donne naissance à de nouvelles combinaisons d'ADN et favorise la variabilité génétique, qui pourrait être le but de la reproduction sexuée. Une ségrégation chromosomique précise dans les deux divisions méiotiques est requise. Chez l'homme, la ségrégation chromosomique dans la méiose féminine est particulièrement sujette aux erreurs (Warburton, 1997; Holubcová *et al.*, 2015; Namgoong, Kim and Christenson, 2018), et de tels défauts entraînent des troubles congénitaux ou des avortements.

De façon similaire à la mitose, un fuseau composé de MTs est assemblé pour séparer de façon identique le matériel génétique en deux cellules filles. Il est important de noter que les ovocytes de nombreuses espèces n'ont pas de centrosomes (Müller-Reichert *et al.*, 2010; Dumont and Desai, 2012; Bennabi, Terret and Verlhac, 2016; Gruss, 2018) et dépendent d'autres voies de nucléation des MTs (chapitre 3). L'une des caractéristiques de la méiose est la nature réductrice de la première division méiotique (MI). Plusieurs mécanismes permettent d'assurer la réduction de moitié de la ploïdie: 1) architecture modifiée des KT sœurs et mono-orientation (Yokobayashi and Watanabe, 2005; Hauf *et al.*, 2007; Sakuno *et al.*, 2011); 2) Cohésine spécialisée et contrôle hiérarchique du clivage de la cohésine par la séparase (Watanabe and Nurse, 1999; Tomoya S. Kitajima *et al.*, 2003; Kitajima, Kawashima and Watanabe, 2004; Miyazaki *et al.*, 2017). Ces mécanismes garantissent que les chromosomes homologues se mono-orientent, c'est-à-dire les KT sœurs, s'attachent aux MTs émanant du même pôle du fuseau. Lorsque le point de contrôle de l'assemblage du fuseau est satisfait, la séparase clive la cohésine entre les bras chromosomiques, mais pas dans la région centromérique, où la cohésine est protégée (Miyazaki *et al.*, 2017). L'allongement du fuseau au cours de l'anaphase B de la méiose I (MI) garantit que les chromosomes homologues bivalents sont suffisamment séparés, et le MI se termine par deux cellules filles qui portent la moitié du nombre de chromosomes par rapport à la cellule mère.

Chez certaines espèces, la méiose I est suivie d'une brève période de repos appelée interkinésie, une forme cryptique d'interphase, dans laquelle les deux noyaux de cellules filles peuvent être observés. La méiose II (MII) est souvent comparée à la mitose, car l'architecture chromosomique est organisée de manière similaire: un fuseau se forme dans chaque noyau et sépare les chromatides sœurs en deux nouvelles cellules filles. Quatre cellules filles contenant la moitié de la quantité initiale de matériel génétique sont ainsi formées en fin de MII.

Dans les ovocytes femelles, la division cellulaire est très asymétrique en MI, et les produits de la méiose I sont contenus dans un œuf mature, contenant un nombre de chromosomes réduit de moitié, et un petit premier corps polaire qui ne joue plus de rôle (Sanders and Jones, 2018). L'ovule mature entre en méiose II, et s'arrête en métaphase jusqu'à ce qu'il soit fécondé par un sperme. La méiose II est également asymétrique et, lors de la fécondation et de la reprise de la division, un embryon et un deuxième corps polaire sont formés.

1.2. La levure fissipare *S. pombe* comme système modèle pour l'analyse de la dynamique du fuseau mitotique et méiotique

La levure fissipare *Schizosaccharomyces pombe* (*S. pombe*) est un organisme eucaryote unicellulaire en forme de bâtonnet qui atteint généralement une longueur de 12 à 14 µm à la division mitotique. En raison de la simplicité de l'organisation de son cytosquelette de MTs, de la conservation évolutive des principaux acteurs de l'assemblage et de la fonction du fuseau, et du fait que, comme chez les eucaryotes supérieurs, plusieurs MT se fixent à un KT (Ding, McDonald and McIntosh, 1993), cet organisme constitue un modèle exceptionnel d'intérêt pour l'étude du fuseau mitotique et méiotique.

