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# The multifunctional spindle midzone in vertebrate cells at a glance

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## ABSTRACT

During anaphase, a microtubule-containing structure called the midzone forms between the segregating chromosomes. The midzone is composed of an antiparallel array of microtubules and numerous microtubule-associated proteins that contribute to midzone formation and function. In many cells, the midzone is an important source of signals that specify the location of contractile ring assembly and constriction. The midzone also contributes to the events of anaphase by generating forces that impact chromosome segregation and spindle elongation; some midzone components contribute to both processes. The results of recent experiments have increased our understanding of the importance of the midzone, a microtubule array that has often been overlooked. This Journal of Cell Science at a Glance article will review, and illustrate on the accompanying poster, the organization, formation and dynamics of the midzone, and discuss open questions for future research.


**KEY WORDS: Midzone, Cytokinesis, Anaphase, Spindle elongation, Microtubule**

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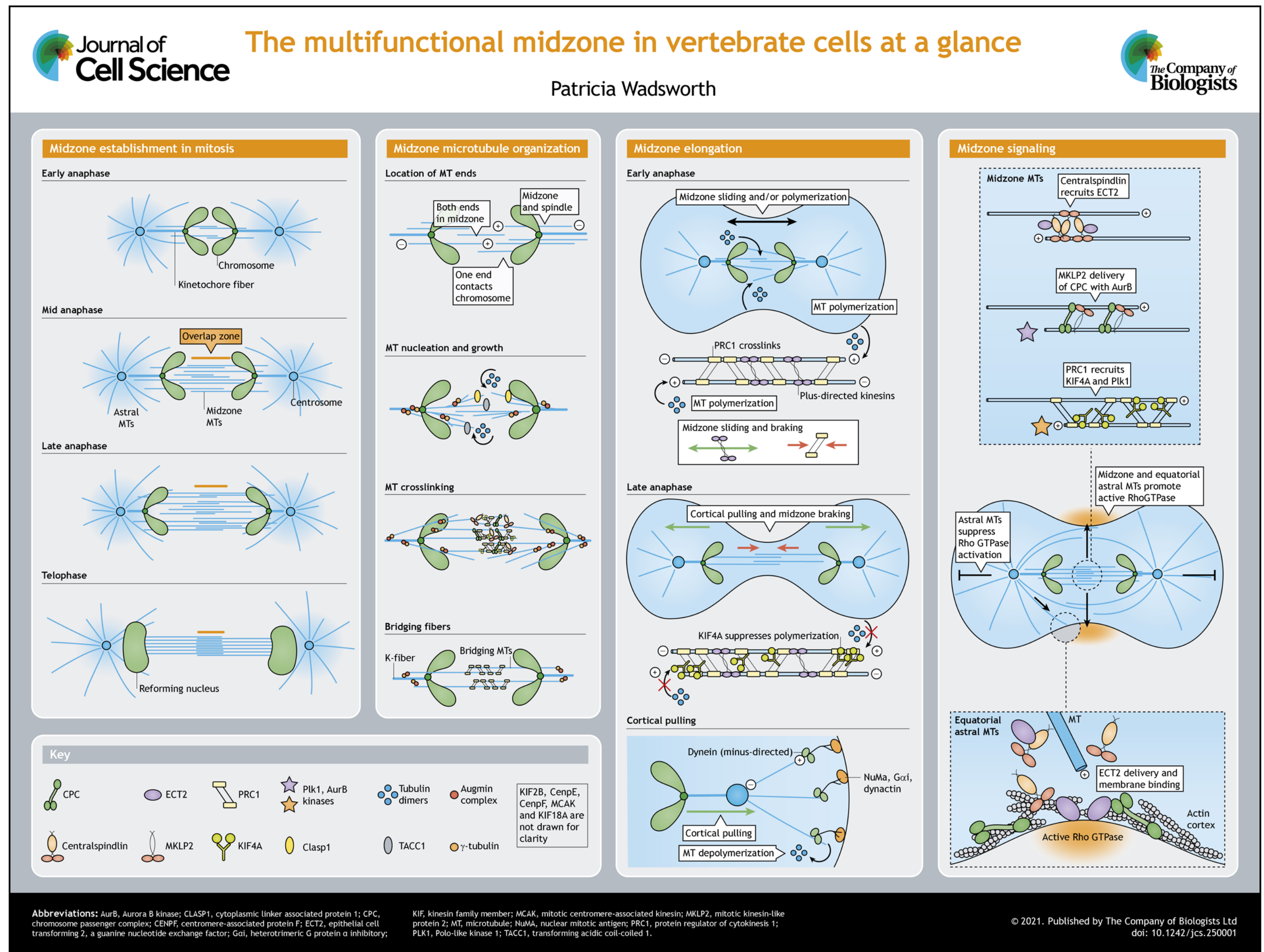
## Introduction

