Microtubule depolymerization and severing during mitosis

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Layman's summary: Without cell division, there would be no life. Without the ability to create more cells, we would for example not be able to grow and develop, to heal injuries or to reproduce. During cell division, chromosomes are first duplicated. Duplicated chromosomes then need to be separated and it is crucial that this happens correctly, so that the two new daughter cells after cell division contain exactly one copy of each chromosome. Mitosis, the process of chromosome segregation, is therefore tightly regulated and controlled. Cytokinesis is the process of separating the mother cell into two daughter cells. In the last step of cytokinesis, abscission, the membrane connection between the two cells is cut and cell division is complete.

Microtubules are hollow cylindrical protein polymers that have many different function within the cell. They are very dynamic, constantly switching between shrinkage and growth, which is tightly regulated by a subset of proteins. During mitosis, microtubules form into a bipolar spindle that facilitates the separation of the chromosomes. In the later phases of mitosis, the spindle needs to be highly dynamic and eventually disassemble again so that abscission can occur to separate the mother cell into two daughter cells. In this review, we describe what proteins are involved in the disassembly of spindle microtubules. Members of the meiotic clade of AAA ATPases can generate breaks. In different situations, this can either have a positive or a negative effect on the total mass of spindle microtubules. They are also important for cutting the spindle microtubules that obstruct abscission. Depolymerizing kinesins of the kinesin-13 family can depolymerize microtubules from both ends. During mitosis, they for example function by 'reeling in' chromosomes to opposite poles of the spindle by depolymerizing the microtubules that are attached to the chromosomes. Depolymerizing kinesins of the kinesin-8 family regulate microtubules in a length dependent manner. They have various functions during mitosis, but in general they keep the length of spindle microtubules within a specific range.

[ABSTRACT] Cell division involves the separation of chromosomes (mitosis) and the cytoplasm (cytokinesis) of a mother cell into two daughter cells. Many of the physical event during mitosis rely on the dynamics of the microtubules within the mitotic bipolar spindle and their constant remodelling. In the last phases of mitosis, the spindle needs to be highly dynamic to segregate the chromosomes and needs to quickly disassemble to allow for abscission of the membrane to complete cytokinesis. Here, we first discuss the microtubule severing enzymes and depolymerizing kinesins involved in spindle microtubule regulation. Second, we discuss their function and regulation over the course of metaphase to abscission. Microtubule severing enzymes of the meiotic clade of AAA ATPases function through removing tubulin dimers from the lattice and can be either positive or negative regulators of total cellular microtubule mass. They play important functions at spindle poles and in disassembling the spindle during cytokinesis. Depolymerizing kinesins of the kinesin-13 family function at both spindle poles and kinetochores to facilitate

microtubule depolymerization. Kinesin-8 family members destabilize microtubules in a length dependent manner and have both a stabilizing and a destabilizing effect on microtubules in different contexts. They have diverse roles over the course of mitosis, but in general regulate the spatial dynamics of microtubules by keeping their length within a specific range.

Introduction

Without cell division, life would not exist. It allows organisms to grow, reproduce and repair or regenerate damaged tissues. Cell division is a stepwise process in which chromosomal DNA is duplicated and equally divided over two daughter cells. It can be divided into two processes; mitosis, the duplication and separation of chromosomes, and cytokinesis, the separation of the cytoplasmic components and division into two daughter cells (Chaigne & Brunet, 2022). Many of the physical events during mitosis rely on microtubules and their constant remodelling.

Microtubules are hollow, cylindrical polymers built from tubulin dimers, which causes them to have a β-tubulin exposed plus and a α-tubulin exposed minus end. In cells, microtubules assemble into many different higher-order arrays that have to continuously be remodelled in order to keep up with the ever-changing needs of the cell over the course of the cell cycle. Therefore, microtubules are highly regulated by many different proteins and by post-translational tubulin modifications (Akhmanova & Kapitein, 2022)

At the start of mitosis, microtubules form into a bipolar mitotic spindle. The mitotic spindle comprises three types of microtubules. Kinetochore microtubules bundled into k-fibres, which attach to the chromosomes, interpolar microtubules, which overlap at the central spindle and form the midzone and astral microtubules, that radiate towards the cell cortex and play a role in spindle positioning (Glotzer, 2009). During each cell cycle, the spindle needs to quickly assemble as well as disassemble. Here, we focus on the impact of microtubule severing and depolymerization on the proper execution of cell division. In the last phases of mitosis, the spindle needs to be highly dynamic to segregate the chromosomes, then disassemble to allow nuclear membrane reformation and finally needs to be broken down to allow for abscission to separate the two newly formed daughter cells. These processes rely on the cooperative action of many microtubules associated proteins, located at kinetochores, centromeres, centrosomes and along the spindle microtubules, that regulate the dynamic behaviour of microtubules in various ways. Coordinated microtubule depolymerization and severing is predominantly performed by two different groups of proteins, depolymerizing kinesins and microtubule severing enzymes (Sharp & Ross, 2012; Wordeman, 2005).

In this review, we first discuss the microtubule severing and depolymerizing proteins involved in spindle microtubule regulation. Second, we describe the role of these proteins at various points in mitosis from metaphase to abscission and discuss how they are linked to other proteins and regulatory mechanisms that play important roles in late mitosis.

Microtubule depolymerizing kinesins with important roles in mitosis

Kinesins are a large group of proteins associated with microtubules that couple mechanical work to ATP hydrolysis. Although kinesins are classified by their possession of a conserved catalytic motor domain, there is lots of sequence variation between different kinesin families which causes the entire superfamily to perform different and specialized functions within the cell (Wordeman, 2005). Of the approximately 40 kinesin genes identified in humans, about half of them play a role in mitosis, highlighting their importance during cell division (Locke et al., 2017). While most classes of kinesins progressively walk over the microtubule lattice, for example to transport cargo or to position organelles, there is also a subset of kinesin proteins families that have other functions, like active microtubule depolymerization (Manning et al., 2007).

Members of the kinesin-13 (fig 1A) and the kinesin-8 (fig2 A) family play essential roles in the regulation of microtubule length during mitosis. Through two distinctly different molecular mechanisms, they actively destabilize or depolymerize spindle microtubules. (Manning et al., 2007; Wordeman, 2005).

Microtubule depolymerizing enzymes of the kinesin-13 family

Although the kinesin13 subfamily is classified as kinesin because they share homology with the conventional kinesin motor domain (Aizawa et al. 1992, Walczak et al. 1996, Wordeman & Mitchison 1995) and by their binding to microtubules in an ATP/ADP-dependent manner (Vale et al. 1985, Walczak et al. 1996, Wordeman & Mitchison 1995), they vastly differ from conventional kinesins both in domain architecture and functional mechanism in four ways. First, whereas most kinesins have their motor domain either at the C or N-terminus, the catalytic domain of kinesin-13 family members is located in the middle of the amino acid sequence (fig 1B). Second, and in line with the location of the catalytic domain, kinesin-13 protein family members are a type of non-motile kinesins and utilize ATP synthesis for active depolymerization instead of translocation along the lattice (Moore & Wordeman, 2004). Third, MCAK can laterally diffuse along the microtubule lattice without need of ATP synthesis and to both microtubule ends in the same extend (Helenius et al., 2006). Based on sequence similarity, it is suspected that other members of the kinesin-13 family possess this rather unique feature as well (Walczak et al., 2013). Lastly, whereas most kinesins are active when bound to cargo (in an open and ADP bound state), kinesin-13 family members appear to be active in an ATP bound state and in a closed conformation (Burns et al., 2014; Ems-McClung et al., 2013; Talapatra et al., 2015).

The human genome encodes four microtubule depolymerizing Kinesin13 family members, Kif2A, kif2B, kif2C/MCAK (from now on referred to as MCAK) and kif24 (Kobayashi et al., 2011; Manning et al., 2007; Ritter et al., 2016). Kif2A, Kif2B and MCAK play important roles during various aspects of mitosis. Although roles of Kinesin13 family members seem to mostly be related to cell division, *C. elegans* Kinesin13 Klp7 plays a role in axon regeneration (Ghosh-Roy et al., 2012) and MCAK has been shown to play a role in cell migration (Braun et al., 2014). The more recently identified Kif24 on the other hand plays an important role in the regulation of ciliogenesis and has to our knowledge not yet been linked to spindle dynamics (Kobayashi et al., 2011; Mashima et al., 2022), which is why this protein is not further discussed in this review.

During mitosis, Kif2A predominantly localizes to centrosomes, and plays a role in spindle assembly and regulation of its dynamics in metaphase and anaphase (Rogers et al., 2004). Kif2B generally has low expression levels in cells (Hood et al., 2012). However, overexpression studies have placed Kif2B at centrosomes, mitotic spindles, kinetochores prior to alignment at the metaphase plate and at the

midbody (Manning et al., 2007; Welburn & Cheeseman, 2012). Based on these localization patterns, it has been suggested that kif2B plays a role in spindle assembly, chromosome movement and segregation and in cytokinesis (Hood et al., 2012; Manning et al., 2007; Ritter et al., 2016). MCAK is the most potent microtubule depolymerase of the kinesin13 family and also by far the best characterized one (Ogawa et al., 2004). During metaphase, it localises at kinetochores, centromeres, centrosomes and astral microtubules, suggesting MCAK depolymerizes different subsets of spindle microtubules from different locations within the spindle. (Helenius et al., 2006; Manning et al., 2007). MCAK can interact with growing microtubule plus end binding, 'tip tracking', protein EB. This suggests that MCAK can be specifically recruited or maintained at growing microtubule ends (Helenius et al., 2006; Wordeman, 2005).

Through various biochemical studies and the solved crystal structure, the molecular mechanism of action for microtubule depolymerization by MCAK is well characterized (Ems-McClung et al., 2013; Talapatra et al., 2015). Although such studies have not been performed on other kinesin-13 members, based on homology and structure similarity it is suspected they function through a similar mechanism (Ritter et al., 2016). MCAKs motor domain has both ATPase and microtubule binding activity (Friel & Howard, 2011). It is located at the centre of the protein and flanked by a positively charged neck region, which promotes the catalytic activity of the motor domain (fig 1B) (Ogawa et al., 2004). The N-terminal domain is important for subcellular localization and not necessary for in vitro activity of MCAK (Ems-McClung et al., 2013; Maney et al., 2001). MCAK acts as a homodimer and dimerization is facilitated through the C-terminal domain (fig 1B). Although monomeric MCAK has depolymerization activity in vitro, its dimerization enhances the depolymerization efficiency and results in a more specific localization to the microtubule ends. (Cooper et al., 2010; Hertzer et al., 2006; Maney et al., 2001; Talapatra et al., 2015; Wang et al., 2015).

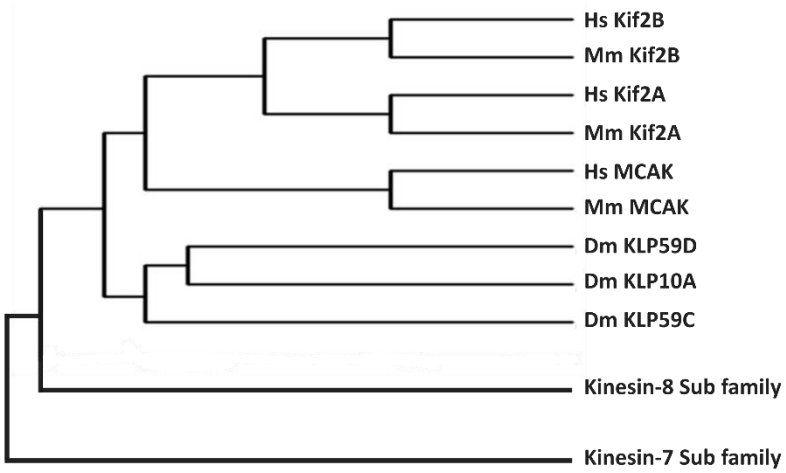
In solution, MCAK is in a closed and ATP bound state, facilitated through binding of one of the C-terminal tails of the dimer to either both motor domains (Talapatra et al., 2015) or to the neck region (Ems-McClung et al., 2013). Motor-domain binding to either the c-terminal tail or to the microtubule lattice is mutually exclusive (Talapatra et al., 2015). Contact with the microtubule lattice is suspected to cause a conformational change in the tubulin binding of the motor domain, allowing the positively charged neck to engage in electrostatic interaction with C-terminal tubulin tails on the microtubule lattice (Burns et al., 2014; Talapatra et al., 2015). This results in ATP hydrolysis and lattice-associated MCAK to obtain an open conformation (Ems-McClung et al., 2013; Talapatra et al., 2015) (fig 1C). The open conformation allows for 1D lateral diffusion over the microtubule lattice, which is important for fast microtubule end targeting of MCAK. Interaction of the tubulin binding site within the motor domain combined with electrostatic repulsion between the negatively charged C-terminal tails of both MCAK and tubulin, are suspected to play an important role in this (Helenius et al., 2006; Talapatra et al., 2015)

The terminal tubulin subunit at both the microtubule plus and minus end can adopt a more curved conformation than the rest of the lattice. Once MCAK reaches a microtubule end, ADP is rapidly exchanged to ATP which results in a conformational change back into a more closed conformation that allows for a high affinity interaction with tubulin (Ems-McClung et al., 2013; Friel & Howard, 2011) (fig 1B). A unique structural element to the kinesin-13 family is a conserved "KVD" motif within the microtubule binding domain. This motif has been shown to play an important role mediating the conformational change and ATP binding through interacting with tubulin (Cooper et al., 2010; Wang et al., 2015). Structural modelling predicts that MCAK, aided by the "KVD" motif, binds at the interface between two tubulin dimers. This tight interaction stabilizes an intermediate curved state of the tubulin dimer (Ogawa et al., 2004), which ultimately results in depolymerization (Ritter et al.,

2016). Burns et al. propose a model for depolymerization in which stabilizing the curved intermediate promotes outward curvature of the tubulin dimer. This strain leads to disruption of the lateral contacts with the other protofilaments, destabilizing the growing plus-end (Burns et al., 2014). Wang et al propose a model in which the stabilized curved intermediate of the terminal tubulin dimer cannot be accommodated in the lattice, resulting in release of a MCAK-tubulin complex that drags along the second tubulin dimer of the same protofilament, resulting in depolymerization (Wang et al., 2015).

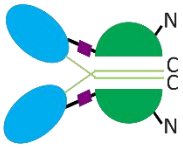
Tubulin detaches from the lattice during polymerization still associated with MCAK. ATP hydrolysis is not necessary for depolymerization (Wang et al., 2015), but triggers a conformational change back to an open conformation again with lower tubulin affinity, resulting in dissociation of MCAK from the tubulin dimer (Wang et al., 2015). Binding of new ATP of MCAK in solution results in the original closed state again (Ems-McClung et al., 2013). MCAK and Kif2A are inactivated through phosphorylation in the neck region by Aurora B kinase, a master regulator of mitosis. They can be reactivated through binding by inner centromere Kin-I stimulator (ICIS) (Knowlton et al., 2009). ICIS can bind to Aurora B, MCAK or Kif2A, regulatory proteins INCENP and TD60 and to the microtubule lattice (Knowlton et al., 2009). It is therefore suggested it functions as a scaffold to facilitate tight spatial and temporal regulation of kinesin-13 mediated microtubule depolymerization within the spindle (Knowlton et al., 2009). MCAK phosphorylation in the neck results in a conformational change in MCAK to a more open confirmation. This more open conformation results in looser association of MCAK with the microtubule lattice, resulting in a higher ‘off rate’ and inhibits the switch to the closed conformation necessary for depolymerization at the microtubule end (Ems-McClung et al., 2013).

A)

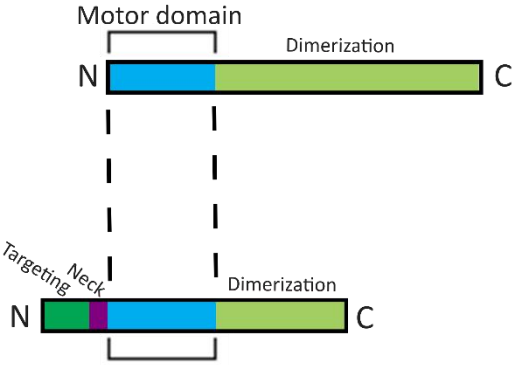


B)

Kinesin-1



Kinesin-13



C)