Lorsqu'elle se trouve dans des conditions riches en nutriments, la levure fissipare *S. pombe* vit principalement comme un haploïde et entre une mitose pour proliférer. Comme les cellules de mammifères, cet organisme suit la progression typique des étapes du cycle cellulaire: G1, S, G2 et M (Mitchison and Creanor, 1971; Nurse, Thuriaux and Nasmyth, 1976). Dans des conditions de carence , notamment en cas de déficit en azote, la levure de fission quitte le cycle cellulaire végétatif et entre dans la voie de la méiose (Harigaya and Yamamoto, 2007). On distingue deux types sexuels dans les cellules de levure *S. pombe* : h + et h-. Si les cellules voisines sont de type sexuel opposé

et sont privées d'azote, elles peuvent fusionner et former un zygote. Le zygote diploïde peut proliférer en tant que diploïde, si un milieu riche en nutriments devient disponible, ou continuer avec des divisions méiotiques. À la fin de la méiose, le zygote aura formé quatre noyaux haploïdes encapsulés par une membrane générant quatre spores haploïdes. Une fois que les conditions sont favorables, les spores haploïdes peuvent germer et recommencer le cycle cellulaire végétatif. Bien que la privation d'azote induise la conjugaison des cellules haploïdes en zygotes, les cellules haploïdes peuvent néanmoins continuer à proliférer par mitose. Cela permet d'étudier les deux types de division cellulaire simultanément, dans les mêmes conditions.

L'assemblage et l'allongement du fuseau sont stéréotypés dans la levure fissipare *S. pombe*, tant dans la mitose que dans la méiose, et se composent de trois étapes distinctes (Nabeshima, Nakagawa, Straight, Murray, *et al.*, 1998). La phase I de l'assemblage du fuseau en prophase se réfère aux étapes initiales de la formation du fuseau, comme par exemple l'établissement de bipolarité. La phase II est marquée par un maintien de la longueur du fuseau à l'état d'équilibre, accompagné d'une congression chromosomique vers la plaque métaphasique. A la fin de la phase II, les chromatides sœurs sont séparées aux pôles opposés. Enfin, en phase III, le fuseau s'allonge rapidement et permet la séparation des chromosomes.

1.2.1. Comparaison de la dynamique du fuseau mitotique et méiotique

La mitose et la méiose ont fait l'objet de recherches approfondies et de nombreuses études ont explore le rôle des moteurs moléculaires, des MAPs et de la dynamique des MT du fuseau. Malgré de nombreux travaux de recherche, aucune étude à ce jour n'a comparé la dynamique du fuseau dans la mitose et la méiose de façon simultanée dans le même système modèle. Très probablement en raison du fait qu'une telle tâche reste techniquement impossible dans de nombreux systèmes, ou

bien qu'il existe trop de facteurs qui pourraient rendre difficile l'interprétation des données. De plus, de telles études chez l'homme sont extrêmement compliquées, car les ovocytes sont considérés comme une ressource biologique précieuse. Compte tenu de la nature fondamentale des processus mitotiques et méiotiques, une comparaison de la dynamique du fuseau mitotique et méiotique serait essentielle pour explorer respectivement les différences et les particularités de chaque type de division cellulaire.

La levure de fission est devenue un bon système modèle pour une telle étude. Son cytosquelette de MTs est simple et bien caractérisé, et les principaux acteurs moléculaires de l'assemblage du fuseau sont conservés de la levure fissipare à l'homme. Il est important de noter que la levure *S. pombe* peut subir une mitose et une méiose dans le même environnement et dans les mêmes conditions, ce qui rend possible une étude comparative de la dynamique du fuseau mitotique et méiotique de façon simultanée.

Dans des expériences conçues pour comparer la dynamique du fuseau mitotique et méiotique, des cellules de types sexuels opposés contenant des composants du fuseau marqué par des molécules fluorescentes ont d'abord été cultivées dans un milieu riche (YE5S) pendant une nuit, puis mélangées sur un milieu pauvre en azote (ME) de façon à induire la conjugaison. Après 16-20 heures, les cellules ont été remises en suspension dans 10 µL de ME liquide et transférées sur des coussins semi-solides composés d'agarose et de milieu malt-extrait (ME) et imagées pendant plusieurs heures. En suivant ce protocole, un champ de cellules contenant un mélange de zygotes et de cellules végétatives a pu être identifié et imagé avec le microscope confocal à disque rotatif (spinning disk).