The mitotic spindle is a bipolar, microtubule-based structure that is responsible for chromosome segregation during cell division. The spindle generates forces to align the chromosomes at the metaphase plate and segregate them during anaphase. The metaphase spindle is



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composed of kinetochore microtubules, which link the chromosomes to the spindle, astral microtubules, which radiate from each spindle pole toward the cell periphery, and overlapping microtubules, which extend from each half spindle toward the spindle midplane, connecting the two halves of the bipolar spindle (McIntosh et al., 2012). As the chromosomes are segregated in anaphase, the microtubules between the segregating chromosomes become more prominent. This microtubule-rich region has been referred to by many different names, including the central spindle, midzone and anaphase spindle. The microtubules that comprise the structure have been called interchromosomal, interpolar, interzonal or midzone microtubules (Green et al., 2012; Glotzer, 2009; Mastronarde et al., 1993). Here, I refer to the structure that forms between the segregating chromosomes as the midzone, and the microtubules within it the midzone microtubules. The midzone plays an important role in specifying the location of contractile ring assembly, resulting in the formation of two separate daughter cells (Green et al., 2012). In telophase, midzone microtubules are compacted into a structure called the midbody or intracellular bridge (Hu et al., 2012), which is the location of abscission, the final step in generating two independent daughter cells (Lee et al., 2012). Note that the midbody forms after the midzone and is a distinct structure that contains complexes required for abscission (Fededa and Gerlich, 2012). In the following sections, the architecture, formation and dynamics of the midzone and the contribution of the midzone to chromosome segregation and cytokinesis will be discussed and illustrated on the poster. Given length constraints the focus will be primarily on vertebrate tissue culture cells.

## Midzone architecture

Electron microscopy (EM) has provided detailed information about the organization of spindle microtubules throughout mitosis (Mastronarde et al., 1993; McDonald et al., 1992; McIntosh and Landis, 1971). In the spindles of cultured vertebrate cells, some microtubules extend from each half-spindle and overlap near the spindle midplane in early mitosis, linking the two halves of the spindle. However, microtubules that extend the entire distance from one spindle pole to the midplane during anaphase are not typically detected in these spindles (McDonald et al., 1992; O'Toole et al., 2020). Instead, EM tomography data show that the midzone is composed of short microtubules that indirectly link the midzone to kinetochore fibers and to the spindle poles (Yu et al., 2019; O'Toole et al., 2020). Some midzone microtubules have both ends within the region between the segregating chromosomes, while others have minus-ends that contact the chromosomes, and some have minus-ends within the half spindle where they closely approach kinetochore fiber microtubules, indicative of an interaction (Yu et al., 2019; O'Toole et al., 2020). Midzone microtubules that closely approach kinetochore fibers in each half spindle correspond to the recently described bridging fibers (Vukušić et al., 2017; Kajtez et al., 2016). Observations by light microscopy (LM) suggest that a single bridging fiber links a pair of sister kinetochore fibers (Polak et al., 2017), although observations using EM suggest that bundles of midzone microtubules that extend into each half spindle closely approach multiple kinetochore fibers (Mastronarde et al., 1993; O'Toole et al., 2020). Both LM and EM further show that as anaphase progresses, the organization of midzone microtubules changes from weakly bundled microtubules with extensive overlap to highly bundled microtubules with a short, well-defined overlap zone (∼2 µm in mid-anaphase and ∼0.5 µm in late anaphase) (Mastronarde et al., 1993; Pamula et al., 2019) (see poster). Finally, during cytokinesis, midzone microtubules are further compacted to form the midbody. In summary, the number and organization of midzone microtubules changes as mitosis progresses so as to establish and maintain a link between the two half-spindles.