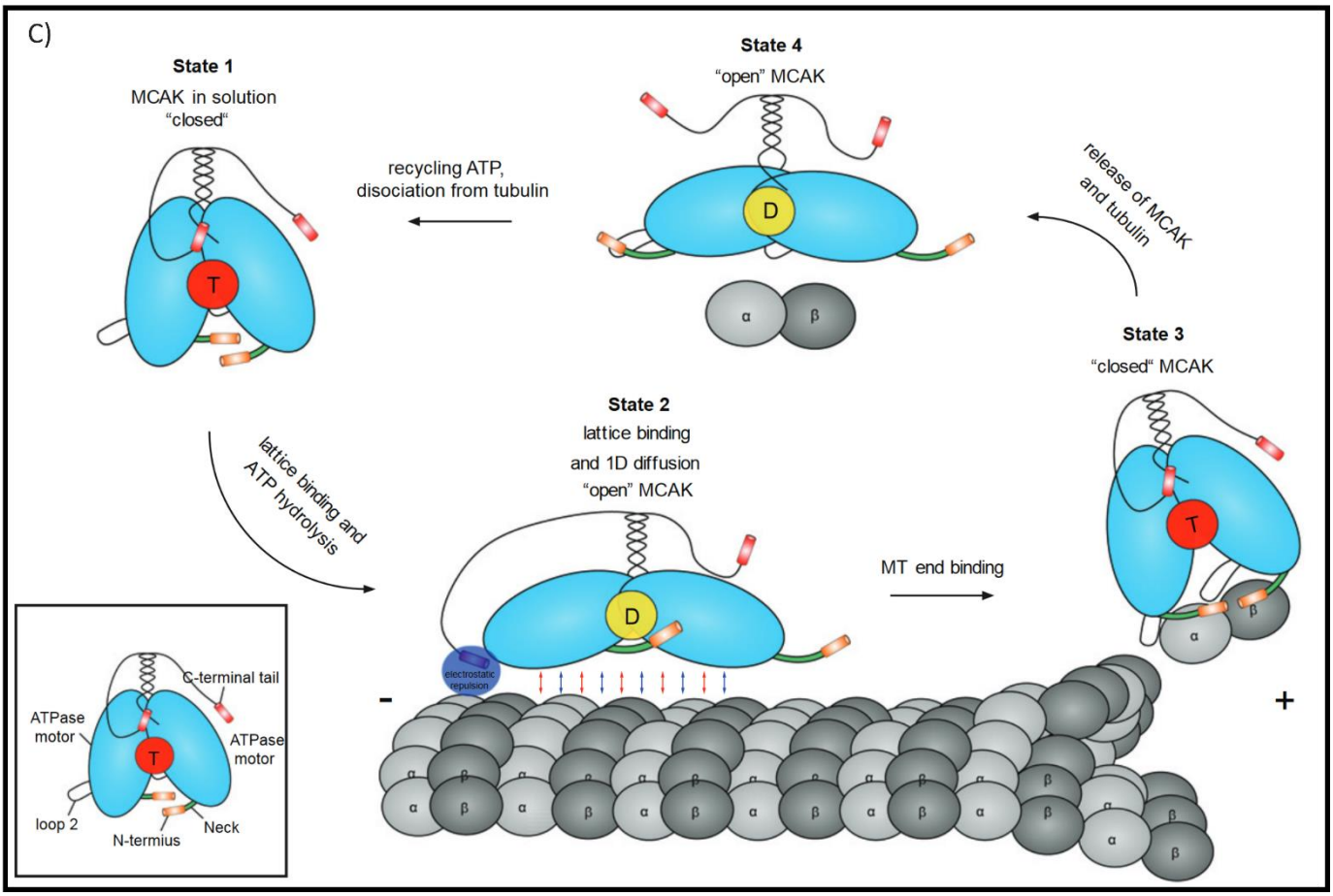


Figure 1 depolymerizing kinesins of the kinesin-13 subfamily (A) Phylogenetic comparison of kinesin-13 genes. Hs; human, Dm; drosophila melanogaster, Mm: mouse, Sc; Saccharomyces cerevisiae. Kinesin-13 is relatively closely related to the kinesin-8 sub family. Kinesin-7 is used as an outgroup. Figure adapted from (*Kinesin Tree – Kinesin*, n.d.; Manning et al., 2007) (B) Schematic representation of human kinesin-13 MCAK domains and dimer conformation, compared with kinesin-1 which has a more conventional kinesin domain architecture. Figure adapted from (Alberts et al., 2014; Ritter et al., 2016; Walczak et al., 2013) (C) Simplified model of microtubule depolymerization by MCAK. In solution, the ATP bound MCAK dimer is in a closed conformation, mediated by the c-terminal tail binding either to the motor domains or to the neck region. Contact with microtubule lattice is suspected to induce a conformational change in the microtubule binding region of the motor domain, resulting in a second conformational change in which the c-terminal tail of MCAK releases the two motor domains, allowing the positively charged neck to disposition and engage electrostatic interaction with the tubulin tails of the microtubule lattice. ATP hydrolysis results in a conformational change to an open configuration of MCAK, that allows for 1D lateral diffusion over the microtubule lattice. At the terminal tubulin subunit of either the plus or minus end, ADP is exchanged for ATP and MCAK converts back to its closed configuration. Linked to ATP hydrolysis, MCAK in complex with a tubulin dimer is released from the microtubule. ATP hydrolysis then results in dissociation from the removed tubulin dimer, preparing MCAK for the next cycle. Circled 'D' and 'T' refer to ADP and ATP bound state, respectively. Figure adapted from (Ritter et al., 2016).

Microtubule depolymerizing enzymes of the kinesin-8 family

The kinesin-8 family forms the second class of microtubule depolymerizing enzymes. In vertebrates, three members are expressed; KIF18A, KIF18B and KIF19 (Locke et al., 2017). Kif18A and yeast and *Drosophila melanogaster* homologues Kip3 and Klp67A have been shown to play essential roles in cell division (Gupta et al., 2006, 2006; Mayr et al., 2007). Kif18A localizes to the tips of microtubules at kinetochores (Mayr et al., 2007), where it plays an important role in chromosome alignment at the metaphase plate (Locke et al., 2017). KIF18B is generally associated with regulation of astral microtubules and for its role in the efficient targeting of MCAK to microtubule plus ends (Tanenbaum et al., 2011) . Kif19 plays a role during ciliogenesis (Niwa et al., 2012). Typically for kinesins with plus-end directed motility, the motor domain is located at the N-terminus (Su et al., 2012) (fig 2B). The microtubule binding site within the motor domain shares some similarities with the kinesin-13 motor domain, as it appears to also be able to make an extra contact with α-tubulin unlike many other kinesin families (Peters et al., 2010; Su et al., 2012). A shared feature of all kinesin8 family members is that they display robust plus end processivity and stay associated for some time with the microtubule once they reach the end, resulting in local accumulation (Su et al., 2012). This high processivity for Kif18A and Kip3 is at least in part facilitated by a C-terminal tail that contains an additional microtubule binding domain (Stumpff et al., 2011; Su et al., 2012). Interestingly, kinesin-8 family members form a length-dependent gradient at the microtubule lattice (Fukuda et al., 2014; Mayr et al., 2007; Stumpff et al., 2008; Varga et al., 2009). As a consequence, the effect of kinesin-8 family members on microtubule dynamics is dependent on the length of the microtubule (Peters et al., 2010; Varga et al., 2009). Various studies suggest these proteins to have both a stabilizing and a destabilizing effect on microtubules in different contexts (Du et al., 2010; Stumpff et al., 2008; Varga et al., 2009).

The best characterized kinesin-8 is budding yeast Kip3. Kip3 is both a plus end-directed motor and a plus end-specific depolymerase, albeit at a slower rate than MCAK (Gupta et al., 2006). After reaching the plus end, Kip3 stays associated with the microtubule. Although the mechanism for retention at the plus end is unclear, it is known to at least in part depend on the C-terminal domain (Su et al., 2011). Two models have been proposed for the working mechanisms of Kip3; the concentration threshold model (Su et al., 2011, 2012) (fig 2C) and the antenna or 'bump-off' model

(Varga et al., 2009) (fig 2D). Following the concentration threshold model, Kip3 motors associate with the growing microtubule lattice at random points and shows progressive plus end motility in an ATP depended manner. As walking speed is faster than microtubule polymerization rate and Kip3 is highly processive (Gupta et al., 2006), Kip3 reaches the growing plus end where it stays associated with the microtubule and accumulates, resulting in a high local concentration of Kip3 that favours microtubule catastrophe (Su et al., 2011). Once a specific threshold of accumulation is reached, the microtubule starts to depolymerize, resulting in the loss of the accumulated Kip3. For the concentration threshold model, the mechanism of how exactly a high concentration of Kip3 at the plus induces catastrophe, is unclear (Su et al., 2012). Suspected is that catastrophe is either promoted through active depolymerization or by capping to prevent further growth and therefore destabilizing the microtubule end (Su et al., 2012). As the microtubule depolymerizes, lattice-associated processive Kip3 keeps reaching the end of the shrinking microtubule. At a lower concentration, Kip3 has a stabilizing effect, resulting in slower depolymerization. The concentration threshold model therefore provides an explanation for both the dual role of Kip3 as a stabilizer and a depolymerizer, as well as the effect of microtubule length on regulation. When added to a longer microtubule, more Kip3A can bind to the lattice, resulting in a quicker accumulation and faster reaching of the threshold. The threshold model fits well with the observed role of Kip3 in vivo, an open question however remains how exactly at high concentrations Kip3 causes depolymerization from the plus end. A possible mechanism for this could be provided by the antenna model.

Based on in vitro studies on chemically stabilized microtubules, Varga et al described the antenna or ‘bump-off’ model (Varga et al., 2009) (fig 2D). Similar as for the concentration threshold model, in the antenna model the association of Kip3 at random places along the lattice combined with high processivity and plus end motility generates a gradient of Kip3 increasing along the lattice towards local accumulation at the plus end (Varga et al., 2009). When a Kip3 motor reaches the terminal end of a protofilament, it removes the terminal tubulin dimer but the kip3-tubulin complex stays bound to the microtubule. Dissociation is then greatly accelerated by a new kip3 motor reaching the microtubule plus-end, "bumping off” the most terminal Kip3 with the tubulin dimer. Alternatively, upon being bumped off by other incoming Kip3 motors, the most terminal one is bumped off still in complex with the tubulin dimer attached to the lattice, and it drags the terminal tubulin dimer with it upon dissociation (Varga et al., 2009). Although the antenna model provides an explanation as to why the Kip3 mediated depolymerization occurs faster for longer microtubules, it does not explain the stabilizing properties observed for kip3. The model also has only been tested on chemically stabilized and not on dynamic microtubules. It is complicated to determine of the bumping off of one kip3 by an incoming one caused through a specific interaction or is just the result of crowding.

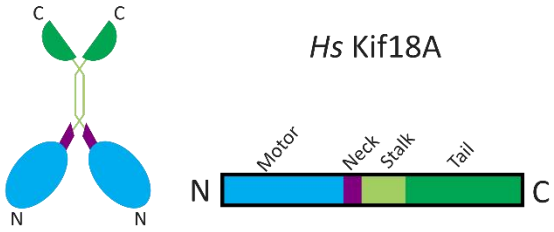
In addition to these models, it has been shown that individual kip3 motors frequently switch between protofilaments during processive walking (Bormuth et al., 2012). This gives rise to the hypothesis that instead of actually pausing at the plus end, Kip3 keeps walking in a non-processive circle once its path is obstructed by either the end of the lattice or by other accumulated Kip3 motors (Bormuth et al., 2012). Torsional force generated during this protofilament switching could destabilize lateral interactions between protofilaments at the plus end and thereby contribute to microtubule depolymerization. These findings therefore propose an alternative or additional mechanism for the missing piece of how plus end accumulation promotes catastrophe in the concentration threshold model. Future studies on dynamic microtubules and in vivo, combined with computational models, could aid in elucidating a complete mechanism for microtubule depolymerization by Kip3.

Whether Kif18A, the human homologue of Kip3A, possesses microtubule depolymerizing abilities remains a topic of debate. Two similar *in vitro* experiments with GMPCPP stabilized microtubules from two different publications disagree about the capabilities of Kif18A to depolymerize microtubules. (Mayr et al., 2007) observed mild GMPCPP microtubule depolymerization, and showed this to be dependent on microtubule length and to be very sensitive to salt concentration. Therefore they suggest Kif18A to be a microtubule depolymerizer through a similar mechanism as Kip3A. (Du et al., 2010) however observed no significant change in GMPCPP microtubule depolymerization *in vitro,* but observed that Kif18A antagonized plus end microtubule polymerization. Based on their results, (Du et al., 2010) suggested an alternative model in which Kif18A acts as a damper on microtubule dynamics, constraining the distance over which plus ends grow and shrink. The mechanism of action of Kif18A is an area for further exploration.

A)

Organism	Kinesin-8 family member names
Homo sapiens	Kif18A, Kif18B, Kif19
Drosophila melanogaster	Klp67A, Kif19A
Caenorhabditis elegans	KLP-13
Schizosaccharomyces pombe	Klp5/6 (heterodimer Klp5 and Klp6)
Saccharomyces cerevisiae	Kip3

B)



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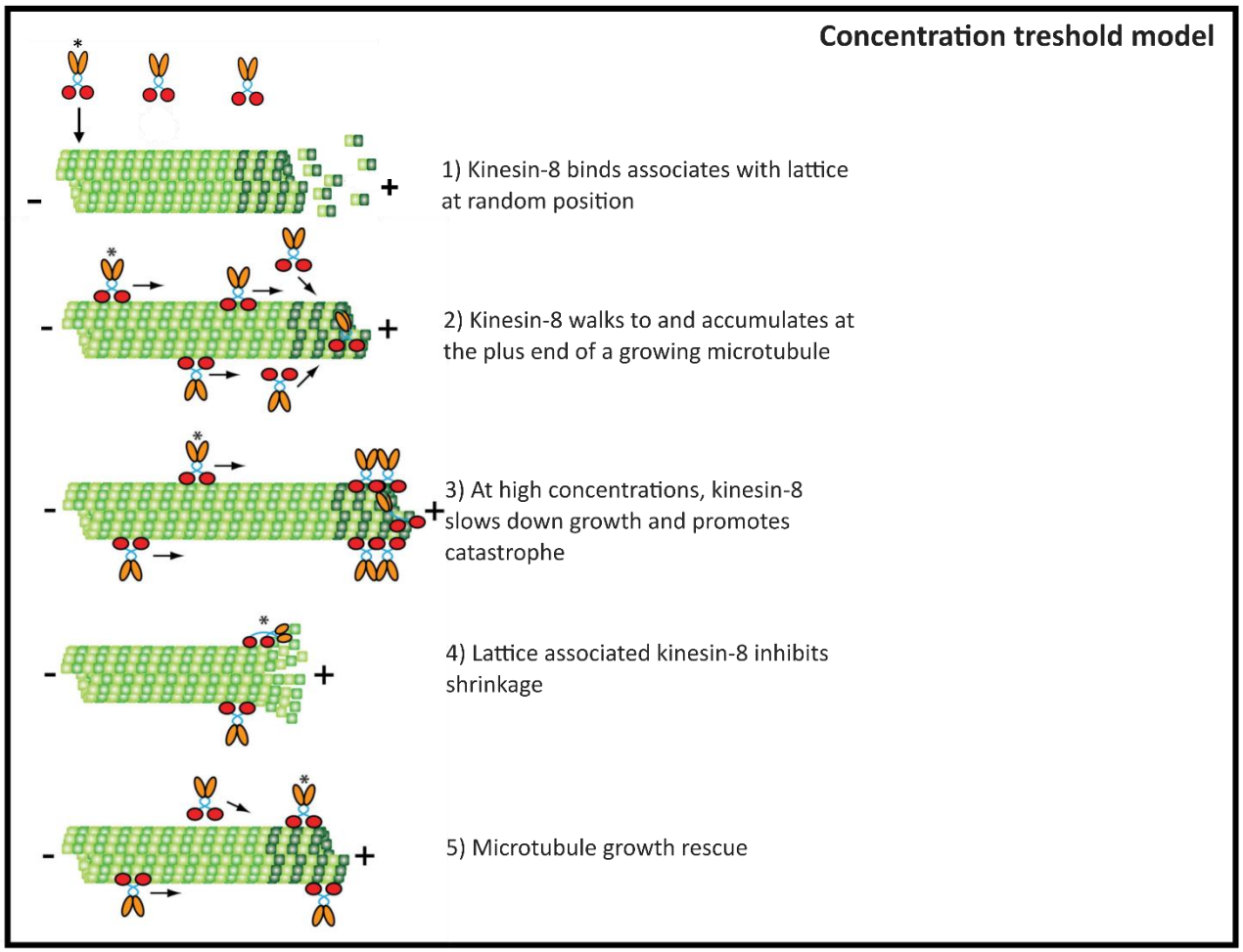

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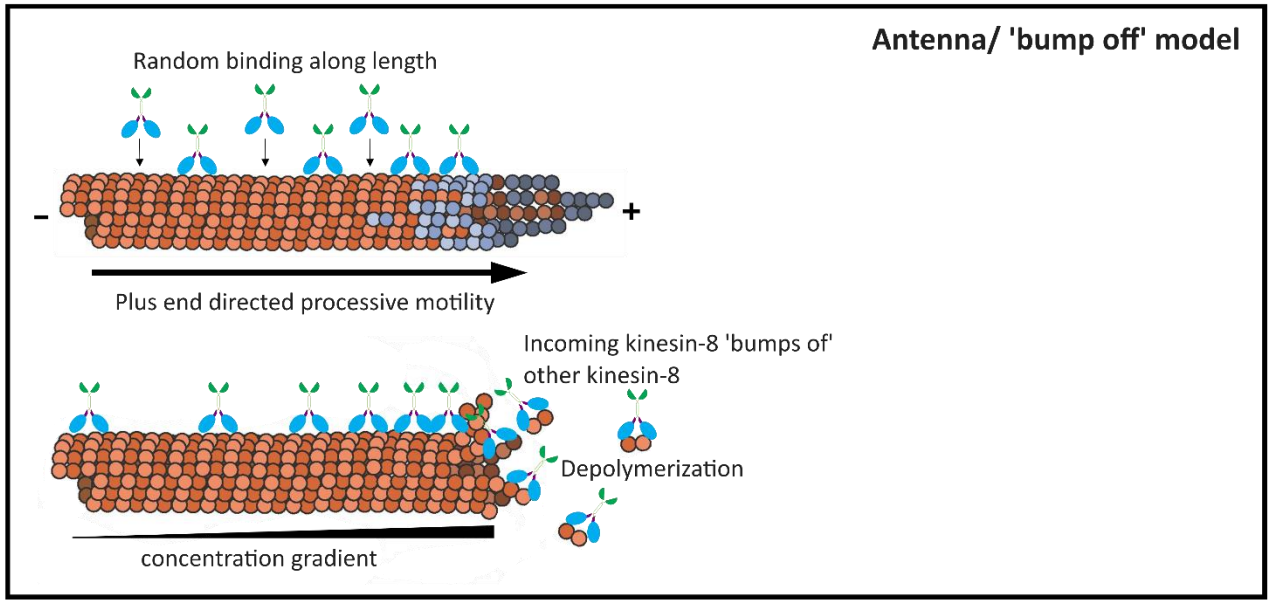


Figure 2 Depolymerizing kinesins of the kinesin-8 subfamily (A) Kinesin-8 family members in different organisms. Adopted from (Walczak et al., 2013). (B) Schematic representation of human kinesin-8 Kif18A domains and dimer conformation. Characteristic for plus-end directed kinesins, the motor domain is located at the N-terminus. Adapted from (Weaver et al., 2011) (C) Simplified mechanism of microtubule depolymerization by Kip3A according to the threshold mechanism. Kip8 binds at a random location at the microtubule lattice and walks processivity towards the plus-end and stays associated there, resulting in local accumulation. The accumulation promotes catastrophe, but the model doesn't describe if this is through a depolymerization or capping mechanism. Catastrophe results in the loss of the high concentration of kip3 at the plus end. During depolymerization, lower concentrations of kip3 are encountered along the lattice by the shrinking end. These lower concentrations slow down depolymerization and promote rescue. Figure adapted from (Su et al., 2011, 2012) (D) Simplified mechanism of microtubule depolymerization by Kip3A according to the antenna or 'bump-off' model. Kip3 associates with the microtubule lattice at a random position and shows processive movement to the plus end, resulting in a concentration gradient along the lattice. At the plus-end kip3 removes the most terminal tubulin dimer but stays associated and is 'bumped off' by other incoming Kip3 proteins. Adapted from (Bowne-Anderson et al., 2013; Su et al., 2012; Varga et al., 2009)

Microtubule severing enzymes of the AAA ATPase protein family

Microtubule severing enzymes are implicated in microtubule dynamics regulation in a wide range of cellular processes, including cell division (Matsuo et al., 2013). The AAA ATPase protein family comprises a large group of proteins represented in all major cellular pathways. Members have one common property, namely the use of energy from ATP hydrolysis to disassemble large complexes through their AAA ATPase domains, such as complex polymeric networks or aggregated and misfolded proteins (Olivares et al., 2015). The microtubule severing enzymes Katanin, Spastin and Fidgetin, together with ESCRT-III filament severing vacuolar protein 4 (VPS4), form the so-called meiotic clade of AAA ATPases (fig 3A) . This clade got its name from its first discovered member, Katanin, which two subunits where identified in a screen for embryonic lethal mutants in *C.elegans* and which depletion resulted in meiotic spindle defects (Mains et al., 1990). Microtubule severing enzymes remove tubulin dimers from the microtubule lattice and are both positive and negative regulators of microtubule mass (fig 3B). If the two generated ends after severing are unstable, the outcome will be microtubule depolymerization. Severing enzymes can therefore function to debranch networks, clear microtubule assemblies and increase the cytoplasmic pool of free tubulin dimers (F. J. McNally & Roll-Mecak, 2018; Sarbanes et al., 2022). If the two new generated ends are stable, for example through the incorporation of GTP tubulin at the severing site and through the aid of microtubule stabilizing proteins, the two severed ends can function as new seeds. In this situation, the net result of severing is actually amplification (Ribbeck and Mitchison, 2006; Roll-Mecak and Vale, 2006). Removal of tubulin subunits by severing enzymes does not necessarily result in lattice breakage. Removed lattice GDP-tubulin can be replaced by GTP-tubulin, creating lattice GTP-tubulin islands. Through this mechanism, severing enzymes can therefore work as rescue factors by creating rescue sites along the lattice (Vemu et al., 2018).