1.2.2. Le double mutant de délétion kinésine-5 /kinésine-14 (*cut7Δpkl1Δ*) comme outil permettant la comparaison des fuseaux mitotiques et méiotiques

Chez *S. pombe*, lors de la phase I de l'assemblage du fuseau, les MTs en croissance sont regroupés en faisceau antiparallèle par la kinesine-5 /Cut7 (Hagan and Yanagida, 1990, 1992a). Le moteur homotétramérique Cut7 dirigé à l'extrémité plus des MTs exerce des forces sur le faisceau antiparallèle et fait glisser les MTs vers l'extérieur, repoussant ainsi les 'spindle pole body' (SPB, équivalent du centrosome chez *S. pombe*) permettant d'établir la bipolarité. Cut7 est une protéine essentielle et il a été montré que les mutants Cut7 thermosensibles assemblent des fuseaux mitotiques monopolaires (Hagan and Yanagida, 1990, 1992a).

Un autre acteur dans les étapes initiales de l'assemblage du fuseau chez *S. pombe* est une protéine motrice dirigée vers l'extrémité "moins" des MTs, la kinésine 14 Pkl1, localisée au SPB et faiblement aux MTs du fuseau aux étapes initiales de l'assemblage du fuseau mitotique (Pidoux, LeDizet and Cande, 1996; Syrovatkina and Tran, 2015; Yukawa, Ikebe and Toda, 2015). Pkl1 forme un complexe avec les protéines Msd1 et Wdr8, important pour l'ancrage du fuseau microtubulaire aux SPBs (Toya *et al.*, 2007; Yukawa, Ikebe and Toda, 2015). De plus, Pkl1 réticule probablement des faisceaux de MTs parallèles pour fournir une stabilité structurelle aux pôles du fuseau en raison de la force exercée par Cut7 vers l'extérieur. Dans les cellules de *S. pombe* dépourvues d'activité de Pkl1, les pôles du fuseau ne sont pas focalisés, ce qui est évident dans la formation de protrusion MT médiée par Cut7, pouvant induire une aneuploïdie (Syrovatkina and Tran, 2015).

Fait intéressant, la double délétion de Pkl1 et de Cut7 permet à la cellule mutante de former un fuseau bipolaire mitotique capable de séparer les chromosomes; de plus, la relation antagoniste de

Cut7 et Pkl1 est largement étudiée dans les fuseaux mitotiques de *S. pombe* (Rincon *et al.*, 2017; Yukawa *et al.*, 2017). Cependant, les zygotes *cut7Δpkl1Δ* sont incapables de poursuivre normalement la méiose. En effet, à la fin de la méiose, les zygotes *cut7Δpkl1Δ* ne produisent pas quatre spores régulières. Ce résultat suggère que les chromosomes de ce double mutant ne peuvent pas être séparés et que la mécanique du fuseau peut être compromise. Ainsi, dans ce travail, il a été étudié pourquoi la méiose échoue alors que la mitose se déroule normalement chez le mutant *cut7Δpkl1Δ* de *S. pombe,* qui a été utilisé comme outil pour étudier les différences dans les fuseaux mitotiques et méiotiques.

2. Resultats

2.1. La Dynamique du fuseau diffère en mitose et en méiose dans la levure fissipare

Afin d'évaluer la dynamique du fuseau dans la mitose et la méiose, nous avons imagé des cellules de levure fissipare *S. pombe* de type sauvage (*wt* - wild type) exprimant l'alpha-2 (α2) tubuline marquée avec mCherry (mCherry-Atb2) et le composant SPB Sid4 marqué avec GFP α2-tubuline (Sid4-GFP), cultivé dans du malt- extrait (ME) afin d'induire la conjugaison. Pour évaluer la dynamique du fuseau, nous avons mesuré la longueur du signal entre les deux SPB à des intervalles de temps d'une minute. Il a été constaté que les fuseaux MI et MII présentaient une progression en trois phases similaires aux fuseaux mitotiques.

La longueur finale du fuseau MI a été augmentée de 25% en moyenne par rapport aux fuseaux mitotiques, et la vitesse d'élongation du fuseau MI en phase III était plus rapide qu'en mitose. Contrairement aux cellules mitotiques végétatives, les zygotes ont passé la plus grande partie du temps d'élongation du fuseau en phase II de MI et MII. Enfin, les zygotes ont passé une proportion similaire de temps dans la phase I de MI et MII, mais la vitesse d'assemblage du fuseau chez les

zygotes était plus faible que dans les cellules mitotiques. Ces différences dans la dynamique du fuseau impliquaient des changements qualitatifs et/ou quantitatifs dans les composants du fuseau, tels que les moteurs moléculaires et les MAPs, ou des changements dans la dynamique MT entre mitose et méiose.