## Midzone formation

As the chromosomes move poleward in anaphase, midzone microtubules become more apparent as they elongate and form bundles. Tracking growing microtubule plus-ends with GFP–EB1 (EB1 is also known as MAPRE1) during anaphase shows that midzone microtubules originate from the chromosome region, not the centrosomes, consistent with a non-centrosome-mediated nucleation mechanism (Uehara and Goshima, 2010). In vertebrate spindles, midzone microtubule formation requires augmin, an eight-subunit protein complex, which binds to the side of a preexisting microtubule, recruits the γ-tubulin ring complex and nucleates a new microtubule, forming a branch (Uehara et al., 2009; Manenica et al., 2020 preprint) (see poster). Mammalian cells depleted of augmin complex subunits or the γ-tubulin adaptor subunit NEDD1, which interacts with augmin, show a severe reduction in the density of midzone microtubules (Courthéoux et al., 2019; Uehara et al., 2009; Uehara and Goshima, 2010). Microtubule depolymerization and regrowth assays demonstrate that *de novo* assembly is important for midzone formation and that NEDD1 and the Ran-regulated protein HURP (also known as DLGAP5) contribute to this process (Courthéoux et al., 2019; Uehara and Goshima, 2010). Together, these results support the view that in vertebrate cells, midzone formation proceeds by centrosome-independent microtubule nucleation and branching in anaphase.

In addition to γ-tubulin-mediated microtubule nucleation, proteins that promote microtubule assembly are also required for midzone microtubule formation. Perhaps the best characterized is CLASP1, a microtubule plus-end-tracking protein that contains TOG domains, which bind tubulin dimers and accelerate microtubule assembly (Pereira et al., 2006). CLASP1 is associated with the kinetochore in early mitosis and relocalizes to the midzone in anaphase (Maton et al., 2015; Liu et al., 2009). Other kinetochore proteins, including KIF2B, MCAK (also known as KIF2C), CenpE, CenpF and KIF18A also relocalize to the midzone in anaphase (Maton et al., 2015; Malaby et al., 2019; Liao et al., 1994; Manning et al., 2007; Lan et al., 2004). CLASP1 interacts with the microtubule-crosslinking protein regulator of cytokinesis 1 (PRC1), an interaction that likely tunes midzone microtubule dynamics (Mani et al., 2020 preprint). Other microtubule-associated proteins that promote midzone microtubule assembly include TACC3, which associates with TOG domain containing proteins (Lioutas and Vernos, 2013) and p150glued (also known as DCTN1), a regulator of dynein (Reboutier et al., 2013). Several microtubule-associated proteins that regulate midzone assembly are downstream targets of the mitotic kinase Aurora A (see Box 1).

Newly formed midzone microtubules are highly dynamic, with turnover half-times, measured using photoactivation of photoactivatable (PA)-GFP-tubulin, of ∼30 s (Vukušić et al., 2017; Yu et al., 2019). In late anaphase and telophase, however, photobleach marks on midzone microtubules recover more slowly than marks elsewhere in the spindle, demonstrating differential stability (Saxton and McIntosh, 1987). Midzone microtubules develop resistance to nocodazole-induced disassembly in late anaphase, another hallmark of microtubule stability (Landino and Ohi, 2016; Murthy and Wadsworth, 2008). In summary, midzone formation in anaphase results from the Aurora A-dependent formation of a new array of microtubules, which assembles in an augmin-dependent manner in the region between the segregating chromosomes.

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**Box 1. Aurora kinases**

Aurora kinases are serine/threonine kinases that contribute to many aspects of cell division. Aurora kinases are composed of a conserved kinase domain and a more divergent N-terminal region that mediates interactions with distinct binding partners (Willems et al., 2018; Hochegger et al., 2013). Aurora A and B are widely expressed, whereas Aurora C expression in restricted to meiotic cells (Willems et al., 2018). Aurora A kinase localizes to centrosomes, and Aurora B localizes to the midzone and cortex in anaphase cells (Hochegger et al., 2013; Afonso et al., 2017). Inhibition of Aurora A kinase activity with small-molecule reagents and chemical genetic approaches results in numerous mitotic defects including abnormal spindles, chromosome misalignment and centrosome defects resulting in aneuploid cells (Hochegger et al., 2013). In addition to regulating early mitotic events, Aurora A phosphorylates microtubule-associated proteins that are important for midzone assembly, including NEDD1, TACC3, HURP and p150glued (Courthéoux et al., 2019; Lioutas and Vernos, 2013; Reboutier et al., 2013). Expression of non-phosphorylatable mutants of these proteins results in defects in midzone formation, demonstrating the importance of Aurora A activity in the spatial and temporal regulation of the midzone.