Discovery and localization of Katanin, Spastin and Fidgetin

Katanin is the first discovered microtubule severing enzyme and was named after the Japanese samurai sword katana. During mitosis, it is localized at spindle poles, the midbody and to our knowledge uniquely in *Drosophila melanogaster*, to kinetochores (Zhang et al., 2007). Katanin consists of two subunits, P60 and P80. P60 contains the AAA ATPase domain, and therefore provides the catalytic activity required for severing. The regulatory P80 subunit is important for targeting to

the centrosome and microtubule crossovers (E. A. Zehr et al., 2020). Although P60 alone is sufficient for microtubule binding and severing, P80 enhances the microtubule binding affinity of Katanin (O'Donnell et al., 2012).

Spastin was originally studied because mutations in its sequence were linked to the neurodegenerative disorder hereditary spastic paraplegia (HSP) and it was found to play an important role during neuronal development and function (Hazan et al., 1999). It was later identified as a microtubule severing enzyme. Many of Spastin's known interaction partners are linked to membrane trafficking (Connell et al., 2009). During mitosis, Spastin localizes to the spindle poles, spindle microtubules, newly formed nuclear envelopes and to the midbody (Reid et al., 2005; Zhang et al., 2007). Four Spastin isoforms are expressed in mammalian cells, of which a 60 kD isoform is generally most strongly expressed over different cell types (Reid et al., 2005).

Fidgetin was discovered after a report of a spontaneous mutation in lab mice linked to a head-shaking 'fidgety' phenotype (Sharp & Ross, 2012). Fidgetin localizes to spindle poles throughout mitosis, where it is proposed to actively suppresses microtubule attachment to the spindle poles through severing (Mukherjee et al., 2012). It is also observed at the spindle midzone during telophase and in *Drosophila melanogaster*, Fidgetin is additionally observed at chromosomes in metaphase (Mukherjee et al., 2012). Human Fidgetin has been shown to depolymerize microtubules in vitro specifically at the minus end (Mukherjee et al., 2012). Off all three severing enzymes, Fidgetin is the least well characterized, and structural and mechanistic data is very limited for this protein.

Molecular mechanism of microtubule severing

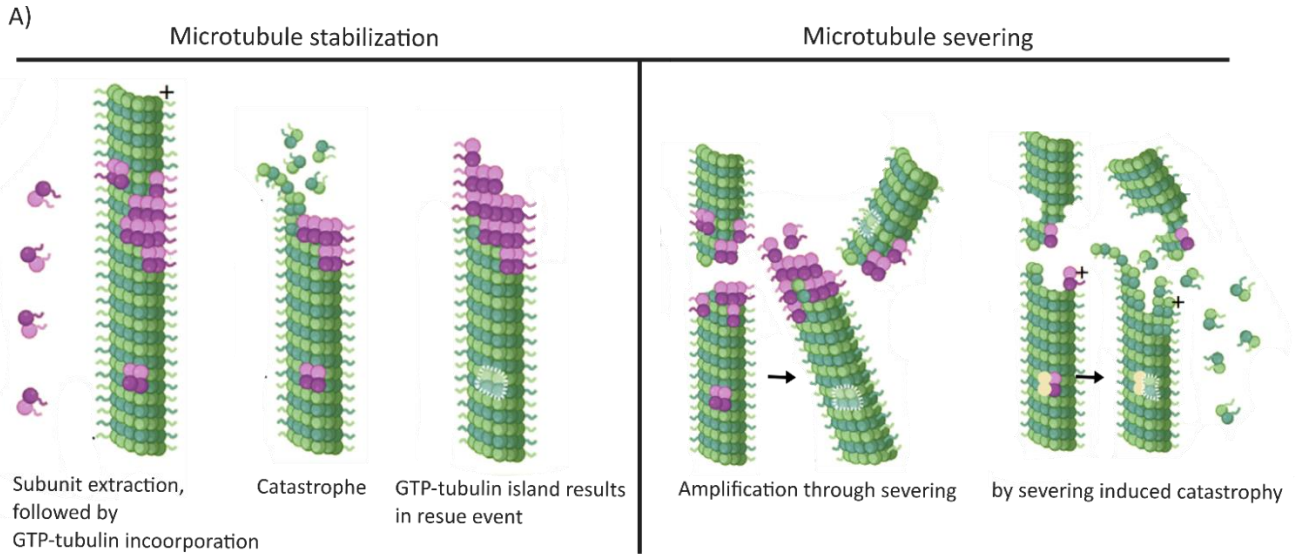
The p60 subunit of Katanin, Spastin and Fidgetin have a very similar domain architecture (fig 3B). All containing the evolutionary conserved AAA ATPase domain that is connected to a microtubule interaction and trafficking (MIT) domain at the N-terminus through a poorly conserved linker (F. J. McNally & Roll-Mecak, 2018). MIT domains have a weak microtubule binding affinity and facilitate interactions with other proteins (Wenzel et al., 2022). In particular, for Spastin the MIT domain facilitates interaction with the ESCRTIII protein CHM1B and for Katanin P60 with ESCRTIII proteins and with its other subunit, P80 (Wenzel et al., 2022). Although the overall structural features of Fidgetin, Katanin and Spastin are highly homologous, which suggest a highly similar molecular mechanism of action for all three severing enzymes, mechanistic and structural work performed on Fidgetin so far is very limited. Therefore, we will focus on Katanin and Spastin.

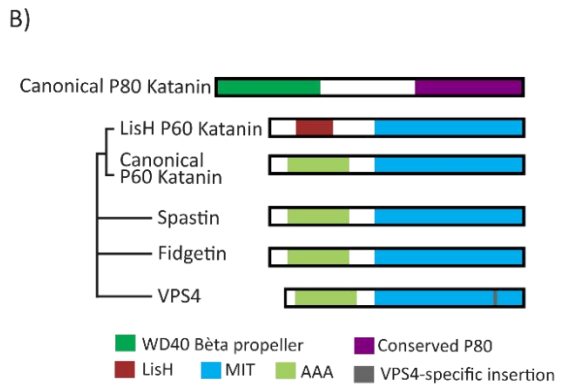
Upon binding of ATP, Katanin and Spastin oligomerize into a hexamer, forming a 14 – 16 nm ring structure around C-terminal tubulin tails on the microtubule (O'Donnell et al., 2012). Hexamerization increases microtubule affinity and catalytic activity. In this conformation, the AAA ATPase domains of Katanin P60 and Spastin face inward and form an internal pore within the hexamer ring through which the C-terminal tail is treaded (E. Zehr et al., 2017; E. A. Zehr et al., 2020). The protein undergoes additional contacts with the microtubule lattice through the MIT domains. The tubulin tail engages in multiple tight interactions with pore loops, conserved positively charged aromatic residues, in the AAA ATPase domains (E. Zehr et al., 2017). Cryo-EM reconstructions of Katanin revealed that the AAA domains of a Katanin hexamer switch between an open spiral an a closed ring conformation, coupled to ATP hydrolysis (E. Zehr et al., 2017). The repeated switching between these two conformation through cycles of ATP hydrolysis, cause the hexamer to 'tug' on the tubulin tail (E. Zehr et al., 2017). Two models exist for how this could lead to the removal of a tubulin dimer. First, the tail could be pulled through the pore, leading to local unfolding and weakening of interdimer bonds and eventually to the removal of the tubulin subunit from the lattice. Second, the hexamer could tightly bind to the tubulin tail and upon ATP hydrolysis bend and straighten to destabilize

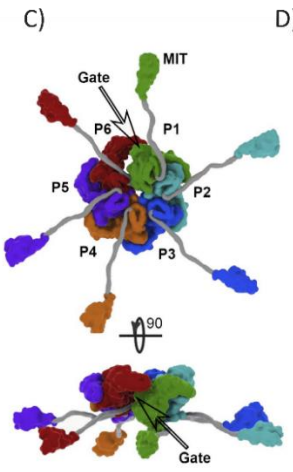
lateral interactions and wedge dimers out of the lattice without unfolding them (Belonogov et al., 2019; E. Zehr et al., 2017) (fig 3E). Both mechanisms are not necessarily mutually exclusive (Belonogov et al., 2019). The hexamer assembly is thought to be stabilized by the microtubule, as both Spastin and Katanin are mostly found as monomers at cellular concentrations in the presence of ATP and the critical concentration for oligomerization can by lowered by addition of microtubules (Tanenbaum et al., 2011).

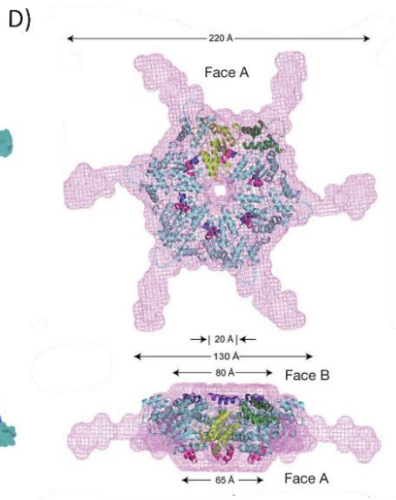
In addition to severing, Katanin is also able to depolymerize microtubules from both ends through a separate mechanism (Belonogov et al., 2019). Unlike for severing, the depolymerization by Katanin is independent of C-terminal tubulin tails and does not require ATP hydrolysis and depolymerization rates are enhanced in the presence of ATP. This suggests that Katanin can bind to microtubules in two different ways and that depolymerization by Katanin is a passive process (Belonogov et al., 2019). The suggested model for Katanin driven microtubule end depolymerization is that simply the binding of Katanin to the interdimer interfaces at the microtubule end destabilizes it (Belonogov et al., 2019). The energy barrier to remove a dimer from the end is less high then for removing a dimer from the lattice, which could explain why this second mechanism of action is not seen along the lattice (Belonogov et al., 2019). Further corroborating this suggested model is that in vitro, observed depolymerization by Katanin decreased after a certain concentration lower than the concentration at which maximal severing was observed (Díaz-Valencia et al., 2011). This potential dual function of Katanin could be regulated through its cellular concentration. End depolymerization has to our knowledge not been reported for Spastin or Fidgetin.

As cells generally express severing enzymes at levels that would allow for complete severing of the entire microtubule mass, tight regulation of their activity and targeting to specific microtubule subsets is crucial (Solowska et al., 2008; Sudo & Baas, 2010). Severing enzymes typically favour stable and longer microtubules (Sarbanes et al., 2022). The post-translational tubulin modifications; detyrosination, (poly)glutamylation and acetylation are characteristic for stable or long lived microtubules, and highly abundant on kinetochore, interpolar and midbody microtubules (Valenstein & Roll-Mecak, 2016). Katanin, but not Spastin, preferably severs microtubules at highly acetylated areas (Sudo & Baas, 2010). How acetylation primes microtubules for Katanin severing is not clear. As the Katanin P80 subunit is important for its localization and generally enhances the severing activity of P60, regulation of expression of P80 may add an additional mechanism of control for Katanin mediated severing (O'Donnell et al., 2012). (Sarbanes et al., 2022). Severing activity of Spastin can be quantitively tuned by microtubule polyglutamylation. Polyglutamylation increases Spastin localization, yet severing activity increases until eight glutamates per tubulin tail, after which it decreases again (Valenstein & Roll-Mecak, 2016). The proposed mechanism for this regulation is that polyglutamylation of microtubules has a positive effect on Spastin recruitment but a negative effect on it's microtubule severing activity. Below the threshold, increased localization still results in increased severing. Above the threshold, Spastin can no longer actively sever but is sequestered by the microtubule, which downregulates its overall cellular activity (Valenstein & Roll-Mecak, 2016). It is suspected that Katanin mediated severing is regulated by polyglutamylation as well. If the threshold for both severing enzymes lies at a different repeat number of glutamates, this could potentially specialize them for microtubule arrays with different glutamylation ranges (Valenstein & Roll-Mecak, 2016). Regulation of severing activity of Fidgetin has to our knowledge not been studied.


A)
Microtubule stabilization
Microtubule severing
Subunit extraction, followed by GTP-tubulin incoorporation
Catastrophe
GTP-tubulin island results in resue event
Amplification through severing
by severing induced catastrophy


B)
Canonical P80 Katanin
LisH P60 Katanin
Canonical P60 Katanin
Spastin
Fidgetin
VPS4
WD40 Bèta propeller
Conserved P80
LisH
MIT
AAA
VPS4-specific insertion


C)
MIT
Gate
P6
P1
P5
P2
P4
P3
90
Gate


D)
220 Å
Face A
20 Å
130 Å
80 Å
Face B
65 Å
Face A

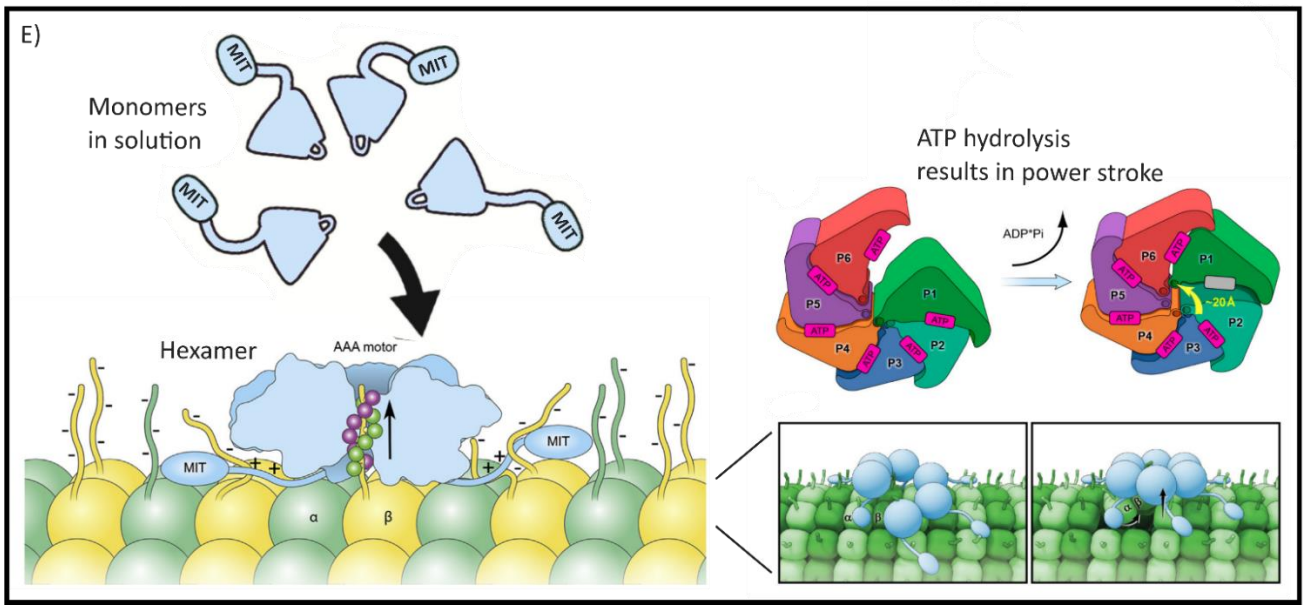

E)
Monomers in solution
MIT
Hexamer
AAA motor
α
β
ATP hydrolysis results in power stroke
ADP*Pi
~20Å

Figure 3 microtubule severing enzymes (A) Different effects on severing on microtubule dynamics. If GDP-tubulin removal by severing enzymes is outpaced by incorporation of new GTP-tubulin dimers, severing enzymes can create 'GTP islands' along the lattice and therefore function through stabilizing the microtubule. If GDP-tubulin removal outpaces GTP-tubulin incorporation, severing enzymes function through creating breaks in the lattice. This either results in catastrophe, or the newly generated ends resume growth. In the latter situation, severing increased the total cellular microtubule mass. Adapted from (Sarbanes et al., 2022). (B) Phylogenetic comparison of the meiotic clade of AAA ATPases and their domain architecture. LisH; lissencephaly type 1-like homology motif, AAA; AAA ATPase domain, MIT; Microtubule-Interacting and Trafficking domain. Adapted from (Roll-Mecak & McNally, 2010). (C) structure of a Katanin hexamer (F. J. McNally & Roll-Mecak, 2018) (C) Structure of a Spastin hexamer (Roll-Mecak & Vale, 2008) (D) Simplified model tubulin dimer removal from lattice by microtubule severing enzymes. Figure is based on Katanin. Katanin monomers form a hexamer around a tubulin tail. Each monomers undergoes multivalent interactions with the microtubule through the AAA ATPase domain, the MIT domain and a neck region. ATP hydrolysis results in a conformation change generates an outward force on the tubulin tail. This cycle is repeated until the lattice contacts are lost and the tubulin dimer dissociates. Figure adapted from (Monroe & Hill, 2016; Sarbanes et al., 2022; Sharp & Ross, 2012).

Microtubule dynamics in mitosis

Mitosis is a multistep and tightly coordinated process in which duplicated chromosomes are segregated and the mother cells splits into two daughter cells. Upon onset of mitosis, the interphase microtubule network needs to be drastically remodelled. The mitotic spindle needs to be assembled, constantly reorganized to comply with the specific mitotic phase, and eventually also disassembled again in order for the interphase network to rebuild (box 1). Therefore, many (microtubule associated) proteins and regulatory mechanisms are needed for coordination. Microtubule severing and depolymerization by previously discussed severing enzymes and microtubule depolymerizing kinesins, under regulation and in cooperation with various regulatory networks and proteins, play a prominent role. There roles are particularly important starting from metaphase, when the initial spindle is assembled and needs to be actively maintained and organized.

Box 1 | Brief overview of mitosis

Mitosis, the process of chromosome segregation, consists of four phases: prophase, metaphase, anaphase and telophase, which are defined based on the appearance of chromosomes under the microscope. Various checkpoints serve as safeguards to ensure successful mitosis. Cyclins control the progression of cells through the cell cycle, through regulation of various kinases that in turn regulate the progression through the different mitotic phases. The cyclin dependent kinases m-CDK, Aurora A and B, polo-like kinases and the anaphase-promoting complex, are master regulators over the course of mitosis.