2.2. L'intégrité du fuseau est compromise spécifiquement en MI dans les zygotes du double mutant *cut7Δpkl1Δ*

Pour étudier la dynamique du fuseau dans la méiose du double mutant *cut7Δpkl1Δ*, nous avons imagé des zygotes *cut7Δpkl1Δ* exprimant mCherry-Atb2 et Sid4-GFP. Contrairement à la mitose, où toutes les cellules *cut7Δpkl1Δ* ont assemblé un fuseau bipolaire, les zygotes *cut7Δpkl1Δ* ont échoué en méiose: 74 ± 2% des zygotes cut7Δpkl1Δ n'ont pas réussi à établir un fuseau bipolaire et à séparer les SPB en MI, comme cela a été observé dans un rapport récent (Shirasugi and Sato, 2019). De plus, parmi les zygotes qui avaient assemblé des fuseaux bipolaires en MI, 8 ± 2% supplémentaires n'ont pas réussi à assembler de fuseau en MII. Une séparation des SPB infructueuse en MII, pourrait résulter d'erreurs dans la MI précédente ou des propriétés intrinsèques des fuseaux *cut7Δpkl1Δ* de MII.

Nous avons ensuite comparé la dynamique des fuseaux de cellules *cut7Δpkl1Δ* et *wt*. En mitose, les fuseaux pré-phasiques III étaient plus courts et l'entrée en phase III était retardé dans le doubles mutant *cut7Δpkl1Δ* comparativement aux cellules *wt*, ce qui corrobore avec les études précédentes (Rincon *et al.*, 2017; Yukawa *et al.*, 2018). Des résultats similaires ont été observés pour la dynamique du fuseau MII. En revanche, la plupart des fuseaux MI sont restés monopolaires. 25%des fuseaux MI *cut7Δpkl1Δ* qui ont réussi à atteindre la bipolarité et à séparer les SPB n'ont pas montré la progression triphasée habituelle de l'allongement du fuseau, car les phases I et II (de

l'allongement du fuseau) n'ont pas pu être distinguées. Combinées, nos données indiquent que l'assemblage et l'allongement du fuseau sont spécifiquement perturbés pendant l'IM des zygotes cut7Δpkl1Δ. Nous concluons que la suppression de Pkl1 ne peut pas compenser entièrement l'absence de Cut7 en MI. Cela suggère que Cut7 et Pkl1 sont régulés différemment en mitose et en MI.

2.3. Le ratio Cut7-à-Pkl1 est plus élevé dans le fuseau MI qu'en mitose

Afin d'évaluer si Cut7 et Pkl1 sont régulés différemment en mitose et en MI, nous avons imagé Cut7 ou Pkl1 marqués avec la GFP et les MT avec mCherry-Atb2 en mitose et en MI dans les cellules *wt*. Nous avons observé que Cut7-GFP était localisé au fuseau et aux pôles du fuseau depuis l'assemblage jusqu'à sa rupture en mitose et en MI. De même, le profil de localisation de Pkl1-3XGFP est apparu inchangé entre la MI et la mitose. Pkl1-3XGFP est localisé aux pôles du fuseau peu de temps après le début de son assemblage, et reste associé aux SPB jusqu'à sa rupture. Pkl1-3XGFP a également été détecté sur le fuseau lors de la transition entre la phase I et la phase II.

Nous avons ensuite mesuré la distribution du signal Cut7-GFP et Pkl1-3XGFP au fuseau pendant la transition de la phase I à la phase II, 1 minute avant que le fuseau n'atteigne une longueur constante qui marque le début de la phase II. L'intensité maximale de Cut7-GFP était plus élevée dans en MI, tandis que l'intensité maximale de Pkl1-3XGFP était similaire en mitose et en MI. De plus, l'intensité totale de Cut7-GFP, calculée en intégrant les profils d'intensité du 'linescan', était élevée en MI comparativement à la mitose, tandis que l'intensité totale de Pkl1-3XGFP est restée similaire dans les deux types de fuseau.