Aurora B kinase is part of the CPC, which localizes to centromeres in early mitosis and to the midzone and cortex in anaphase (Vagnarelli and Earnshaw, 2004). At the centromere, Aurora B destabilizes incorrectly attached kinetochores in early mitosis through the phosphorylation of the microtubule-destabilizing kinesin MCAK and several kinetochore proteins (Hochegger et al., 2013). Following anaphase onset, the CPC is relocalized to the midzone microtubules, establishing a spatial gradient of Aurora B kinase activity in anaphase cells (Afonso et al., 2014; Fuller et al., 2008). The Aurora B gradient regulates the timing of nuclear envelope reformation, thus preventing entry into telophase prior to chromosome segregation to nascent daughter cells (Afonso et al., 2014).

Aurora B plays a critical role in midzone formation and cytokinesis, as evidenced by the number of midzone proteins that are regulated by the kinase. Phosphorylation of KIF4A by midzone-localized Aurora B promotes its interaction with PRC1 and regulates the microtubule growth-regulating activity of the kinesin (Nunes Bastos et al., 2013; Bastos, Cundell, and Barr, 2014). Aurora B phosphorylation of KIF2A also contributes to midzone microtubule length regulation (Uehara et al., 2013). Both the MgcRacGAP and MKLP1 subunits of centralspindlin are regulated by Aurora B and this regulation is important for centralspindlin clustering, enhancing motility and accumulation of centralspindlin at microtubule plus-ends, antiparallel overlaps and the cell cortex, and cytokinesis (Guse et al., 2005; Neef et al., 2006; Minoshima et al., 2003; Douglas et al., 2010; Basant et al., 2015; Minoshima et al., 2003; Hutterer et al., 2009. Inhibition of Aurora B during late anaphase further demonstrates that Aurora B is important for completion of cytokinesis (Guse et al., 2005).

## Regulation of midzone organization and cytokinetic signaling

PRC1, the chromosome passenger complex (CPC) and centralspindlin play important roles in the organization and stabilization of newly assembled midzone microtubules and contribute to specifying the location of contractile ring assembly (Fededa and Gerlich, 2012; Glotzer, 2009). These molecules are referred to by different names depending on the experimental system; here I use the nomenclature commonly used for vertebrate cells.

The CPC is composed of the mitotic kinase Aurora B (see Box 1), the centromere-binding protein INCENP and two additional subunits, survivin and borealin (symbols BIRC5 and CDCA8, respectively), that contribute to CPC localization (Vagnarelli and Earnshaw, 2004). The CPC relocalizes from centromeres in early mitosis to the midzone in anaphase as CDK1 activity decreases (Earnshaw and Cooke, 1991). Midzone localization is promoted by MKLP2 (also known as KIF20A), which binds the INCENP subunit of CPC (Gruneberg et al., 2004; Adriaans et al., 2020; Serena et al., 2020; Landino et al., 2017).

Centralspindlin is a heterotetramer comprised of two molecules of a plus-end-directed kinesin 6 motor protein, MKLP1 (also known as KIF23), and two molecules of a Rho GTPase-activating protein (GAP), called MgcRacGAP (RACGAP1) in vertebrate cells; both components are important for localization to and bundling of midzone microtubules (Basant et al., 2015; Hutterer et al., 2009; Mishima et al., 2002). Clustering of centralspindlin is regulated by Aurora B kinase (Box 1).

PRC1, a member of the conserved MAP65 family, forms dimers that preferentially bind and crosslink antiparallel microtubules (Subramanian et al., 2010). PRC1 localizes to antiparallel microtubules in metaphase cells and binding is enhanced in anaphase as the activity of CDK1 drops (Polak et al., 2017; Pamula et al., 2019; Asthana et al., 2020 preprint; Mollinari et al., 2002; Zhu and Jiang, 2005). The length of the antiparallel microtubule overlap zone, marked by PRC1, shortens during anaphase as antiparallel microtubules slide relative to each other (Pamula et al., 2019; Asthana et al., 2020 preprint). PRC1 binds to the chromokinesin KIF4A and recruits it to the midzone (Zhu and Jiang, 2005; Kurasawa et al., 2004). KIF4A suppresses elongation of microtubule plus-ends contributing to midzone length regulation (see Box 2) (Hu et al., 2011) (Nunes Bastos et al., 2013). PRC1 also binds to the mitotic kinase PLK1, localizing it to the midzone, to the microtubule-associated protein CLASP1, and to CenpE and MKLP1 (Kurasawa et al., 2004; Neef et al., 2007; Mani et al., 2020 preprint; Zhu and Jiang, 2005). PRC1 undergoes rapid turnover in the midzone (36 s in first ∼2 min of anaphase) that slows as anaphase progresses (Pamula et al., 2019; Asthana et al., 2020 preprint).