The two centrosomes, the main microtubule organizing centres of the cell, are located close to the nuclear envelope during interphase. Interphase cells often have a very organized microtubule network, which is required to maintain specific shapes or specialized structures, like cilia.

During **prophase**, the interphase microtubule network is remodelled, and the mitotic spindle starts to assemble. Aided by kinesin-5 sliding microtubules, the two centrosomes translocate from the nuclear envelope to the opposite sides of the cell and start to nucleate microtubules to form the bipolar spindle. The spindle contains three types of microtubules; kinetochore microtubules and interpolar microtubules point towards the opposite pole, and astral microtubules point backwards towards the cell cortex. Simultaneously, the duplicated chromosomes or sister chromatids, start to condense. CDK1 and Cyclin B are important regulators of the earlier mitotic events.

In **prometaphase,** the nuclear envelope abruptly and swiftly breaks down and spindle microtubules start to attach to the kinetochores of the sister chromatids. Attached microtubules bundle into **k-fibres** and chromosomes start to move.

Metaphase is characterized by the alignment of the chromosomes at the metaphase plate. Although the spindle is now at steady state length, individual microtubules remain dynamic. Cells cannot exit metaphase before all sister chromatids are attached correctly and the spindle assembly checkpoint (SAC) is satisfied.

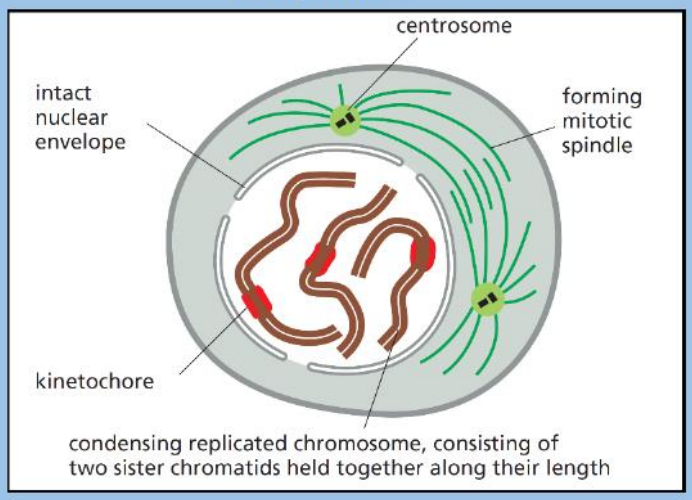
Anaphase can start after the SAC checkpoint is reached, the anaphase-promoting complex (APC/C) signals for loss of cohesions between the two sister chromatids. Anaphase can be divided into two processes, anaphase A and anaphase B. Anaphase A describes the movement of the separated sister chromatids, now again referred to as chromosomes, to the opposite poles. This requires shortening of k-fibres. Anaphase B describes the moving apart of the two poles, largely facilitated through sliding of interpolar microtubules facilitated by various motor proteins at the region of overlap at the midzone. Between the segregating chromosomes, a crosslinked microtubule and protein-based apparatus termed the central spindle starts to form.

During **telophase,** chromosomes arrive at the opposite poles and decondense. New nuclear envelopes form and the spindle is disassembled.

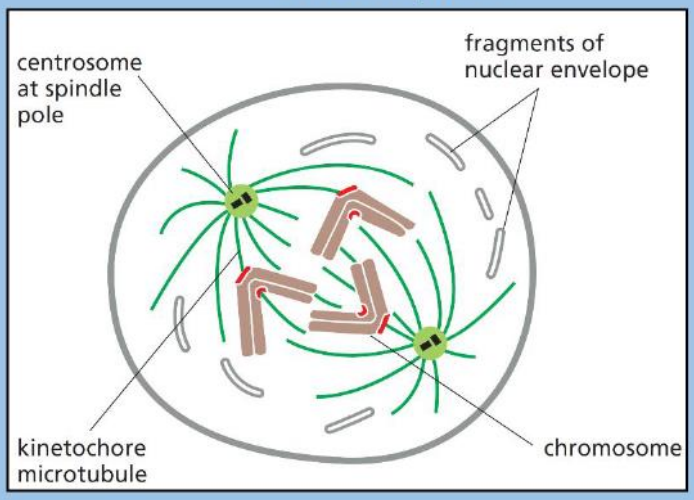
The start of **cytokinesis,** the process of separating the mother cell into two daughter cells, is marked by the formation of the central spindle and overlaps with telophase. An actomyosin contractile ring forms at the site of the central spindle and divides the cytoplasm into two. During **abscission**, the final stage of cytokinesis, the membrane between the two daughter cells is cut. Aurora B kinase, which is part of the chromosome passenger complex, is an important regulator during cytokinesis.

Box 1 | continued

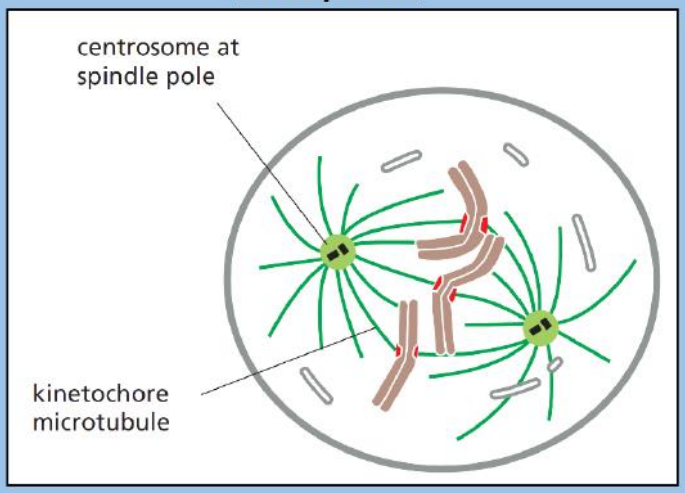
Prophase



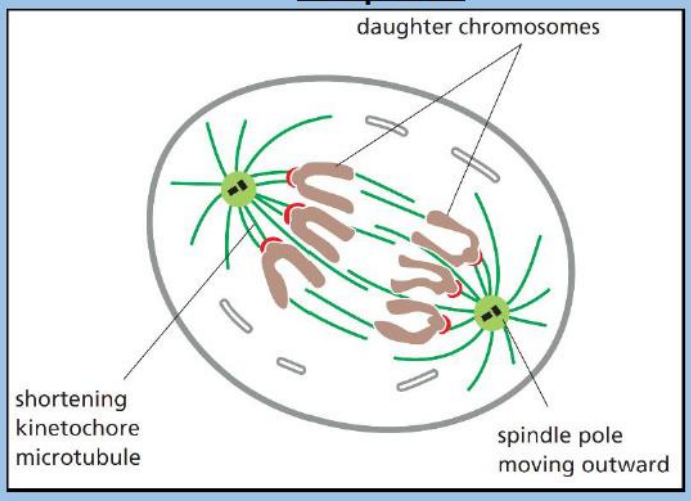
Prometaphase



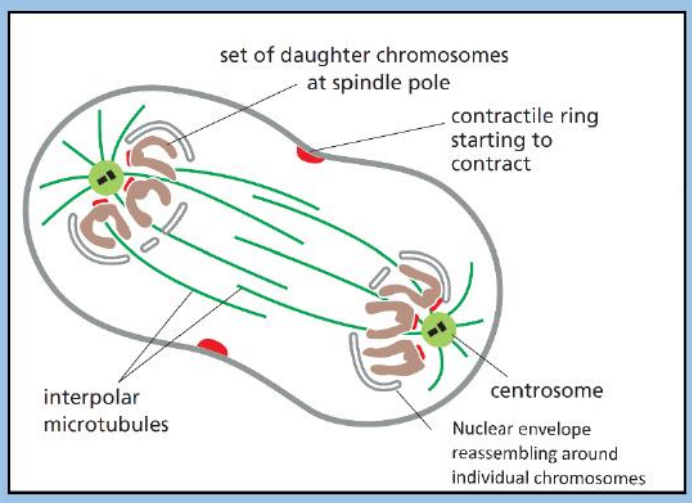
Metaphase



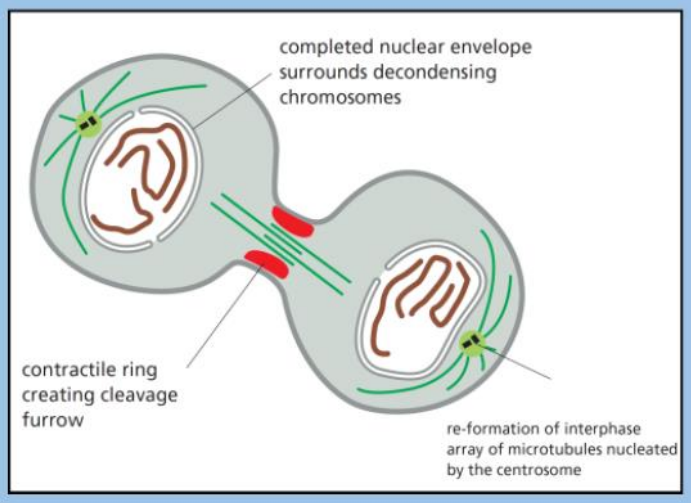
Anaphase



Telophase



Cytokinesis



Text and figures of box 1 are adapted from Alberts et al., 2014

Metaphase

During metaphase, the kinetochores of the chromosomes are attached to bundles of spindle microtubules from opposing poles, from then on often referred to as k-fibres. Proper attachment of both sister chromatids to opposites poles and their alignment at the metaphase plate is required for satisfying the spindle assembly checkpoint (SAC) to complete metaphase.

Although the spindle is at a steady state length during metaphase and is robust as it has the ability to generate and sustain mechanical forces, individual spindle microtubules remain highly dynamic (Barisic et al., 2021). A process of simultaneous depolymerization at minus ends at the spindle poles and plus end polymerization at kinetochores gives rise to constant net inward flux of tubulin while maintaining the microtubule at constant length (Fig 4 A,B). This phenomenon, referred to as 'poleward flux', is an evolutionary conserved mechanism and creates tension in the spindle and on chromosomes which is important for proper metaphase plate alignment, spindle stability and SAC satisfaction (Rogers et al., 2004). Through regulation of microtubule length and dynamics, microtubule severing enzymes and depolymerization kinesins play important roles in generating poleward flux and in correct kinetochore to spindle pole attachment and alignment.

Poleward flux within the steady-state metaphase spindle

Poleward flux describes the movement of a tubulin dimer within a microtubule towards the minus end (fig 4B). It is the net result of balanced kinesin-13 mediated depolymerization at microtubule minus ends at the poles, combined with polymerization at microtubule plus ends at the midzone and at kinetochores. Flux appears to be a process unique to k-fibre and interpolar spindle microtubules, as it has not been observed in astral microtubules or in the interphase microtubule network (Rogers et al., 2005; Zhai et al., 1995). Poleward flux rates are not constant and change between the different stages of mitosis in cultured cells (Rogers et al., 2005).

Although the phenomenon of poleward flux has been studied for multiple decades, the exact molecular mechanism from which the required force originates has been a topic of debate. Two main models are proposed to drive poleward flux. In the first model, force required for flux is produced at the spindle poles through microtubule depolymerization and is independent of plus-end dynamics (reviewed by Rogers et al., 2005). This model is challenged by experiments performed in *Drosophila melanogaster* S2 cells, where flux was still observed after detachment of stable microtubule minus ends from the spindle pole (Maiato et al., 2004). The model is however reinforced by microsurgery experiments in grasshopper spermatocytes that were cut in halve to create two spindles. Here, poleward chromosome movement was completed in the generated monopolar half-spindles (Alsop & Zhang, 2003). In the second model, force for poleward flux originates from tension induced by sliding of interpolar microtubules, which is transferred to k-fibre microtubules through crosslinking (reviewed by Barisic & Rajendraprasad, 2021) (fig 4A). Minus end depolymerization at the pole is then viewed as a mere response to this outward force and not as a driver of flux, suggesting that poleward flux can be uncoupled from minus end depolymerization at the pole (Barisic et al., 2021). Noteworthy is that both models developed from experiments with different model systems, thus they may not need to inherently contradict each other. Different systems can possibly implement different mechanisms for generating poleward flux.

A recent study by Steblyanko et al. provides strong evidence that in mammalian cells, force for poleward flux is generated as described by the second model. Their study showed that the combined action of 4 motor proteins together with different microtubule crosslinkers provides the forces important for generating poleward flux in different stages of mitosis (Steblyanko et al., 2020).

Plus-end directed kinesins Kif4A located at chromosome arms and EG5 and KIF15 at interpolar microtubules at the midzone, slide interpolar microtubules poleward to generate the outward force for poleward flux (Steblyanko et al., 2020). Cross-linking proteins like NuMA and HSET transfer this force to k-fibre microtubules (Steblyanko et al., 2020). Outward movement of both interpolar and k-fibre microtubules is then counterbalanced by polymerization at the microtubule plus end, aided by +TIPs such as CLASPs, and depolymerization at the minus ends by kinesin-13 Kif2A. The result is a metaphase spindle under tension but at a constant length (Barisic et al., 2021; Steblyanko et al., 2020). Kinesin-13 MCAK is located at kinetochores (Shao et al., 2015). It is hypothesized that poleward flux regulates the balance between MCAK-mediated depolymerization and plus end polymerization of k-fibre plus ends in steady state length metaphase spindles. Namely, generated pulling forces on kinetochores are thought to counteract MCAK mediated plus end depolymerization of k-fibres by providing a bias for plus end polymerization (Barisic et al., 2021; Steblyanko et al., 2020). This theory is in line with studies showing that either over expression of MCAK or depletion of CLASP result in shorter spindles (Steblyanko et al., 2020).

Microtubule severing enzymes also play important roles in poleward flux. Abnormal spindle-like microcephaly associated protein (ASPM) forms a complex with Katanin, enhancing the latter's activity (Jiang et al., 2017). The complex localizes to spindle poles, and is suspected to contribute to poleward flux in two ways (Jiang et al., 2017). First, preferential minus-end binding of ASPM result in a high local concentration of the Katanin-ASPM complex at spindle poles, where Katanin then severs microtubules. Second, ASPM binding to severed microtubule ends prevent their growth, and this can be potentiated by Katanin. The Katanin-ASPM complex therefore might prime microtubules at spindle poles for depolymerization, as blocked microtubules make more suitable substrates for KIF2A, promoting poleward flux (Jiang et al., 2017) (fig 4C). In support of this theory is that MCAK can efficiently accumulate at stable, but not at growing microtubule ends without any additional factors (Montenegro Gouveia et al., 2010; Tanenbaum et al., 2011). Similar to ASPM, WDR6, a microcephaly associated proteins who's function and localization remain under debate, and Katanin also mutually enhance each other's Localization to spindle poles (Guerreiro et al., 2021; Huang et al., 2021). ASPM or WDR6 facilitated localization of Katanin are independent of each other (Guerreiro et al., 2021).

Human and *Drosophila melanogaster* Fidgetin and Spastin have been linked to poleward flux through a suspected similar mechanisms as Katanin, as depletion results in slower flux rates during metaphase (Mukherjee et al., 2012; Zhang et al., 2007). Fidgetin is thought to release microtubules from γ-TurC shortly after nucleation at spindle poles (Mukherjee et al., 2012). Released microtubules can then quickly be stabilized by different MAPs, to allow incorporation into the spindle and become accessible as substrate for kinesin-13 family members driven depolymerization. Based on the fact that Fidgetin shows minus-end specific depolymerization of *in vitro* reconstituted microtubules, it could alternatively also depolymerize microtubules directly at spindle poles to contribute to poleward flux (Mukherjee et al., 2012). As simultaneous knockdown of severing proteins has a high lethality rate, it is unfortunately difficult to assess if Katanin, Spastin and Fidgetin have distinct or partially redundant functions at the spindle poles (Sharp & Ross, 2012). Interestingly, *Drosophila melanogaster* Katanin does not contribute to metaphase poleward flux (Zhang et al., 2007). Regulatory mechanisms for generation and maintenance of poleward flux could therefore differ between species.

Roles of microtubule depolymerization during chromosome alignment

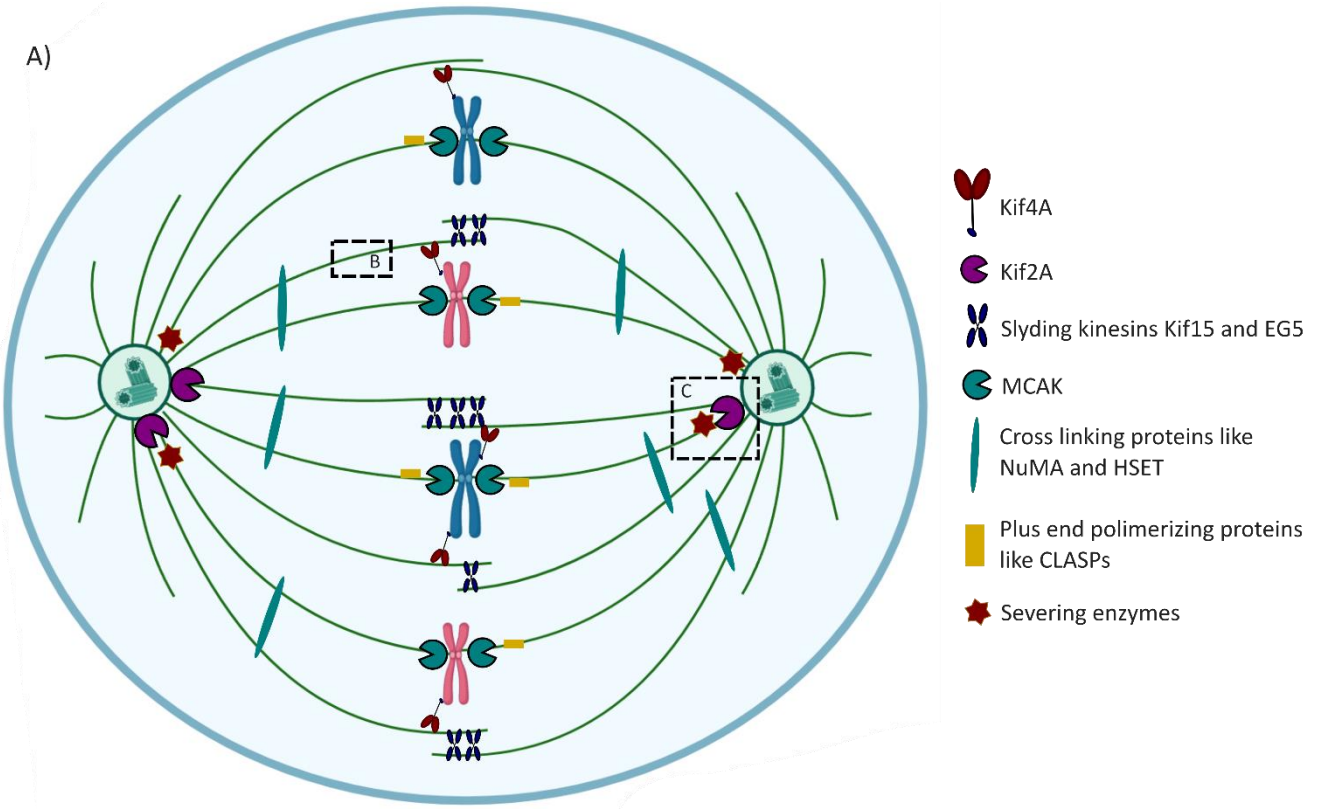
Correct attachment of sister kinetochores to opposite poles and alignment at the metaphase plate during metaphase is crucial for satisfying the SAC and for accurate chromosome segregation during anaphase. Chromosomes can be connected to the spindle poles in three ways; I) amphiletic attachment, correct attachment in which the kinetochores of both sister chromatids are connected to a different pole, II) syntelic, both kinetochores are attached to the same pole and III) merotelic, in which a single kinetochore is attached to both poles. As syntelic and merotelic attachment result in inaccurate chromosome segregation during anaphase, kinetochore-microtubule attachment needs to be both tightly regulated and corrected during metaphase (Lan et al., 2004).