Parce que l'intensité totale des MTs est différente dans fuseaux mitotiques et en MI, nous avons normalisé les valeurs d'intensité obtenues pour Cut7-GFP et Pkl1-3XGFP aux valeurs d'intensité

des MTs. De cette façon, nous avons constaté que la concentration relative de Cut7 restait inchangée, tandis que la concentration relative de Pkl1-3XGFP par MT était plus faible en MI que celle observée en mitose. Cela peut expliquer pourquoi la suppression de Pkl1 ne peut pas compenser la suppression de Cut7 en MI, et suggère qu'un facteur supplémentaire pourrait contrecarrer l'activité de Cut7 pendant la phase II de MI pour maintenir l'équilibre des forces.

2.4. La fonction de la kinésine 14 Klp2 exercée sur le fuseau est distincte en MI et en mitose

Afin d'identifier des de nouveaux composants du fuseau qui pourraient contrecarrer les forces dépendantes de Cut7 en méiose, nous avons systématiquement délété les moteurs et les MAPs candidat-e-s dans le fond génétique *cut7Δpkl1Δ*, croisé les triples mutants et évalué le nombre de spores. De toutes les protéines testées, seule la suppression de la kinésine-14 Klp2 a induit la formation de zygotes qui ont produit quatre spores typiques de façon plus fréquente (44 ± 2% des zygotes *cut7Δpkl1Δklp2Δ* ont produit quatre spores versus 20 ± 1% dans le fond génétique/les doubles mutants de délétion *cut7Δpkl1Δ*).

L'imagerie des cellules vivantes a révélé que si seulement 26 ± 2% des zygotes *cut7Δpkl1Δ* ont réussi à établir la bipolarité du fuseau et à séparer les SPB, 49 ± 2% des zygotes *cut7Δpkl1Δklp2Δ* ont assemblé des fuseaux bipolaires en MI. La comparaison de la dynamique du fuseau de MI a révélé que non seulement la bipolarité du fuseau a été rétablie, mais la dynamique d'élongation du fuseau habituellement tri-phasique a été partiellement complémentée chez les zygotes *cut7Δpkl1Δklp2Δ*. Cela différait fortement des zygotes *cut7Δpkl1Δ* dans lesquels la dynamique du fuseau tri-phasique n'était pas observée. Nos analyses du fuseau mitotique ont montré que la suppression de Klp2 complémentait partiellement le temps nécessaire pour atteindre la phase III,

mais cela n'a pas permis de restaurer la longueur du fuseau de phase III dans les cellules *cut7Δpkl1Δ*. Ce résultat était différent de celui noté dans les fuseaux de MI du triple mutant *cut7Δpkl1Δklp2Δ,* où la longueur du fuseau de pré-phase III a été partiellement restaurée. L'ensemble de ces résultats suggèrent que la kinésine-14 Klp2 contrecarre les forces de poussée vers l'extérieur dépendant de Cut7 et représente un nouvel acteur dans le maintien de l'équilibre des forces du fuseau de MI au cours de la phase II.

Nous avons marqué Klp2 avec la GFP pour étudier sa localisation dans les fuseaux mitotiques et de MI. L'imagerie de Klp2-GFP a montré sa localisation le long du fuseau, dès son assemblage jusqu'au début de la phase III dans la mitose comme en MI (Mana-Capelli *et al.*, 2012; Yukawa *et al.*, 2018). L'analyse 'linescan' de Klp2-GFP a montré des pics d'intensité maximale plus élevés en MI, indiquant une augmentation de la quantité totale de Klp2-GFP en MI par rapport à celle observée en mitose. Cependant, la concentration normalisée de Klp2-GFP par rapport aux MTs du fuseau est restée inchangée. Nous avons également remarqué que Klp2-GFP se localisait plus intensément aux pôles du fuseau en MI par rapport à la mitose.

2.5. La suppression de la dynamique des MTs restaurela bipolarité du fuseau de MI dans les zygotes *cut7Δpkl1Δ*

Considérant que la délétion de klp2 n'a restauré que partiellement la bipolarité du fuseau dans les zygotes *cut7Δpkl1Δ*, nous avons recherché des facteurs supplémentaires pouvant participer à l'équilibre des forces des fuseaux de phase II de MI. Nous soupçonnions que la dynamique des MTs pouvait être régulée de façon différente entre la mitose et la méiose. Étant donné que les limitations d'imagerie actuelles ne nous permettent pas de mesurer la dynamique des MTs individuels du fuseau, où il existe de nombreux MT réticulés, nous avons plutôt mesuré la dynamique des

faisceaux individuels de MTs avant le début de la mitose et pendant la période de pause entre MI et MII.