Depletion of PRC1 inhibits the formation of midzone microtubule bundles, although the CPC and centralspindlin still localize to the disorganized midzone and furrows ingress (Mollinari et al., 2002; Zhu and Jiang, 2005). Cells depleted of KIF4A display elongated and disorganized midzone microtubules with ingressing furrows (Hu et al., 2011). In contrast, cells depleted of centralspindlin or the CPC show defects in midzone formation and cytokinesis (Basant et al., 2015; Douglas et al., 2010; Guse et al., 2005; Yüce et al., 2005). Reduction of both centralspindlin and CPC results in a more severe cytokinesis defect, revealing independent contributions to furrowing in mammalian cells and *Caenorhabditis elegans* (Lewellyn et al., 2011; Kitagawa et al., 2013).

## Cytokinetic signaling by the midzone

It has been well established that microtubules play a key role in specifying the location of contractile ring formation, but the specific subset of microtubules that are required has been the topic of much debate (Rappaport, 1996). For example, classic experiments using toroidal shaped echinoderm eggs showed that two arrays of astral microtubules that lacked an intervening midzone array of microtubules are sufficient to induce furrowing (Rappaport, R., 1996). Experiments performed in mammalian cells, however, showed that midzone microtubules, and not astral microtubules, are continually required for cytokinesis (Cao and Wang, 1996; Wheatley and Wang, 1996). More recent work has also shown that cortically localized, midzone-independent signals promote furrow formation (Kotýnková et al., 2016). In *C. elegans*, severing the connection between the spindle midzone and aster showed that two signals are generated, an astral- and a midzone-derived signal, each giving rise to a furrow (Bringmann and Hyman, 2005). Polar astral microtubules suppress contractility at the non-equatorial cortex (Green et al., 2012; Murthy and Wadsworth, 2008).

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**Box 2. *In vitro* reconstitution of microtubule overlap zones**

*In vitro* reconstitution experiments with purified components have been used to explore the properties of individual midzone proteins and to reconstitute overlapping microtubule arrays. Experiments with human proteins show that PRC1 binds the lattice of individual stabilized microtubules whereas the kinesin-4, family protein KIF4A, decorates microtubule tips (Subramanian et al., 2013). When both proteins are added together, they accumulate at microtubule plus-ends forming an end tag, the size of which depends on microtubule length and PRC1 concentration (Subramanian et al., 2013). Addition of KIF4A to antiparallel microtubules crosslinked by PRC1 results in microtubule sliding, the velocity of which is proportional to initial overlap length; sliding stalls as the end tags collide, forming an overlap zone of defined length (Wijeratne and Subramanian, 2018). Experiments performed with *Xenopus* proteins show that PRC1 autonomously bundles antiparallel microtubules that continue to elongate at their plus-ends (Bieling et al., 2010). Xklp1, the *Xenopus* kinesin-4, shows processive motion to microtubule plus-ends, and suppresses microtubule growth without inducing catastrophe (Bieling et al., 2010). Addition of both PRC1 and Xklp1 results in the formation of overlap zones whose length depends on the ratio of Xklp1 to PRC1; overlap length is dynamic and responds to changes in XKlp1 concentration (Bieling et al., 2010). Thus, a system comprising microtubules, PRC1 and a kinesin-4 results in microtubules crosslinked in an antiparallel configuration, suppression of microtubule plus-end growth, and overlap zones of defined length.

Dynamic microtubules in solution with PRC1 and KIF4A also form antiparallel arrays (Hannabuss et al., 2019). As observed with immobilized microtubules, overlap length is sensitive to the ratio of KIF4A to PRC1. Remarkably, under the *in vitro* conditions used, the length of the overlap zones was similar to those observed in anaphase cells. Microtubule plus-ends in the overlap zone are non-growing, although minus ends remain dynamic (Hannabuss et al., 2019).