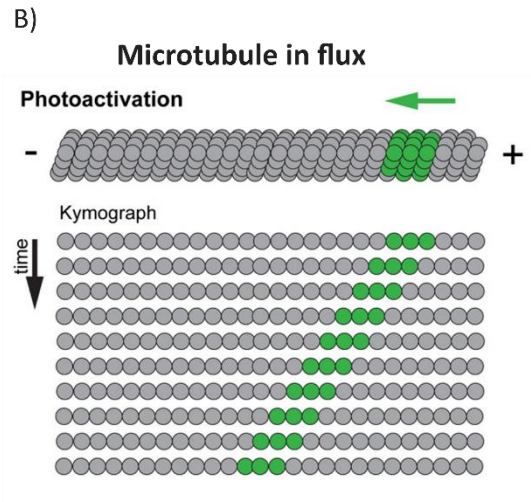
Microtubule depolymerizing enzymes of the kinesin-13 family play a vital role in detaching wrongly attached microtubules from kinetochores to ensure amphiletic attachment (fig 4D). Kinesin-13 activity is regulated through master cell division regulator Aurora B kinase and through changes in tension within the centromere (Bakhoum et al., 2009; Shao et al., 2015). In prometaphase and metaphase, Aurora B is located at the centromeres with a decreasing concentration gradient towards the kinetochores (Shao et al., 2015). Aurora B can recruit, activate and retain Kif2B at kinetochores, but exclusively during prometaphase (Bakhoum et al., 2009). Centromeres are under relatively low tension at this time, so the concentration gradient reaches far enough outwards. Kif2B destabilizes the attachment of microtubules through active depolymerization (Bakhoum et al., 2009). Kif2B expression levels are very low (Bakhoum et al., 2009) and this is thought to allow for microtubules to attach to the kinetochores during prometaphase but not to form stable connections (Bakhoum et al., 2009; Lan et al., 2004). Simultaneously, Aurora B recruits MCAK to the centromeres and puts it in an inactive state through phosphorylation of S194 in the neck region (Knowlton et al., 2006; Lan et al., 2004; Shao et al., 2015). Aurora B also activates the kinase PLK1 at the centromere (Shao et al., 2015). In part because of poleward flux, correct attachment and orientation of chromosomes creates tension at the centromeres (Bakhoum et al., 2009; Matos et al., 2009). Kinesin-8 Kif18A is mammalian is crucial for generating enough kinetochore tension to satisfy the SAC, but the molecular mechanism behind this requires further exploration (Janssen et al., 2018). Increased tension stretches the centromeres apart. Therefore, due to the concentration gradient, Aurora B concentration at the kinetochores decreases as tension increases (Liu et al., 2009). This results in the loss of Kif2B at the kinetochores, as Aurora B can no longer retain it there (Bakhoum et al., 2009). Secondly, this also separates MCAK and PLK1 from Aurora B. This results in activation of MCAK through phosphorylation by PLK1 and allows for further correction in microtubule to kinetochore attachment (Shao et al., 2015). Once proper attachment and alignment at the metaphase plate are achieved, the majority of PLK1 has to be removed from the kinetochores in order to maintain stable microtubule to kinetochore connections and to satisfy the SAC (Beck et al., 2013). This is achieved through ubiquitination of PLK1 by E3 ubiquitin ligase Cullin 3 in complex with the adaptor protein KLHL22, which starts to accumulate as stable microtubule kinetochore connections start to form (Beck et al., 2013). The fate of MCAK once proper chromosome attachment is achieved, is an area that requires further study. Although Aurora B and MCAK localize at higher levels to not yet aligned and to aligned but merotelically attached centromeres (Knowlton et al., 2006), there is no direct experimental evidence for specific Aurora B mediated targeting of the correction machinery to syntelic or merotelic attached chromosomes. Due to the orientation of chromosomes with kinetochores facing towards one of the spindle poles, they are biased to be captured by microtubules originating from that pole. Therefore, Bakhoum et al. propose a model where there is no specific depolymerization of incorrect attached microtubules, but rather that randomly destabilizing microtubule attachments will eventually favour accumulation of properly attached microtubules

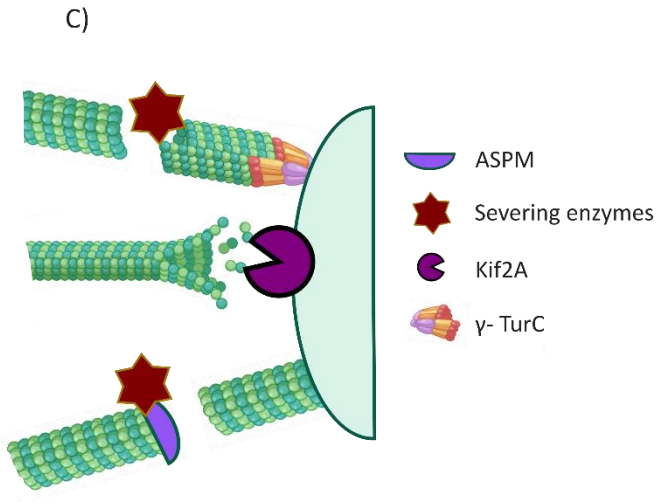
without the need to specifically target the correction machinery to mal-oriented kinetochores (Bakhoum et al., 2009; Liu et al., 2009).

The spatial separation between Aurora B and its targets at the centromere and kinetochores through tension also regulates other proteins involved in regulation of stable microtubule-to-kinetochore attachment. For example, spatial separation due to tension allows crosslinker protein NDC80, to stabilize microtubule to kinetochore attachments (Asbury, 2017). It was shown that without centromere tension, which is present in properly attached and aligned chromosomes at the metaphase plate, no stable connections between microtubules and kinetochores can be obtained (Liu et al., 2009). This highlights the importance of by poleward flux generated tension and the complexity of regulation during mitosis.

In the process of congression, chromosomes keep oscillating over the metaphase plate until the sudden loss of sister chromatid cohesion marking anaphase onset (Mayr et al., 2007, Skibbens et al., 1993). Kif18A dampens chromosome oscillations at metaphase plate alignment by increasing the switches between the direction of the oscillations and by decreasing overall movement velocity (Du et al., 2010) Although the exact molecular mechanism behind this has not yet been elucidated, a hypothesised model is that when a chromosome oscillates further over the metaphase plate, more Kif18A will accumulate at the tip of the k-fibre microtubules and induce depolymerization, bringing the chromosome back towards the metaphase plate. This model is in line with the threshold model and the concentration and microtubule length dependent depolymerization behaviour of kinesin-8 family members as discussed in a previous paragraph. This model is also in line with the observation by Stumpff et al. that kif18A often accumulates at a higher level at one of the sister kinetochores at a chromosome (Stumpff et al., 2008).


A)
B
C
Kif4A
Kif2A
Slyding kinesins Kif15 and EG5
MCAK
Cross linking proteins like NuMA and HSET
Plus end polimerizing proteins like CLASPs
Severing enzymes


B)
Microtubule in flux
Photoactivation
-
+
Kymograph
time


C)
ASPM
Severing enzymes
Kif2A
γ- TurC

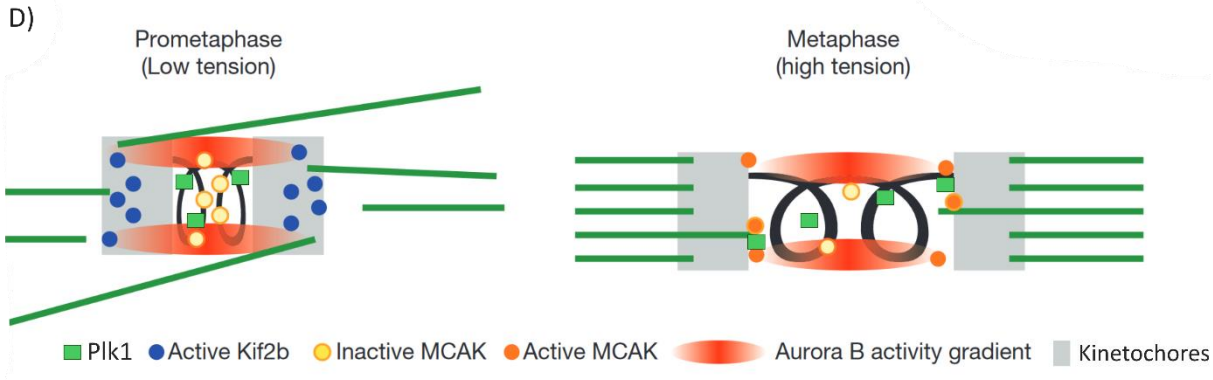

D)
Prometaphase
(Low tension)
Metaphase
(high tension)
Plk1
Active Kif2b
Inactive MCAK
Active MCAK
Aurora B activity gradient
Kinetochores

Figure 4 poleward flux within the steady-state metaphase spindle (A) Simplified schematic illustration of the Regulation of poleward flux in steady state length metaphase spindle. Kif4A localized at chromosome arms, together with crosslinking and sliding kinesins EG5 and KIF15 at interpolar microtubules, create an outward force which is transferred to k-fibres by crosslinking proteins such as NuMA and HSET. This outward force is counterbalanced by minus end depolymerization at the poles Kif2A and plus end polymerization aided by +tips such as CLASP. Together, this results in poleward flux. Severing enzymes additionally contribute to flux at the spindle poles. Adapted from (Barisic & Rajendraprasad, 2021; Sharp & Ross, 2012; Steblyanko et al., 2020)(B) Schematic representation of microtubule flux as observed using photoactivation-based microscopy that relies on laser activation of photoactivatable fluorescent protein-tagged tubulin. Adapted from (Barisic & Rajendraprasad, 2021) (C) Close-up of (A) displaying dynamics at the spindle pole in more detail. Katanin, together with minus end stabilizing proteins like ASPM, primer microtubule minus ends for kinesin-13 mediated depolymerization. Additionally, severing at the poles regulates spindle microtubule number by freeing γ-TurC and through creating new seeds for microtubule growth. Adapted from (Barisic & Rajendraprasad, 2021; Sharp & Ross, 2012; Steblyanko et al., 2020). (D) Model for establishing correct kinetochore to microtubule attachments. A lower inter-kinetochore tension, Aurora B recruits and activates Kif2B which destabilizes microtubule attachments to the kinetochores, keeps MCAK in an inactive state and recruits Plk1. At higher inter-kinetochore tension, the Aurora B gradient does no longer reach far enough outward to retain Kif2B at the kinetochores. MCAK and PLk1 now also fall out of the concentration gradient, resulting in Plk1 mediated activation of MCAK. MCAK destabilizes microtubule attachments. Adapted from (Bakhoum et al., 2009; Shao et al., 2015).

Anaphase

During anaphase, sister chromatids separate and move towards the spindle poles (box 1). Anaphase consists of two partly simultaneous yet mechanistically distinct processes, Anaphase A and Anaphase B. During Anaphase A, chromosomes translocate towards the two spindle poles through a mechanism of microtubule disassembly referred to as 'Pacman-flux'. At kinetochores, microtubule plus ends of k-fibres are actively depolymerized. Thereby the chromosome is 'chewing' its way polewards, and this motility is referred to as Pacman. Simultaneously, k-fibre microtubule minus ends are also actively depolymerized, and therefore drag the chromosomes poleward, which is referred to as flux (Zhang et al., 2007) The ratio to which Pacman and flux contribute to chromosome movement largely varies between species. In *Drosophila melanogaster*, Pacman and flux contribute equally to chromosome movement, and the rate of depolymerization at spindle poles is similar to the rate observed during metaphase (Zhang et al., 2007). In mammalian cells, the ratio of contribution of Pacman and flux to net poleward movement is usually 1 to 3 (Barisic & Rajendraprasad, 2021; Zhai et al., 1995). In contrast, budding and fission yeast display no flux and poleward chromosome movement solely depends on Pacman (Sagolla et al., 2003).

Anaphase B is the spatial separation of the two opposite spindle poles by elongation through the sliding spindle microtubules of opposite poles, for which the forces are generated by motor proteins. Poleward movement can be facilitated by active microtubule depolymerization at the spindle pole, at the kinetochore or through a combination of both.

Chromosome segregation in Anaphase A

As during anaphase A the chromosomes need to move outward towards the opposing poles, dynamics of poleward flux need to be remodelled from maintaining steady state to shortening of k-fibres. This can be achieved by converting the plus end polymerization at kinetochores into active depolymerization (Pacman), no longer balancing out the depolymerization at the minus ends (flux) and resulting in poleward movement of the chromosomes. In mammalian cells, k-fibre microtubule depolymerization by kif2A located at the spindle poles is the main driver of the flux component.

(Barisic & Rajendraprasad, 2021; Ganem et al., 2005). Yet the mechanism behind the Pacman component, k-fibre plus end depolymerization, remains largely unclear for mammalian cells (Barisic & Rajendraprasad, 2021).

The Pacman-flux model for chromosome segregation is better characterised in *Drosophila melanogaster* (Fig 5A). Kinesin-13 KLP59C facilitates the k-fibre plus end depolymerization at kinetochores and KLP10A minus end depolymerization at the spindle poles (Rogers et al., 2004). Dynein plays an important role by pushing the microtubule plus end towards the kinetochore (Rogers et al., 2004). To our knowledge solely in *Drosophila melanogaster*, Katanin localizes to and is upregulated at kinetochores during Anaphase A where it promotes Pacman (Zhang et al., 2007). It is proposed that Katanin has a similar function here as severing enzymes at the spindle poles, namely through removing plus end caps from the microtubule through severing to make them suitable substrates for microtubule depolymerizing kinesins (Zhang et al., 2007). Although no specific Pacman-inhibiting microtubule plus end capping complexes have been identified, there are many known microtubule plus end associated proteins such as CLIPs, EBs and CLASPs, that have stabilizing properties (Akhmanova & Kapitein, 2022). It has however not been determined if such proteins inhibit kinesin-13 driven microtubule depolymerization. Severing near the plus end could also function to decouple k-fibre microtubules from spindle pole microtubules, for example through the severing of interpolar microtubules bound by chromosome arm bound sliding kinesins (Zhang et al., 2007). Alternatively, Katanin could directly facilitate Pacman through severing without involvement of kinetochore located depolymerizing kinesins. However, studies demonstrating the contribution of different kinesin-13 members to Pacman in different cell types through knockout and knockdown experiments do not support this theory (Rogers et al., 2004; Zhang et al., 2007)

Destabilization of k-fibres through severing of microtubules at the spindle poles is crucial for proper chromosome segregation (Guerreiro et al., 2021). This is highlighted by the observation that depletion of WDR62, which facilitates proper Katanin recruitment at the spindle poles in mammalian cells, results in asynchronous anaphase due to lagging chromosomes as a consequence of reduced poleward flux rates (Guerreiro et al., 2021). Reduced flux rates due to inefficient severing are likely a result of too little substrate available for depolymerizing kinesins.

Kinetochores can remain attached to the plus end depolymerizing k-fibre microtubules through tethering proteins. In mammalian cells, the most important tether is the Ndc80c complex (Asbury, 2017). Ndc80c itself is stably anchored to the outer kinetochore layer, where together with other tethering proteins like KNL1 it forms clusters that bind and anchor k-fibres to the kinetochore (Cheeseman & Desai, 2008). Additionally and to a lesser extent, conventional motor proteins also play roles in microtubule to kinetochore coupling, and the degree of contribution highly varies between species (Asbury, 2017)

Central spindle formation

At anaphase onset, the mitotic spindle undergoes major structural transformations. Astral microtubules nucleate from the poles and grow towards the cortex and the central spindle starts to form at the midzone between the poleward moving chromosomes. The central spindle consists of partially overlapping bundles of microtubules of similar lengths that lie parallel to the division axis (Yu et al., 2019). Many microtubule bundling proteins such as PRC1 or centralspindlin are located at the central spindle. The central spindle is a key regulator over the course of cytokinesis. It locally activates small GTPase RhoA which triggers the formation of the acto-myosin contractile ring (Uehara et al., 2013). After furrow ingression the central spindle forms the midbody, which functions as a binding platform for many proteins and lipids that play a role in abscission. Some central spindle

microtubules originate from the spindle poles, but many nucleate de novo from k-fibres or chromosomes. Aurora B kinase, forms a concentration gradient fading outward at the central spindle (Uehara et al., 2013). Kinesin-13 Kif2B locates to the central spindle microtubule minus ends and its activity can be inhibited by Aurora B phosphorylation. Therefore, following the concentration gradient of Aurora B, Kif2B is more active the further away it is from the middle of the central spindle, keeping the central spindle microtubules at a steady state length (Uehara et al., 2013).