Nous avons constaté que les faisceaux MT rétrécissaient plus lentement et croissaient plus vite chez les zygotes, mais atteignaient une longueur maximale plus courte que les faisceaux dans les cellules végétatives. De plus, les faisceaux de MTs ont subi une “catastrophe” deux fois plus fréquente chez les zygotes que dans les cellules végétatives. En général, les faisceaux de MTs méiotiques sont plus dynamiques que les faisceaux de MTs mitotiques. Bien que non mesurés dans les fuseaux, ces résultats suggèrent que la dynamique des MTs est régulée différemment dans les cellules mitotiques et les zygotes. Nous avons donc testé si une modification de la dynamique des MTs pouvait influencer l'assemblage du fuseau de MI et la formation de spores chez les zygotes *cut7Δpkl1Δ*.

Nous avons perturbé la dynamique des MTs en utilisant de faibles doses de carbamate de méthyl benzimidazole (MBC), une molécule qui inhibe la polymérisation des MTs et réprime leur dynamique (Yenjerla *et al.*, 2009; Vela-Corcía *et al.*, 2018). L'accouplement de zygotes sur des boîtes de ME avec 5 µg /ml de MBC a entraîné une fréquence légèrement inférieure de zygotes contenant quatre spores. En revanche, les zygotes *cut7Δpkl1Δ* ont produit quatre spores plus fréquemment avec l'ajout de MBC (44 ± 1% avec l'ajout de MBC par rapport à 20 ± 1% en absence de MBC). L'imagerie des cellules vivantes a montré que l'ajout de 5 µg /ml MBC permettait un assemblage du fuseau bipolaire en MI dans 80 ± 4% des zygotes *cut7Δpkl1Δ*, contre 26 ± 2% des zygotes *cut7Δpkl1Δ* en absence de MBC.

La dynamique du fuseau *Cut7Δpkl1Δ* après ajout de MBC a montré un début retardé de la phase III de la MI ainsi qu’un fuseau MI phase I / phase II plus court, similaire à ce qui a été observé lors de la progression mitotique chez le double mutant *cut7Δpkl1Δ* en l'absence de MBC. Contrairement

aux fuseaux *cut7Δpkl1Δklp2Δ*, les fuseaux *cut7Δpkl1Δ* MI en présence de 5 µg /ml de MBC n'ont pas présenté de progression en trois phases de l'allongement du fuseau. Cela suggère que le mécanisme de restauration dépendant de MBC est distinct de celui sur lequel repose/dont dépend la déplétion de Klp2.

Enfin, l'ajout de faibles doses de MBC ne semble pas avoir d'impact sur la progression mitotique. Nous concluons que dans la phase II de la MI, la dynamique des MTs contrecarre les forces de poussée vers l'extérieur dépendantes de Cut7 avec Klp2 pour atteindre un bon équilibre des forces et permettre la formation de fuseaux bipolaires fonctionnelles.

3. Discussion

Dans ce travail, nous avons analysé les différences entre la dynamique du fuseau mitotique et méiotique dans la levure de fission. Nous avons constaté que la quantité totale de MTs, ainsi que la quantité totale de moteurs moléculaires étudiés (à l'exception de la kinésine-14 Pkl1), étaient plus élevées en MI que dans la mitose. Dans la levure de fission, la longueur du fuseau varie en fonction de la taille des cellules (Cortés et al., 2018; Krüger et al., 2019). Un mécanisme de mise à l'échelle simple pourrait donc expliquer l'augmentation des longueurs finales de fuseau dans toutes les phases de MI, ainsi que l'augmentation de la vitesse de la phase III de MI (Krüger et al., 2019). Parce que les zygotes sont diploïdes et plus grands, ils contiennent probablement davantage de MTs, de moteurs et de MAP pour organiser un fuseau. L'augmentation moyenne de 25% de la longueur finale du fuseau de MI par rapport au fuseau mitotique pourrait être facilement expliquée par l'augmentation du volume cellulaire et la disponibilité accrue des composants du fuseau.

Nous avons en outre constaté que dans la MI, contrairement à la mitose, la plus grande partie du temps était consacrée à la phase II (tableau 1). En MI, les chromosomes homologues bivalents sont

maintenus ensemble par la cohésine et les chiasmes (Watanabe and Nurse, 1999; Molnar *et al.*, 2003; Tomoya S Kitajima *et al.*, 2003). La nécessité temporelle de terminer la résolution des chiasmas pourrait expliquer pourquoi la phase II de la MI est prolongée par rapport à la mitose.