Frictional forces, produced by PRC1 molecules crosslinking antiparallel microtubules, have been measured using an optical trap assay (Gaska et al., 2019). These *in vitro* assays show that frictional forces scale with the number of PRC1 molecules in the overlap and with the velocity of microtubule sliding, but are independent of overlap length and PRC1 density (Gaska et al., 2019; Alfieri et al., 2020). Experiments in which kinesin motors were employed to generate microtubule sliding identifies different regimes of sliding behavior that result from the accumulation of PRC1 near microtubule ends (Alfieri et al., 2020). Together, these *in vitro* results suggest that the number and organization of PRC1 molecules and the magnitude of forces applied to the midzone will impact spindle elongation behavior *in vivo*.

Despite the debate regarding which population(s) of microtubules are required for signaling in a given cell type, the data are consistent with the conclusion that localized, cortical activation of the small GTPase Rho (RhoA) is an essential feature of the signaling process (Su et al., 2011; Wagner and Glotzer, 2016). Rho activation and contractile ring assembly occur at the cell cortex, an F-actin-rich layer subjacent to the plasma membrane. GTP-bound Rho (Rho-GTP) activates downstream molecules, including formins and myosin II, which promote F-actin assembly and cortical contractility, respectively (Fededa and Gerlich, 2012).

Rho activation requires a Rho GTP exchange factor (GEF), called ECT2 in mammalian cells, which is recruited to midzone microtubules by binding to the MgcRacGAP subunit of centralspindlin (Yüce et al., 2005). PLK1-dependent phosphorylation of MgcRacGAP is required for its interaction with ECT2 (Wolfe et al., 2009; Burkard et al., 2009; Petronczki et al., 2007). The ECT2 C-terminus contains plekstrin homology and basic domains, which mediate an interaction of ECT2 with the membrane and are essential for cytokinesis (Su et al., 2011; Kotýnková et al., 2016). Depletion of ECT2 results in cytokinetic failure, demonstrating the importance of Rho activation (Yüce et al., 2005). Additionally, a photoactivation-medited recruitment of ECT2 to the plasma membrane is sufficient to stimulate furrowing, even in non-mitotic cells and at non-equatorial locations in mitotic cells (Wagner and Glotzer, 2016).

The GAP domain of the MgcRacGAP subunit of centralspindlin is also required for cytokinesis, but the GAP target is debated. Some data support the view that the GAP activity is directed toward Rho, thus maintaining a flux of Rho activity that is thought to be important for the highly restricted zone of active Rho observed in dividing cells (Miller and Bement, 2009). Other work shows that the GAP activity is directed toward the related GTPase, Rac (Rac1), and indirectly leads to Rho activation (Canman et al., 2008; Bastos et al., 2012). In vertebrate cells, specificity of the GAP domain for Rac or Rho can also be regulated by phosphorylation (Minoshima et al., 2003) (Box 1).

Although clearly visualized on midzone microtubules, centralspindlin oligomers also localize to the cell membrane (Box 1) (Adriaans et al., 2019; Basant et al., 2015). Centralspindlin is delivered to the equatorial cortex by astral microtubules prior to furrowing (D'Avino et al., 2006; Nishimura and Yonemura, 2006) (see poster). Recent work shows that centralspindlin tracks growing astral microtubule plus-ends in frog and fly cells, suggesting a possible mechanism for delivery to cortical sites (Breznau et al., 2017; Verma and Maresca, 2019). Like centralspindlin, the CPC localizes to midzone microtubules and the cortex in anaphase (Earnshaw and Cooke, 1991). Cortical localization is mediated by INCENP binding to actin (Landino et al., 2017). MKLP2 has also been shown to bind to cortical myosin (Kitagawa et al., 2013).

Together, these results demonstrate that the CPC and centralspindlin accumulate not only on overlapping midzone microtubules but also on microtubule plus-ends and at the membrane, where they can locally activate ECT2, which is a key step in specifying the location of contractile ring formation (von Dassow, 2009).