Anaphase B

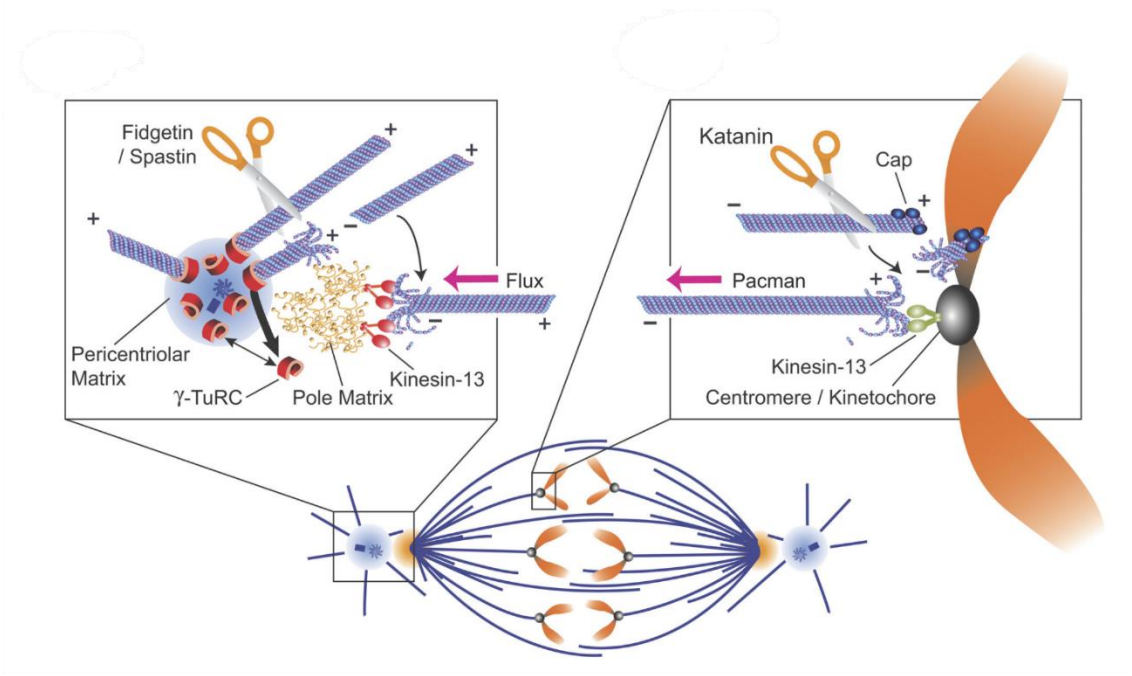
Anaphase B describes the process of spindle elongation to distance the two spindle poles further from each other. As describes previously, in metaphase and anaphase A motor proteins slide the interpolar microtubules over each other but this outward movement is counteracted through depolymerization at the microtubule minus ends. Upon Anaphase B onset, the ratio between plus end polymerization and depolymerization at interpolar microtubules needs to shift toward polymerization to allow for net microtubule growth so that the sliding of the interpolar microtubules by motor proteins at the midzone can produce forces to move apart the two spindle poles. To allow for poleward chromosome movement (Anaphase A), Spindle elongation has a slower speed then poleward movement in many systems (Yu et al., 2019)

After degradation of cyclin B signals for anaphase B onset, poleward flux within the interpolar microtubules is highly reduced through the inhibition of depolymerization from the poles (fig 5B). In *Drosophila melanogaster*, this is regulated through increased localization of the microtubule end binding and stabilizing protein Patronin (Wang et al., 2013). Cyclin B degradation leads to a further uncharacterized modification of Patronin, which enhances its localization and activity at inter polar microtubules (Wang et al., 2013). Patronin thereby protects the microtubules from kinesin-13 mediated depolymerization, shutting down the minus-end component of poleward flux (Goodwin & Vale, 2010; Pavlova et al., 2019; Wang et al., 2013). The plus-end polymerization at the midzone continues, resulting in a net increase in microtubule length. This leads to the recruitment of more cross-linker proteins like the Ase1-PRC1-MAP65 family, in turn resulting in a more robust and midzone. Motor proteins like kinesin-5 family members continue with actively sliding the interpolar microtubules, but as this force is no longer counterbalanced, the spindle poles start to move apart to elongate the spindle further. Patronin caps and protects interpolar microtubule ends all over the spindle and not just at the poles, resulting in further stabilization and strengthening (Pavlova et al., 2019; Wang et al., 2013). It needs to be determined if CAMSAPs, the human homologues of Patronin, have similar function during Anaphase B. CAMSAP1 is the only isoform that localizes to mitotic spindles and its loss only results in a slight reduction in spindle length (Hueschen et al., 2017; Pavlova et al., 2019). Severing at the poles by Katanin during anaphase B to create new seeds for microtubule growth is thought to ensure enough microtubules are present in the spindle to allow for the sliding forces to be generated (K. McNally et al., 2006).

Anaphase B spindle elongation scales with cell size (Rizk et al., 2014). As kinesin-8 family members promote microtubule depolymerization in a length dependent manner (discussed in previous paragraph), they are likely candidates to aid in regulating spindle elongation. Kinesin-8 members are required for successful progression through metaphase in HeLa and *Drosophila melanogaster* S2 cells, hence studying their role during anaphase B can be challenging (Mayr et al., 2007; Rizk et al., 2014). Therefore, the role of kinesin-8 kip3 in regulating Anaphase B spindle elongation has been characterized in budding yeast, as here metaphase can be completed successfully independent of kip3 (Rizk et al., 2014). The midzone converts from a dynamic state to a relatively non-dynamic state after it has reached its maximal length (Rizk et al., 2014). Likely through destabilizing interpolar microtubule plus-ends in a length dependent manner, kip3 limits the region of overlap of interpolar

microtubules at the midzone. This in turn could limit the maximal outward pushing force generated by sliding motor proteins so that enough force can be generated to overcome cytoplasmic resistance but not cell-cortex resistance during spindle elongation, scaling spindle elongation to cell size (Rizk et al., 2014). Alternatively, length dependent microtubule depolymerization by Kip 3 could increasingly suppress polymerization at the midzone over the course of elongation (Rizk et al., 2014).

A)



B)

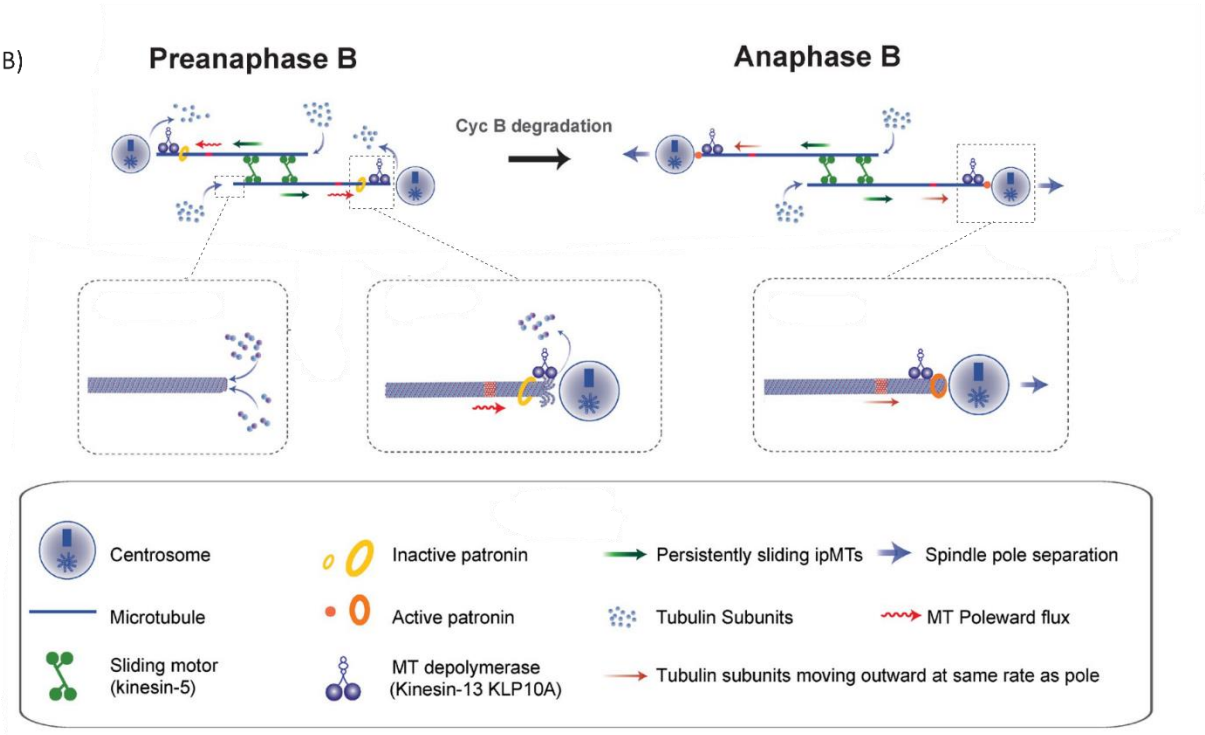


Figure 5 *Drosophila Melanogaster* Anaphase A and B spindle dynamics (A) Simplified model of the 'Pacman-flux' machinery as characterized for *Drosophila Melanogaster* specifically. At the spindle poles, Spastin and Fidgetin sever microtubules attached to γ-TurC to turn them into suitable substrates for Kinesin-13. At kinetochores, Katanin severs microtubules near to plus end. It is suspected that this removes capping proteins that stabilize the plus ends. Adapted from (Zhang et al., 2007) (B) Simplified model of induction of Anaphase B onset in *Drosophila Melanogaster.* Before Anaphase B onset, minus ends at the poles are depolymerized by kinesin-13 KLP10A to counteract outward force generated by kinesin-5 sliding apart interpolar microtubules (poleward flux). Upon cyclin B degradation, Patronin get's activated at the spindle poles and stabilizes interpolar microtubule minus ends and protects them from KLP10A. This allows for spindle elongation. Adapted from (Wang et al., 2013).

Nuclear envelope resealing

During nuclear envelope reassembly, leftover microtubule bundles still associated with chromatin discs locally prevent membrane sealing (Vietri et al., 2015). At these sites, CHMP7 recruits CHMP4B which in turn recruits the rest of the ESCRT-III machinery. Simultaneously, IST1, a component of the ESCRT-III machinery, recruits Spastin which severs the leftover microtubules to enable ESCRT-III to seal the membrane and close the holes (Vietri et al., 2015). This ESCRT-III based membrane sealing is thought to be a distinct process and not a continuum of abscission, as these two events are dependent on different upstream factors (Vietri et al., 2015).

Telophase

During telophase, the final phase of mitosis, sister chromatids have reached the opposing poles. A new nuclear envelope forms, and spindle pole microtubules start to disassemble. Spindle disassembly relies at least in part on microtubule severing and depolymerizing enzymes. Spindle disassembly is important for nuclear envelope formation and provides free tubulin dimers needed for organization of the interphase microtubule network. Cytokinesis describes the process of the separation of the cytoplasmic components and division into two daughter cells. It starts with the separation of the cytoplasm by an actomyosin contractile ring. Further contraction leads to a thin cytoplasmic bridge connecting the two daughter cells. The bridge is full of former spindle microtubules and its centre is formed by an electron dense and protein rich structure termed the midbody. During abscission, the last step of cytokinesis, this bridge is cut. Microtubules are cut through severing and the membrane is cut by ESCRT machinery. After abscission, cell division is completed.

Mitotic spindle breakdown after chromosome segregation

After the chromosomes are successfully segregated during anaphase, the bulk of mitotic spindle is broken down. This is a swift and irreversible process, as interpolar microtubules are no longer detectable 2 min after the abrupt onset of disassembly (Xu et al., 2022). Although spindle assembly has been extensively researched, disassembly is poorly understood. Most of the current knowledge stems from studies in budding yeast.

In budding yeast, spindle disassembly is coordinated by three distinct pathways, together referred to as the spindle disassembly network (Woodruff et al., 2010). In the first pathway, cross-linking MAPs are degraded to facilitate separation and destabilization of the two spindle halves. The mitotic exit network (MEN) stimulates association of the anaphase-promoting complex (APC) with its co-factor Cdh1 (Woodruff et al., 2010). This results in ubiquitination followed by degradation of multiple microtubule crosslinking or midzone proteins, including Ase1 (yeast PRC1 homologue), Cin8 (yeast

BimC homologue). The mechanism of activation by MEN of this pathway is not clear. In the second pathway, Ipl1 (yeast Aurora B homologue), through phosphorylation inactivates the interpolar microtubule stabilizing protein bim1 (yeast EB1 homologue) and activates the spindle destabilizing protein She1. In the third pathway, kinesin-8 Kip3 depolymerizes interpolar microtubules and actively prevents their regrowth, finishing spindle disassembly (Woodruff et al., 2010). The implementation of three distinct pathways could facilitate the swift switch-like onset and irreversible disassembly of the spindle.

The spindle disassembly network found in yeast has not been observed in mammalian cells, suggesting that mammalian cells possess different mechanisms for spindle disassembly (Xu et al., 2022). However, considering the high degree of conservation of aforementioned proteins between different eukaryotes, one could speculate that some of these mechanisms are also found in mammalian cells. For example, the APC complex was observed to concentrate at the central spindle and to destabilize PRC1 (Peters et al., 2006).

A recent study discovered reduction-oxidation as a mechanism for spindle disassembly (Xu et al., 2022). Due to the highly dynamic and reversible properties of cellular redox in physiological conditions, especially in comparison with other covalent tubulin modifications, it perfectly matches the requirements for the regulation of the swift turnover of spindles (Xu et al., 2022). Cysteine-rich interactor of PDZ-domain protein 'CRIPT' , a postsynaptic protein that links synaptic membrane proteins to microtubules, was incidentally discovered to also play a crucial role in spindle disassembly (Xu et al., 2022). CRIPT localizes to spindle poles and the midzone, where it undergoes redox modification within its conserved CXXC motifs. Direct interaction of CRIPT with spindle microtubules results in a putative thiol-disulfide redox exchange with tubulin subunits, leading to immediate microtubule depolymerization (Xu et al., 2022).All cysteine residues of lattice incorporated tubulin dimers are accessible to cytoplasmic ross. Therefore, tubulin dimers appear to not be able to sense normal cytoplasmic ROS levels directly but require additional proteins like CRIPT for redox mediated regulation. (Xu et al., 2022). CRIPT might have a higher reactivity for H_2O_2 then tubulin, could therefore sense oscillations in cytoplasmic ROS, and transfer this to tubulin. How exactly CRIPT activity itself is regulated during mitosis and how it can transfer redox signals to tubulin, requires further exploration. Cell cycle coordination has been linked to intracellular redox oscillations before (Conour et al., 2004; Patterson et al., 2019). Combined with the exciting link between CRIPT redox transfer and rapid spindle disassembly, intracellular redox poses a novel mechanism of mitotic regulation.

Microtubule severing during cleavage furrow ingression

Katanin P60 is an important regulator of central spindle dynamics. In telophase, Katanin P60, but not P80, starts to relocate from the spindle poles to the central spindle (Matsuo et al., 2013). During cleavage furrow ingression, it localizes in a ring-shaped manner to the gap between the contractile ring and the microtubule bundles of the central spindle. The mechanism behind this localization is unknown. As the central spindle is a protein dense region and covered with spindle-binding proteins, Katanin P60 is thought to not be able to properly access microtubules (Matsuo et al., 2013). As the actomyosin-ring contracts, Katanin P60 gets forced to accumulate and to make close contact with the microtubule bundles. This enables Katanin to sever the microtubules, clearing the way for further ingression of the actomyosin-contractile ring (Matsuo et al., 2013). During cytokinesis, Katanin is thought to prevent re-elongation of midbody microtubules (Matsuo et al., 2013).

Abscission

After the contraction of the actomyosin contractile ring ('primary constriction') is finished, the formed cellular bridge is further narrowed ('secondary constriction') which involves reorganization of the cytoskeleton, membrane remodelling and the removal of F-actin. Abscission of the cytoplasmic bridge separates the two daughter cells and is the final step of cytokinesis. Abscission can be divided into two separate steps that are controlled by distinct molecular machineries; severing of microtubules in the bridge, and cutting of the membrane (Advedissian et al., 2023). Microtubule severing during abscission is performed by Spastin (Connell et al., 2009). The final cut of the membrane is facilitated by the endosomal sorting complexes required for transport "ESCRT" protein machinery, which consists of three main protein complexes, ESCRT-I, ESCRT-II and ESCRT-III that function through membrane remodelling in various cellular processes (reviewed elsewhere (Vietri et al., 2020)). Human ESCRT-III consists of many proteins and plays a key role in abscission by cutting the membrane of the cytoplasmic bridge.

ESCRT-III gets recruited by the midbody to the cellular bridge at the beginning of abscission. Through polymerization of different members, it start to form circles and then spirals extending outward from the midbody and eventually pinching the membrane (reviewed elsewhere, (Kodba & Chaigne, 2023)). This site of constriction is often referred to as either 'secondary ingression' or 'abscission site' and is typically located about 1 to 2 μm from the midbody (Advedissian et al., 2023). Before the ESCRT machinery can cut at the abscission site, microtubules and actin at the cut site first need to be cleared for membrane fusion to be possible (Connell et al., 2009). Spastin gets recruited to the midbody through interaction with ESCRT-III protein CHMP1B through its MIT domain (Wenzel et al., 2022). Midbody localization of Spastin is independent of interaction with microtubules, as deletion of the MIT but not of the MTB (microtubule binding) domain inhibits localization (Connell et al., 2009). Spastin first accumulates in a double-ring structure around the stembody, the middle segment of the midbody. Then, as ESCRT-III polymerizes outward, Spastin localizes as a cone on one side of the midbody and eventually concentrates at the tip of the ESCRT-III cone before microtubules are severed. This localization pattern is consistent with the interaction with CHMP1B being important for Spastin localization (Advedissian et al., 2023). Microtubule severing, but not the membrane cut, is linked to F-actin dynamics at the abscission site (Advedissian et al., 2023) (fig 8). Before microtubule severing during abscission, a transiently enriched pool of through ARP2/3 branched actin under regulated by actin depolymerizer cofilin-1 is observed at the abscission site. Cofilin-1 and Arp 2/3 co-localize with CHMP1B at the tip of the spiralizing ESCRT-III cone, which is also the site of Spastin during microtubule severing. It is hypothesized that F-actin and Arp2/3 promote microtubule severing during abscission by forming a transient branched F-actin barrier at the abscission site, that limits the distance to which the ESCRT-III cone can extend away from the midbody and thereby ensuring proper Spastin localization for microtubule severing through arresting the ESCRT-III complex. Cofilin-1 regulates this F-actin pool. As cofilin-1, Arp2/3 are lost immediately after microtubules are severed, it is indicated that branched F-actin depolymerizes concomitantly with the microtubule severing and that this is important for the cutting of the membrane by ESCRT-III (Advedissian et al., 2023).

HIPK2 is a highly conserved tyrosine-regulated serine/ threonine kinase, involved in various cellular processes (Pisciottani et al., 2019). It is recruited to the midbody through phosphorylation by AuroraB. HIPK2 phosphorylates Spastin at S268 in the linker between the MIT and MBD and this is necessary for Spastin localization, as either depletion of HIPK2 or mutation of S268 result in Spastin no longer being able to be observed at the midbody and abscission is impaired (Pisciottani et al., 2019). At this point, it remains unclear why phosphorylation of Spastin at S268 is essential for

midbody localization, especially how this is linked to its recruitment by CHMP1B. Current theories are that it phosphorylation could either stabilize interactions of Spastin and its interacting proteins, or that phosphorylation has a stabilizing effect ensuring for sufficient Spastin concentrations for localization at the end of abscission (Pisciottani et al., 2019). Additionally to Spastin, HIPK2 also contributes to abscission by phosphorylation of extrachromosomal H2B, which like HIPK2 also locates to the midbody in an aurora B dependent manner, and contributes to the formation of the abscission site, upstream of the localization of the ESCRT-III machinery. (Monteonofrio et al., 2018, 2019).