Les fuseaux de MI ont une organisation spécifique des chromosomes bivalents: la cohésine se situe entre les chromosomes homologues, l'existence de chiasmes ainsi que des changements dans l'architecture des kinétochores (Watanabe and Nurse, 1999; Molnar *et al.*, 2003; Kitajima, Kawashima and Watanabe, 2004; Yokobayashi and Watanabe, 2005). Nous avons examiné si cette organisation pouvait influencer l'assemblage du fuseau dans les cellules cut7Δpkl1Δ, mais la suppression individuelle de Rec8 (cohésine spécifique de la méiose), Moa1 (protéine du kinétochore méiotique), Rec11 (cohésion chromatide sœur méiotique) ou Rec12 (endonucléase de recombinaison méiotique) n'a pas restaurer le phénotype du fuseau MI *cut7Δpkl1Δ* ainsi que le nombre de zygotes produisant les quatre spores typiques. Cela indique que la différence observée entre les fuseaux mitotiques et ceux de MI ne vient pas simplement de l'organisation des chromosomes. Cependant, une étude publiée par Shirasugi et Sato lors de la révision de cet article a démontré qu'une double suppression de Rec12 et Moa1 a partiellement complémenté la bipolarité du fuseau observe dans les cellules/double mutant *cut7Δpkl1Δ* (Shirasugi and Sato, 2019).

Contrairement aux autres composants du fuseau testés dans ce travail, la concentration de kinésine-14 Pkl1 par rapport aux MTs du fuseau est plus faible en MI qu'en mitose. La régulation de la diminution de cette concentration relative reste inconnue. Il a été récemment publié qu'une légère surexpression de la kinésine-14 HSET au cours de la MI accélérait la bipolarisation du fuseau pendant la phase I, et entraînait des pôles de fuseau plus focalisés, passant essentiellement de la MI à un fuseau similaire à celui de la mitose (Bennabi *et al.*, 2018). Ceci a engendré un alignement

chromosomique aberrant. Il est tentant de penser qu'une régulation de la baisse de la concentration en kinésine-14 pourrait être conservée évolutivement de la mitose à la méiose I.

Nous avons identifié Klp2 comme un antagoniste supplémentaire de Cut7 en MI. Il a été démontré qu'au cours de la mitose, l'attachement de Klp2 aux SPBs peut, dans une certaine mesure, remplacer le rôle de Pkl1 dans l'ancrage du fuseau et la génération de force, indiquant que dans une telle situation, Klp2 peut efficacement s'opposer aux forces extérieures Cut7 (Yukawa *et al.*, 2018). Étant donné que Klp2-GFP est d'avantage localisé aux pôles du fuseau en MI qu'en mitose, nous proposons que la fonction Klp2 puisse différer en MI par rapport à la mitose, et qu'elle joue un rôle important dans l'établissement de la bipolarité du fuseau en contrecarrant les forces de poussée vers l'extérieur exercées par Cut7 pour compenser la augmentation du rapport Cut7-à-Pkl1. La manière dont Klp2 est régulée et les facteurs qui influencent ses fonctions restent des questions ouvertes.

Une autre découverte importante de notre étude était la possibilité de restaurer la bipolarité du fuseau de MI des cellules cut7Δpkl1Δ en modifiant la dynamique des MTs avec de faibles doses de MBC (Yenjerla et al., 2009; Cortés et al., 2018; Carazo-Salas et al., 2005). Notre hypothèse est que la dynamique des MTs peut être améliorée en MI phase I / phase II par rapport à la phase mitotique I / phase II. Dans tous les cas, le fait que la suppression de la dynamique MT dans les zygotes *cut7Δpkl1Δ* restaure la bipolarité du fuseau en MI, et modifie également le résultat de la méiose *wt*-zygote, mais sans perturber le résultat de la mitose, indique que la formation du fuseau MI est extrêmement sensible aux altérations de la dynamique des MTs et que cette régulation fine de la dynamique des MTs est crucial pour le succès de la méiose. Les facteurs qui modifient la dynamique des MTs entre mitose et méiose restent à étudier.

Dans les cellules végétatives, les centromères sont regroupés dans l'enveloppe nucléaire, en dessous du SPB (Hironori *et al.*, 1993). Chez les zygotes, un bouquet de télomères se forme avant les divisions méiotiques, ce qui place les télomères sous le SPB et les centromères plus loin (Chikashige *et al.*, 1994; Fennell *et al.*, 2015). Une possibilité est que, parce que les centromères / kinétochores sont moins accessibles pour l'attachement aux MTs plus éloignés des SPB en MI, une augmentation de la dynamique des MTs augmenterait la probabilité que les MTs trouvent et s'attachent aux kinétochores (Kirschner and Mitchison, 1986; Heald and Khodjakov, 2015). De plus, il a été démontré que le mouvement de pivotement des MTs autour des pôles du fuseau accélère la capture des kinétochores dans la mitose (Kalinina *et al.*, 2013). Compte tenu de la diminution de la concentration de Pkl1 aux pôles du fuseau en MI, il est plausible de supposer que les MTs peuvent être organisés de manière plus lâche au SPB et que plus de MT sont nucléées pendant la MI (Olmsted *et al.*, 2014). Cela pourrait entraîner une plus grande plage de mouvements de pivotement des MTs, ce qui augmenterait les chances de rencontrer et d'attacher un kinétochore.

Ces résultats ouvrent de nouvelles questions sur la façon dont les moteurs, les MAPs et la dynamique des MTs sont modifiés entre mitose et méiose. Étant donné le degré élevé de conservation évolutive des acteurs moléculaires impliqués dans l'assemblage du fuseau, il sera très intéressant d'étendre cette étude à d'autres organismes afin de développer notre compréhension globale de la mitose et de la méiose.

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RÉSUMÉ

La mitose est un type de division cellulaire qui sert à la prolifération des cellules, tandis que la méiose produit des cellules sexuelles, qui sont utilisées dans la reproduction sexuelle d'un organisme. Dans les deux cas, une machine à base de microtubules appelée le fuseau sépare parfaitement les chromosomes.

Dans cette étude, la dynamique du fuseau mitotique et méiotique a été caractérisée et comparée simultanément dans la levure à fission. La comparaison de la dynamique des fuseaux a permis de déterminer qu'il existe trois types de fuseaux distincts avec des caractéristiques distinctives. Un mutant de levure à fission déficient en kinésine-5 Cut7 et en kinésine-14 Pkl1 a été utilisé comme outil pour identifier la source des différences dans la dynamique du fuseau mitotique et méiotique. Nous révélons que la concentration de Pkl1 est réduite en fuseaux méiotiques I par rapport aux fuseaux mitotiques, et identifions la kinésine-14 Klp2 comme la molécule qui coopère avec Pkl1 en antagonisant Cut7 dans la méiose I. De plus, nous avons constaté que la suppression de la dynamique des microtubules dans cut7Δpkl1Δ zygotes restaure bipolarité du fuseau, faisant valoir que les microtubules sont plus dynamiques dans les fuseaux de la méiose I que dans les fuseaux mitotiques.

En résumé, ce travail montre que les fuseaux mitotiques et méiotiques sont intrinsèquement différents, et leurs différences proviennent de la kinésine-14 et de la régulation de la dynamique des microtubules.

MOTS CLÉS

Mitose, méiose, fuseau, kinésine, microtubule

ABSTRACT

Mitosis is a cell division type that serves for proliferation of cells, while meiosis produces sex cells, which are used in the sexual reproduction of an organism. In both cases, a microtubule-based machine called a spindle flawlessly separates the chromosomes.

In this study, mitotic and meiotic spindle dynamics have been characterized and compared simultaneously in fission yeast. Spindle dynamics comparison ascertained that there are three distinct spindle types with distinguishing features. A fission yeast mutant deficient for kinesin-5 Cut7 and kinesin-14 Pkl1 was used as a tool to identify the source of the differences in mitotic and meiotic spindle dynamics. We reveal Pkl1 concentration is reduced in meiotic I compared to mitotic spindles, and identify kinesin-14 Klp2 as the molecule that co-operates with Pkl1 in antagonizing Cut7 in meiosis I. Furthermore, we found that suppressing microtubule dynamics in cut7Δpkl1Δ zygotes restores spindle bipolarity, arguing that microtubules are more dynamic in meiosis I spindles than in mitotic spindles.

In summary, this work shows mitotic and meiotic spindles are inherently different, and their differences stem from kinesin-14s and MT dynamics regulation.

KEYWORDS

Mitosis, meiosis, spindle, kinesin, microtubule