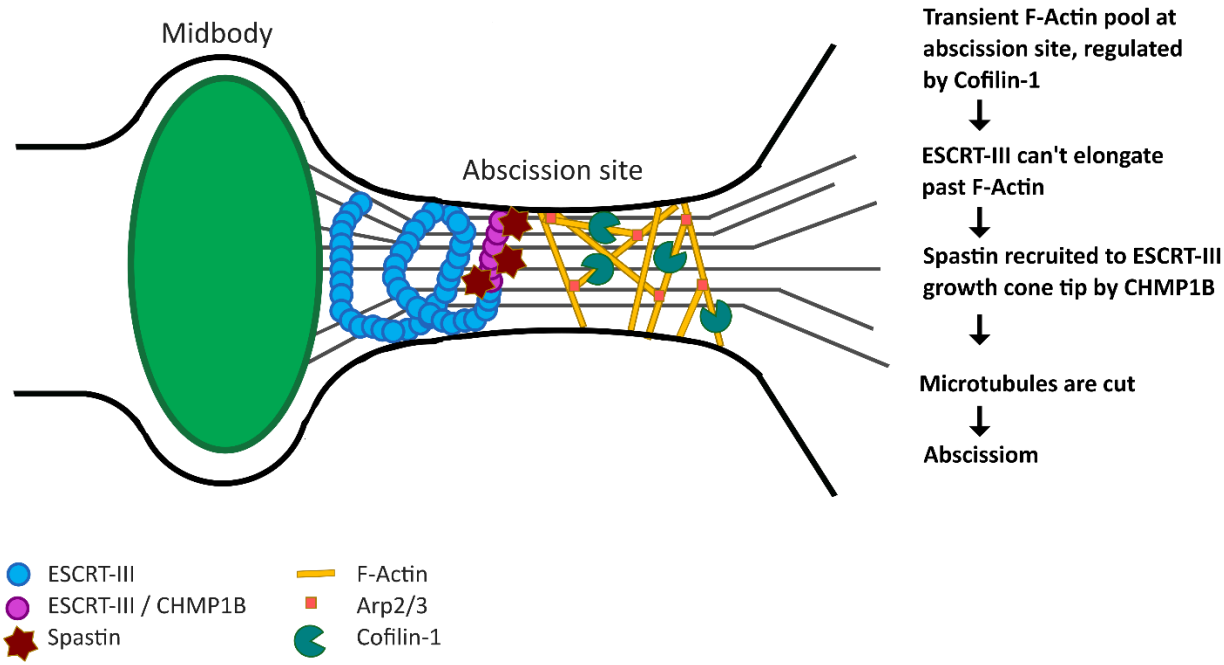


Figure 8 Abscission ESCRT-III filaments spiral outward from the stembody at the abscission site. A network of branched F-actin, regulated by Cofilin1 and Arp2/3, forms a physical barrier to limit the dimensions of ESCRT polymerization at the bridge. This facilitates proper stable recruitment and accumulation of Spastin at the ESCRT-III cone, which allows for microtubule severing.

Concluding remarks

A central question in mitosis is how the microtubules of the spindle are organized and regulated to ensure faithful chromosome separation. Microtubule severing and depolymerization are important for keeping up with the demand for continuous remodelling of the mitotic spindle, specifically during the later mitotic phases. In this review, we have attempted to give an overview of the proteins involved in microtubule severing and depolymerization in mitosis.

Multiple structural and biochemical studies of AAA ATPase microtubule severing enzymes and the depolymerizing kinesin-8 and kinesin-13 families have provided a general idea of their molecular mechanism of action. However, not every protein within this subfamilies is characterized to the same extend. Moreover, for all three protein families multiple and slightly different molecular mechanisms are proposed that often still contain a few unclarities. Additional studies, like single molecule imaging, super resolution in vivo studies and computational modelling, can contribute to further resolving how these proteins function in regards to microtubules at the molecular level.

Although the cellular roles of microtubule severing enzymes and the depolymerizing kinesin-8 and kinesin-13 families have been extensively studied in the last decades, a complete overview of their specific functions and regulation during mitosis in mammalian cells is still lacking. Many studies are performed in cells paused at a specific mitotic phase. Less is known about the regulation of the proteins during the transition to the next mitotic stage. For example, it has been established that MCAK plays an important role in correcting chromosome attachments during metaphase. However, how MCAK activity is downregulated to allow for SAC satisfaction, and if MCAK plays a role in the Pacman component of poleward chromosome movement during anaphase A, still remains unclear.

Different organisms can differently regulate spindle dynamics. For example, katanin plays an important role in the Pacman component of poleward chromosome movement in anaphase A in *Drosophila Melanogaster,* but is not observed at kinetochores in other organisms (Zhang et al., 2007) and the contributions of the Pacman and flux components for chromosome segregation during anaphase A largely vary between species. This highlights that if a specific mechanism of regulation by a severing or depolymerizing enzyme is elucidated in one organism, this isn't necessarily a universal or conserved regulatory mechanism over different species. For example, *Drosophila Melanogaster* Patronin inhibits depolymerization of microtubule minus ends during anaphase B to allow spindle elongation (Pavlova et al., 2019). It however still needs to be determined if CAMSAPS, the human homologues of Patronin, have a similar function. Another example is that Kip3 is crucial for scaling spindle elongation to cell size in yeast (Rizk et al., 2014), but this has not been studied in other organisms.

The microtubule severing and depolymerizing proteins discussed in this review often have important functions during multiple phases of mitosis. This makes in vivo studies in later mitotic phases difficult, as knockout or knockdown of these proteins often causes cell cycle arrests earlier on. Novel in vivo and in vitro approaches could shed more light on the function and regulation of these protein in later mitotic stages, specifically in telophase and cytokinesis.

References

Advedissian, T., Frémont, S., & Echard, A. (2023). *Cytokinetic abscission requires actin-dependent microtubule severing* (p. 2023.12.11.571051). bioRxiv. https://doi.org/10.1101/2023.12.11.571051

Akhmanova, A., & Kapitein, L. C. (2022). Mechanisms of microtubule organization in differentiated animal cells. *Nature Reviews Molecular Cell Biology*, *23*(8), Article 8. https://doi.org/10.1038/s41580-022-00473-y

Alsop, G. B., & Zhang, D. (2003). Microtubules are the only structural constituent of the spindle apparatus required for induction of cell cleavage. *The Journal of Cell Biology*, *162*(3), 383–390. https://doi.org/10.1083/jcb.200301073

Asbury, C. L. (2017). Anaphase A: Disassembling Microtubules Move Chromosomes toward Spindle Poles. *Biology*, *6*(1), Article 1. https://doi.org/10.3390/biology6010015

Bakhoum, S. F., Thompson, S. L., Manning, A. L., & Compton, D. A. (2009). Genome stability is ensured by temporal control of kinetochore–microtubule dynamics. *Nature Cell Biology*, *11*(1), Article 1. https://doi.org/10.1038/ncb1809

Barisic, M., & Rajendraprasad, G. (2021). Mitotic poleward flux: Finding balance between microtubule dynamics and sliding. *BioEssays*, *43*(8), 2100079. https://doi.org/10.1002/bies.202100079

Barisic, M., Rajendraprasad, G., & Steblyanko, Y. (2021). The metaphase spindle at steady state – Mechanism and functions of microtubule poleward flux. *Seminars in Cell & Developmental Biology*, *117*, 99–117. https://doi.org/10.1016/j.semcdb.2021.05.016

Beck, J., Maerki, S., Posch, M., Metzger, T., Persaud, A., Scheel, H., Hofmann, K., Rotin, D., Pedrioli, P., Swedlow, J. R., Peter, M., & Sumara, I. (2013). Ubiquitylation-dependent localization of PLK1 in mitosis. *Nature Cell Biology*, *15*(4), 430–439. https://doi.org/10.1038/ncb2695

Belonogov, L., Bailey, M. E., Tyler, M. A., Kazemi, A., & Ross, J. L. (2019). Katanin catalyzes microtubule depolymerization independently of tubulin C-terminal tails. *Cytoskeleton (Hoboken, N.J.)*, *76*(3), 254–268. https://doi.org/10.1002/cm.21522

Bormuth, V., Nitzsche, B., Ruhnow, F., Mitra, A., Storch, M., Rammner, B., Howard, J., & Diez, S. (2012). The Highly Processive Kinesin-8, Kip3, Switches Microtubule Protofilaments with a Bias toward the Left. *Biophysical Journal*, *103*(1), L4–L6. https://doi.org/10.1016/j.bpj.2012.05.024

Bowne-Anderson, H., Zanic, M., Kauer, M., & Howard, J. (2013). Microtubule dynamic instability: A new model with coupled GTP hydrolysis and multistep catastrophe. *BioEssays*, *35*(5), 452–461. https://doi.org/10.1002/bies.201200131

Braun, A., Dang, K., Buslig, F., Baird, M. A., Davidson, M. W., Waterman, C. M., & Myers, K. A. (2014). Rac1 and Aurora A regulate MCAK to polarize microtubule growth in migrating endothelial cells. *The Journal of Cell Biology*, *206*(1), 97–112. https://doi.org/10.1083/jcb.201401063

Bruce Alberts, Johnson, A., Walter, P., Raff, M., Lewis, J., & Roberts, K. (2014). Molecular biology of the cell, Ch16. In *Molecular biology of the cell* (6th ed., p. 1292). GARL.

Burns, K. M., Wagenbach, M., Wordeman, L., & Schriemer, D. C. (2014). Nucleotide exchange in dimeric MCAK induces longitudinal and lateral stress at microtubule ends to support

depolymerization. *Structure (London, England : 1993)*, *22*(8), 1173–1183. https://doi.org/10.1016/j.str.2014.06.010

Chaigne, A., & Brunet, T. (2022). Incomplete abscission and cytoplasmic bridges in the evolution of eukaryotic multicellularity. *Current Biology*, *32*(8), R385–R397. https://doi.org/10.1016/j.cub.2022.03.021

Cheeseman, I. M., & Desai, A. (2008). Molecular architecture of the kinetochore–microtubule interface. *Nature Reviews Molecular Cell Biology*, *9*(1), Article 1. https://doi.org/10.1038/nrm2310

Connell, J. W., Lindon, C., Luzio, J. P., & Reid, E. (2009). Spastin Couples Microtubule Severing to Membrane Traffic in Completion of Cytokinesis and Secretion. *Traffic*, *10*(1), 42–56. https://doi.org/10.1111/j.1600-0854.2008.00847.x

Cooper, J. R., Wagenbach, M., Asbury, C. L., & Wordeman, L. (2010). Catalysis of the microtubule on-rate is the major parameter regulating the depolymerase activity of MCAK. *Nature Structural & Molecular Biology*, *17*(1), Article 1. https://doi.org/10.1038/nsmb.1728

Díaz-Valencia, J. D., Morelli, M. M., Bailey, M., Zhang, D., Sharp, D. J., & Ross, J. L. (2011). Drosophila Katanin-60 Depolymerizes and Severs at Microtubule Defects. *Biophysical Journal*, *100*(10), 2440. https://doi.org/10.1016/j.bpj.2011.03.062

Du, Y., English, C. A., & Ohi, R. (2010). The kinesin-8 Kif18A dampens microtubule plus-end dynamics. *Current Biology: CB*, *20*(4), 374–380. https://doi.org/10.1016/j.cub.2009.12.049

Ems-McClung, S. C., Hainline, S. G., Devare, J., Zong, H., Cai, S., Carnes, S. K., Shaw, S. L., & Walczak, C. E. (2013). Aurora B Inhibits MCAK Activity through a Phosphoconformational Switch that Reduces Microtubule Association. *Current Biology*, *23*(24), 2491–2499. https://doi.org/10.1016/j.cub.2013.10.054

Friel, C. T., & Howard, J. (2011). The kinesin-13 MCAK has an unconventional ATPase cycle adapted for microtubule depolymerization. *The EMBO Journal*, *30*(19), 3928–3939. https://doi.org/10.1038/emboj.2011.290

Fukuda, Y., Luchniak, A., Murphy, E. R., & Gupta, M. L. (2014). Spatial control of microtubule length and lifetime by opposing stabilizing and destabilizing functions of Kinesin-8. *Current Biology: CB*, *24*(16), 1826–1835. https://doi.org/10.1016/j.cub.2014.06.069

Ganem, N. J., Upton, K., & Compton, D. A. (2005). Efficient mitosis in human cells lacking poleward microtubule flux. *Current Biology: CB*, *15*(20), 1827–1832. https://doi.org/10.1016/j.cub.2005.08.065

Ghosh-Roy, A., Goncharov, A., Jin, Y., & Chisholm, A. D. (2012). Kinesin-13 and tubulin posttranslational modifications regulate microtubule growth in axon regeneration. *Developmental Cell*, *23*(4), 716–728. https://doi.org/10.1016/j.devcel.2012.08.010

Glotzer, M. (2009). The 3Ms of central spindle assembly: Microtubules, motors and MAPs. *Nature Reviews. Molecular Cell Biology*, *10*(1), 9–20. https://doi.org/10.1038/nrm2609

Goodwin, S. S., & Vale, R. D. (2010). Patronin regulates the microtubule network by protecting microtubule minus ends. *Cell*, *143*(2), 263–274. https://doi.org/10.1016/j.cell.2010.09.022

Guerreiro, A., De Sousa, F., Liaudet, N., Ivanova, D., Eskat, A., & Meraldi, P. (2021). WDR62 localizes katanin at spindle poles to ensure synchronous chromosome segregation. *The Journal of Cell Biology*, *220*(8), e202007171. https://doi.org/10.1083/jcb.202007171

Gupta, M. L., Carvalho, P., Roof, D. M., & Pellman, D. (2006). Plus end-specific depolymerase activity of Kip3, a kinesin-8 protein, explains its role in positioning the yeast mitotic spindle. *Nature Cell Biology*, *8*(9), Article 9. https://doi.org/10.1038/ncb1457

Helenius, J., Brouhard, G., Kalaidzidis, Y., Diez, S., & Howard, J. (2006). The depolymerizing kinesin MCAK uses lattice diffusion to rapidly target microtubule ends. *Nature*, *441*(7089), Article 7089. https://doi.org/10.1038/nature04736

Hood, E. A., Kettenbach, A. N., Gerber, S. A., & Compton, D. A. (2012). Plk1 regulates the kinesin-13 protein Kif2b to promote faithful chromosome segregation. *Molecular Biology of the Cell*, *23*(12), 2264–2274. https://doi.org/10.1091/mbc.E11-12-1013

Huang, J., Liang, Z., Guan, C., Hua, S., & Jiang, K. (2021). WDR62 regulates spindle dynamics as an adaptor protein between TPX2/Aurora A and katanin. *The Journal of Cell Biology*, *220*(8), e202007167. https://doi.org/10.1083/jcb.202007167

Hueschen, C. L., Kenny, S. J., Xu, K., & Dumont, S. (2017). NuMA recruits dynein activity to microtubule minus-ends at mitosis. *eLife*, *6*, e29328. https://doi.org/10.7554/eLife.29328

Janssen, L. M. E., Averink, T. V., Blomen, V. A., Brummelkamp, T. R., Medema, R. H., & Raaijmakers, J. A. (2018). Loss of Kif18A Results in Spindle Assembly Checkpoint Activation at Microtubule-Attached Kinetochores. *Current Biology: CB*, *28*(17), 2685-2696.e4. https://doi.org/10.1016/j.cub.2018.06.026

Jiang, K., Rezabkova, L., Hua, S., Liu, Q., Capitani, G., Altelaar, A. F. M., Heck, A. J. R., Kammerer, R. A., Steinmetz, M. O., & Akhmanova, A. (2017). Microtubule minus-end regulation at spindle poles by an ASPM–katanin complex. *Nature Cell Biology*, *19*(5), Article 5. https://doi.org/10.1038/ncb3511

Kinesin Tree – Kinesin. (n.d.). Retrieved February 11, 2024, from https://sites.duke.edu/kinesin/kinesin-tree/

Knowlton, A. L., Lan, W., & Stukenberg, P. T. (2006). Aurora B Is Enriched at Merotelic Attachment Sites, Where It Regulates MCAK. *Current Biology*, *16*(17), 1705–1710. https://doi.org/10.1016/j.cub.2006.07.057

Knowlton, A. L., Vorozhko, V. V., Lan, W., Gorbsky, G. J., & Stukenberg, P. T. (2009). ICIS and Aurora B Coregulate the Microtubule Depolymerase Kif2a. *Current Biology*, *19*(9), 758–763. https://doi.org/10.1016/j.cub.2009.03.018

Kobayashi, T., Tsang, W. Y., Li, J., Lane, W., & Dynlacht, B. D. (2011). Centriolar Kinesin Kif24 Interacts with CP110 to Remodel Microtubules and Regulate Ciliogenesis. *Cell*, *145*(6), 914–925. https://doi.org/10.1016/j.cell.2011.04.028

Kodba, S., & Chaigne, A. (2023). Delayed abscission in animal cells – from development to defects. *Journal of Cell Science*, *136*(13), jcs260520. https://doi.org/10.1242/jcs.260520

Lan, W., Zhang, X., Kline-Smith, S. L., Rosasco, S. E., Barrett-Wilt, G. A., Shabanowitz, J., Hunt, D. F., Walczak, C. E., & Stukenberg, P. T. (2004). Aurora B Phosphorylates Centromeric MCAK and Regulates Its Localization and Microtubule Depolymerization Activity. *Current Biology*, *14*(4), 273–286. https://doi.org/10.1016/j.cub.2004.01.055

Liu, D., Vader, G., Vromans, M. J. M., Lampson, M. A., & Lens, S. M. A. (2009). Sensing chromosome bi-orientation by spatial separation of aurora B kinase from kinetochore substrates. *Science (New York, N.Y.)*, *323*(5919), 1350–1353. https://doi.org/10.1126/science.1167000

Locke, J., Joseph, A. P., Peña, A., Möckel, M. M., Mayer, T. U., Topf, M., & Moores, C. A. (2017). Structural basis of human kinesin-8 function and inhibition. *Proceedings of the National Academy of Sciences*, *114*(45), E9539–E9548. https://doi.org/10.1073/pnas.1712169114

Maiato, H., Rieder, C. L., & Khodjakov, A. (2004). Kinetochore-driven formation of kinetochore fibers contributes to spindle assembly during animal mitosis. *The Journal of Cell Biology*, *167*(5), 831–840. https://doi.org/10.1083/jcb.200407090

Mains, P. E., Kemphues, K. J., Sprunger, S. A., Sulston, I. A., & Wood, W. B. (1990). Mutations affecting the meiotic and mitotic divisions of the early Caenorhabditis elegans embryo. *Genetics*, *126*(3), 593–605. https://doi.org/10.1093/genetics/126.3.593

Maney, T., Wagenbach, M., & Wordeman, L. (2001). Molecular dissection of the microtubule depolymerizing activity of mitotic centromere-associated kinesin. *The Journal of Biological Chemistry*, *276*(37), 34753–34758. https://doi.org/10.1074/jbc.M106626200

Manning, A. L., Ganem, N. J., Bakhoum, S. F., Wagenbach, M., Wordeman, L., & Compton, D. A. (2007). The kinesin-13 proteins Kif2a, Kif2b, and Kif2c/MCAK have distinct roles during mitosis in human cells. *Molecular Biology of the Cell*, *18*(8), 2970–2979. https://doi.org/10.1091/mbc.e07-02-0110

Mashima, Y., Nohira, H., Sugihara, H., Dynlacht, B. D., Kobayashi, T., & Itoh, H. (2022). KIF24 depletion induces clustering of supernumerary centrosomes in PDAC cells. *Life Science Alliance*, *5*(11). https://doi.org/10.26508/lsa.202201470

Matos, I., Pereira, A. J., Lince-Faria, M., Cameron, L. A., Salmon, E. D., & Maiato, H. (2009). Synchronizing chromosome segregation by flux-dependent force equalization at kinetochores. *Journal of Cell Biology*, *186*(1), 11–26. https://doi.org/10.1083/jcb.200904153

Matsuo, M., Shimodaira, T., Kasama, T., Hata, Y., Echigo, A., Okabe, M., Arai, K., Makino, Y., Niwa, S.-I., Saya, H., & Kishimoto, T. (2013). Katanin p60 Contributes to Microtubule Instability around the Midbody and Facilitates Cytokinesis in Rat Cells. *PLOS ONE*, *8*(11), e80392. https://doi.org/10.1371/journal.pone.0080392

Mayr, M. I., Hümmer, S., Bormann, J., Grüner, T., Adio, S., Woehlke, G., & Mayer, T. U. (2007). The human kinesin Kif18A is a motile microtubule depolymerase essential for chromosome congression. *Current Biology: CB*, *17*(6), 488–498. https://doi.org/10.1016/j.cub.2007.02.036

McNally, F. J., & Roll-Mecak, A. (2018). Microtubule-severing enzymes: From cellular functions to molecular mechanism. *Journal of Cell Biology*, *217*(12), 4057–4069. https://doi.org/10.1083/jcb.201612104

McNally, K., Audhya, A., Oegema, K., & McNally, F. J. (2006). Katanin controls mitotic and meiotic spindle length. *Journal of Cell Biology*, *175*(6), 881–891. https://doi.org/10.1083/jcb.200608117

Monroe, N., & Hill, C. P. (2016). Meiotic Clade AAA ATPases: Protein Polymer Disassembly Machines. *Journal of Molecular Biology*, *428*(9, Part B), 1897–1911. https://doi.org/10.1016/j.jmb.2015.11.004

Montenegro Gouveia, S., Leslie, K., Kapitein, L. C., Buey, R. M., Grigoriev, I., Wagenbach, M., Smal, I., Meijering, E., Hoogenraad, C. C., Wordeman, L., Steinmetz, M. O., & Akhmanova, A. (2010). In vitro reconstitution of the functional interplay between MCAK and EB3 at microtubule plus ends. *Current Biology: CB*, *20*(19), 1717–1722. https://doi.org/10.1016/j.cub.2010.08.020

Monteonofrio, L., Valente, D., Ferrara, M., Camerini, S., Miscione, R., Crescenzi, M., Rinaldo, C., & Soddu, S. (2018). HIPK2 and extrachromosomal histone H2B are separately recruited by Aurora-B for cytokinesis. *Oncogene*, *37*(26), 3562–3574. https://doi.org/10.1038/s41388-018-0191-6

Monteonofrio, L., Valente, D., Rinaldo, C., & Soddu, S. (2019). Extrachromosomal Histone H2B Contributes to the Formation of the Abscission Site for Cell Division. *Cells*, *8*(11), 1391. https://doi.org/10.3390/cells8111391

Moore, A., & Wordeman, L. (2004). The mechanism, function and regulation of depolymerizing kinesins during mitosis. *Trends in Cell Biology*, *14*(10), 537–546. https://doi.org/10.1016/j.tcb.2004.09.001

Mukherjee, S., Valencia, J. D. D., Stewman, S., Metz, J., Monnier, S., Rath, U., Asenjo, A. B., Charafeddine, R. A., Sosa, H. J., Ross, J. L., Ma, A., & Sharp, D. J. (2012). Human Fidgetin is a microtubule severing the enzyme and minus-end depolymerase that regulates mitosis. *Cell Cycle*, *11*(12), 2359–2366. https://doi.org/10.4161/cc.20849

Niwa, S., Nakajima, K., Miki, H., Minato, Y., Wang, D., & Hirokawa, N. (2012). KIF19A is a microtubule-depolymerizing kinesin for ciliary length control. *Developmental Cell*, *23*(6), 1167–1175. https://doi.org/10.1016/j.devcel.2012.10.016

O'Donnell, L., Rhodes, D., Smith, S. J., Merriner, D. J., Clark, B. J., Borg, C., Whittle, B., O'Connor, A. E., Smith, L. B., McNally, F. J., de Kretser, D. M., Goodnow, C. C., Ormandy, C. J., Jamsai, D., & O'Bryan, M. K. (2012). An Essential Role for Katanin p80 and Microtubule Severing in Male Gamete Production. *PLoS Genetics*, *8*(5), e1002698. https://doi.org/10.1371/journal.pgen.1002698

Ogawa, T., Nitta, R., Okada, Y., & Hirokawa, N. (2004). A Common Mechanism for Microtubule Destabilizers—M Type Kinesins Stabilize Curling of the Protofilament Using the Class-Specific Neck and Loops. *Cell*, *116*(4), 591–602. https://doi.org/10.1016/S0092-8674(04)00129-1

Pavlova, G. A., Razuvaeva, A. V., Popova, J. V., Andreyeva, E. N., Yarinich, L. A., Lebedev, M. O., Pellacani, C., Bonaccorsi, S., Somma, M. P., Gatti, M., & Pindyurin, A. V. (2019). The role of Patronin in Drosophila mitosis. *BMC Molecular and Cell Biology*, *20*(1), 7. https://doi.org/10.1186/s12860-019-0189-0

Peters, C., Brejc, K., Belmont, L., Bodey, A. J., Lee, Y., Yu, M., Guo, J., Sakowicz, R., Hartman, J., & Moores, C. A. (2010). Insight into the molecular mechanism of the multitasking kinesin-8 motor. *The EMBO Journal*, *29*(20), 3437–3447. https://doi.org/10.1038/emboj.2010.220

Pisciottani, A., Biancolillo, L., Ferrara, M., Valente, D., Sardina, F., Monteonofrio, L., Camerini, S., Crescenzi, M., Soddu, S., & Rinaldo, C. (2019). HIPK2 Phosphorylates the Microtubule-Severing Enzyme Spastin at S268 for Abscission. *Cells*, *8*(7), Article 7. https://doi.org/10.3390/cells8070684

Reid, E., Connell, J., Edwards, T. L., Duley, S., Brown, S. E., & Sanderson, C. M. (2005). The hereditary spastic paraplegia protein spastin interacts with the ESCRT-III complex-associated endosomal protein CHMP1B. *Human Molecular Genetics*, *14*(1), 19–38. https://doi.org/10.1093/hmg/ddi003

Ritter, A., Kreis, N.-N., Louwen, F., Wordeman, L., & Yuan, J. (2016). Molecular insight into the regulation and function of MCAK. *Critical Reviews in Biochemistry and Molecular Biology*, *51*(4), 228–245. https://doi.org/10.1080/10409238.2016.1178705

Rizk, R. S., DiScipio, K. A., Proudfoot, K. G., & Gupta, M. L., Jr. (2014). The kinesin-8 Kip3 scales anaphase spindle length by suppression of midzone microtubule polymerization. *Journal of Cell Biology*, *204*(6), 965–975. https://doi.org/10.1083/jcb.201312039

Rogers, G. C., Rogers, S. L., Schwimmer, T. A., Ems-McClung, S. C., Walczak, C. E., Vale, R. D., Scholey, J. M., & Sharp, D. J. (2004). Two mitotic kinesins cooperate to drive sister chromatid separation during anaphase. *Nature*, *427*(6972), 364–370. https://doi.org/10.1038/nature02256

Rogers, G. C., Rogers, S. L., & Sharp, D. J. (2005). Spindle microtubules in flux. *Journal of Cell Science*, *118*(6), 1105–1116. https://doi.org/10.1242/jcs.02284

Roll-Mecak, A., & McNally, F. J. (2010). Microtubule-severing enzymes. *Current Opinion in Cell Biology*, *22*(1), 96–103. https://doi.org/10.1016/j.ceb.2009.11.001

Roll-Mecak, A., & Vale, R. D. (2008). Structural basis of microtubule severing by the hereditary spastic paraplegia protein spastin. *Nature*, *451*(7176), 363–367. https://doi.org/10.1038/nature06482

Sagolla, M. J., Uzawa, S., & Cande, W. Z. (2003). Individual microtubule dynamics contribute to the function of mitotic and cytoplasmic arrays in fission yeast. *Journal of Cell Science*, *116*(Pt 24), 4891–4903. https://doi.org/10.1242/jcs.00796

Sarbanes, S. L., Zehr, E. A., & Roll-Mecak, A. (2022). Microtubule-severing enzymes. *Current Biology*, *32*(19), R992–R997. https://doi.org/10.1016/j.cub.2022.08.046

Shao, H., Huang, Y., Zhang, L., Yuan, K., Chu, Y., Dou, Z., Jin, C., Garcia-Barrio, M., Liu, X., & Yao, X. (2015). Spatiotemporal dynamics of Aurora B-PLK1-MCAK signaling axis orchestrates kinetochore bi-orientation and faithful chromosome segregation. *Scientific Reports*, *5*, 12204. https://doi.org/10.1038/srep12204

Sharp, D. J., & Ross, J. L. (2012). Microtubule-severing enzymes at the cutting edge. *Journal of Cell Science*, *125*(11), 2561–2569. https://doi.org/10.1242/jcs.101139

Solowska, J. M., Morfini, G., Falnikar, A., Himes, B. T., Brady, S. T., Huang, D., & Baas, P. W. (2008). Quantitative and functional analyses of spastin in the nervous system: Implications for hereditary spastic paraplegia. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *28*(9), 2147–2157. https://doi.org/10.1523/JNEUROSCI.3159-07.2008

Steblyanko, Y., Rajendraprasad, G., Osswald, M., Eibes, S., Barisic, M., & Helder, M. (2020). *Microtubule poleward flux in human cells is driven by the coordinated action of four kinesins*. https://doi.org/10.15252/embj.2020105432

Stumpff, J., Du, Y., English, C. A., Maliga, Z., Wagenbach, M., Asbury, C. L., Wordeman, L., & Ohi, R. (2011). A tethering mechanism controls the processivity and kinetochore-microtubule plus-end enrichment of the kinesin-8 Kif18A. *Molecular Cell*, *43*(5), 764–775. https://doi.org/10.1016/j.molcel.2011.07.022

Stumpff, J., von Dassow, G., Wagenbach, M., Asbury, C., & Wordeman, L. (2008). The kinesin-8 motor Kif18A suppresses kinetochore movements to control mitotic chromosome alignment. *Developmental Cell*, *14*(2), 252–262. https://doi.org/10.1016/j.devcel.2007.11.014

Su, X., Ohi, R., & Pellman, D. (2012). MOVING IN FOR THE KILL: MOTILE MICROTUBULE REGULATORS. *Trends in Cell Biology*, *22*(11), 567–575. https://doi.org/10.1016/j.tcb.2012.08.003

Su, X., Qiu, W., Gupta, M. L., Pereira-Leal, J. B., Reck-Peterson, S. L., & Pellman, D. (2011). Mechanisms Underlying the Dual-Mode Regulation of Microtubule Dynamics by Kip3/Kinesin-8. *Molecular Cell*, *43*(5), 751–763. https://doi.org/10.1016/j.molcel.2011.06.027

Sudo, H., & Baas, P. W. (2010). Acetylation of microtubules influences their sensitivity to severing by katanin in neurons and fibroblasts. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *30*(21), 7215–7226. https://doi.org/10.1523/JNEUROSCI.0048-10.2010

Talapatra, S. K., Harker, B., & Welburn, J. P. (2015). The C-terminal region of the motor protein MCAK controls its structure and activity through a conformational switch. *eLife*, *4*, e06421. https://doi.org/10.7554/eLife.06421

Tanenbaum, M. E., Macurek, L., van der Vaart, B., Galli, M., Akhmanova, A., & Medema, R. H. (2011). A Complex of Kif18b and MCAK Promotes Microtubule Depolymerization and Is Negatively Regulated by Aurora Kinases. *Current Biology*, *21*(16), 1356–1365. https://doi.org/10.1016/j.cub.2011.07.017

Uehara, R., Tsukada, Y., Kamasaki, T., Poser, I., Yoda, K., Gerlich, D. W., & Goshima, G. (2013). Aurora B and Kif2A control microtubule length for assembly of a functional central spindle during anaphase. *Journal of Cell Biology*, *202*(4), 623–636. https://doi.org/10.1083/jcb.201302123

Valenstein, M. L., & Roll-Mecak, A. (2016). Graded Control of Microtubule Severing by Tubulin Glutamylation. *Cell*, *164*(5), 911–921. https://doi.org/10.1016/j.cell.2016.01.019

Varga, V., Leduc, C., Bormuth, V., Diez, S., & Howard, J. (2009). Kinesin-8 Motors Act Cooperatively to Mediate Length-Dependent Microtubule Depolymerization. *Cell*, *138*(6), 1174–1183. https://doi.org/10.1016/j.cell.2009.07.032

Vemu, A., Szczesna, E., Zehr, E. A., Spector, J. O., Grigorieff, N., Deaconescu, A. M., & Roll-Mecak, A. (2018). Severing enzymes amplify microtubule arrays through lattice GTP-tubulin incorporation. *Science*, *361*(6404), eaau1504. https://doi.org/10.1126/science.aau1504

Vietri, M., Radulovic, M., & Stenmark, H. (2020). The many functions of ESCRTs. *Nature Reviews Molecular Cell Biology*, *21*(1), Article 1. https://doi.org/10.1038/s41580-019-0177-4

Vietri, M., Schink, K. O., Campsteijn, C., Wegner, C. S., Schultz, S. W., Christ, L., Thoresen, S. B., Brech, A., Raiborg, C., & Stenmark, H. (2015). Spastin and ESCRT-III coordinate mitotic spindle disassembly and nuclear envelope sealing. *Nature*, *522*(7555), Article 7555. https://doi.org/10.1038/nature14408

Walczak, C. E., Gayek, S., & Ohi, R. (2013). Microtubule-depolymerizing kinesins. *Annual Review of Cell and Developmental Biology*, *29*, 417–441. https://doi.org/10.1146/annurev-cellbio-101512-122345

Wang, H., Brust-Mascher, I., Civelekoglu-Scholey, G., & Scholey, J. M. (2013). Patronin mediates a switch from kinesin-13-dependent poleward flux to anaphase B spindle elongation. *The Journal of Cell Biology*, *203*(1), 35–46. https://doi.org/10.1083/jcb.201306001

Wang, W., Shen, T., Guerois, R., Zhang, F., Kuerban, H., Lv, Y., Gigant, B., Knossow, M., & Wang, C. (2015). New Insights into the Coupling between Microtubule Depolymerization and ATP Hydrolysis by Kinesin-13 Protein Kif2C. *The Journal of Biological Chemistry*, *290*(30), 18721–18731. https://doi.org/10.1074/jbc.M115.646919

Weaver, L. N., Ems-McClung, S. C., Stout, J. R., LeBlanc, C., Shaw, S. L., Gardner, M. K., & Walczak, C. E. (2011). Kif18A Uses a Microtubule Binding Site in the Tail for Plus-End Localization and Spindle Length Regulation. *Current Biology*, *21*(17), 1500–1506. https://doi.org/10.1016/j.cub.2011.08.005

Wenzel, D. M., Mackay, D. R., Skalicky, J. J., Paine, E. L., Miller, M. S., Ullman, K. S., & Sundquist, W. I. (2022). Comprehensive analysis of the human ESCRT-III-MIT domain interactome reveals new cofactors for cytokinetic abscission. *eLife*, *11*, e77779. https://doi.org/10.7554/eLife.77779

Woodruff, J. B., Drubin, D. G., & Barnes, G. (2010). Mitotic spindle disassembly occurs via distinct subprocesses driven by the anaphase-promoting complex, Aurora B kinase, and kinesin-8. *Journal of Cell Biology*, *191*(4), 795–808. https://doi.org/10.1083/jcb.201006028

Wordeman, L. (2005). Microtubule-depolymerizing kinesins. *Current Opinion in Cell Biology*, *17*(1), 82–88. https://doi.org/10.1016/j.ceb.2004.12.003

Xu, K., Wang, C., Keinänen, K., Li, H., & Cai, C. (2022). Mitotic spindle disassembly in human cells relies on CRIPT having hierarchical redox signals. *Journal of Cell Science*, *135*(18), jcs259657. https://doi.org/10.1242/jcs.259657

Yu, C.-H., Redemann, S., Wu, H.-Y., Kiewisz, R., Yoo, T. Y., Conway, W., Farhadifar, R., Müller-Reichert, T., & Needleman, D. (2019). Central-spindle microtubules are strongly coupled to chromosomes during both anaphase A and anaphase B. *Molecular Biology of the Cell*, *30*(19), 2503–2514. https://doi.org/10.1091/mbc.E19-01-0074

Zehr, E. A., Szyk, A., Szczesna, E., & Roll-Mecak, A. (2020). Katanin Grips the β-Tubulin Tail through an Electropositive Double Spiral to Sever Microtubules. *Developmental Cell*, *52*(1), 118-131.e6. https://doi.org/10.1016/j.devcel.2019.10.010

Zehr, E., Szyk, A., Piszczek, G., Szczesna, E., Zuo, X., & Roll-Mecak, A. (2017). Katanin spiral and ring structures shed light on power stroke for microtubule severing. *Nature Structural & Molecular Biology*, *24*(9), 717–725. https://doi.org/10.1038/nsmb.3448

Zhai, Y., Kronebusch, P. J., & Borisy, G. G. (1995). Kinetochore microtubule dynamics and the metaphase-anaphase transition. *Journal of Cell Biology*, *131*(3), 721–734. https://doi.org/10.1083/jcb.131.3.721

Zhang, D., Rogers, G. C., Buster, D. W., & Sharp, D. J. (2007). Three microtubule severing enzymes contribute to the “Pacman-flux” machinery that moves chromosomes. *Journal of Cell Biology*, *177*(2), 231–242. https://doi.org/10.1083/jcb.200612011