## Midzone contribution to chromosome segregation and spindle elongation

In addition to generating signals for cytokinesis, the midzone elongates during anaphase, thus contributing to chromosome segregation (see poster). This contribution was first shown more than 30 years ago by cutting midzone microtubules using either a microneedle (Kronebusch and Borisy, 1982) or laser ablation (Aist et al., 1993, 1991). The results showed that damaging the midzone in either fungal or marsupial cells resulted in an increase in the rate and extent of spindle pole motion. In contrast, ablation of a centrosome, which reduced the number of associated astral microtubules, stopped motion of the damaged pole, while the sister pole accelerated (Aist et al., 1993). Similar results were also reported following laser ablation of the midzone in *C. elegans* embryos (Grill et al., 2001). These results led to the conclusion that midzone microtubules restrict spindle elongation and that astral microtubules contribute to force generation in diverse cell types.

Although these experiments demonstrate that the midzone mechanically links the two halves of the spindle and restricts elongation, other work provides evidence that sliding of antiparallel midzone microtubules generates forces to drive chromosome segregation. Force generation by midzone microtubule bridging fibers has been demonstrated in human cells in which a pair of chromosomes and their associated bridging fiber was isolated from the rest of the spindle by severing the connection between one kinetochore fiber and its associated pole (Vukušić et al., 2017). If the bridging fiber linking these sister chromosomes is subsequently cut in anaphase, the motion of the distal chromosome is greatly

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reduced, demonstrating that bridging fiber microtubules contribute to chromosome segregation (Vukušić et al., 2017). Experiments that involved more extensive laser-mediated cutting across the width of the midzone in early anaphase resulted in a temporary cessation of chromosome segregation in both *C. elegans* meiotic spindles and human cells, demonstrating that intact midzone microtubules promote chromosome segregation (Vukušić et al., 2017; Yu et al., 2019). Force generation by the midzone has also been demonstrated in other systems, including diatom spindles (Leslie and Pickett-Heaps, 1983) and yeast (Khodjakov et al., 2004; Tolić-Nørrelykke et al., 2004). Together, these results support a model in which force generated by midzone microtubules drives chromosome motion during early anaphase.

Experimental perturbation of proteins that crosslink midzone microtubules, including non-motor and motor microtubule-binding proteins, provides insight into the mechanism by which the midzone restricts and/or promotes chromosome motion and spindle elongation. Perhaps one of the best examples of this duality is the kinesin-5 motor, KIF11, chains, which forms bipolar tetramers that crosslink antiparallel microtubules generating force for centrosome separation and maintenance of metaphase spindle length (Kapitein et al., 2005; Mann and Wadsworth, 2019). In human cells, spindle elongation is not altered following inhibition of kinesin-5 (Vukušić et al., 2021; Hu et al., 2011), although a modest increase in the rate of spindle elongation has been reported in pig epithelial cells (Collins et al., 2014). In other cell types, kinesin-5 family members can restrict spindle elongation acting as a brake (*C. elegans*) (Saunders et al., 2007) or contribute to elongation (*Drosophila melanogaster* and *Saccharomyes cerevisae*) (Straight et al., 1998; Brust-Mascher et al., 2004; 2009; Wang et al., 2015).

The antiparallel microtubule crosslinker PRC1 also impacts spindle elongation in mammalian cells. Depletion of PRC1 results in a greater extent and rate of chromosome segregation, which is more pronounced later in anaphase (Vukušić et al., 2021; Pamula et al., 2019; Kurasawa et al., 2004). In *Drosophila,* PRC1 homologs also contribute to spindle elongation by regulating microtubule stability and midzone kinesin motor proteins (Wang et al., 2015). Similarly, experimental perturbation of midzone assembly by depletion of either PRC1 or MKLP1 leads to faster chromosome segregation in *C. elegans (*Maton et al., 2015). In human cells, inhibition of kinesin-5 in combination with depletion of either PRC1 or KIF4A reduced spindle elongation, indicating that crosslinkers and motors work together to regulate anaphase motion (Vukušić et al., 2021).

The observation that cutting the midzone or depleting midzone microtubule crosslinkers enhances spindle elongation reveals that, at least in some systems, forces generated external to the spindle contribute to spindle elongation. In metaphase, spindle positioning is mediated by pulling forces generated by interactions of astral microtubules with dynein motors anchored to the cortex by a conserved multi-subunit complex comprised of Gαi (encoded by *GNAI1*), LGN (also known as GPSM2) and NuMA (also known as NUMA1) (Du and Macara, 2004; Grill et al., 2003). In anaphase, dynein and NuMA accumulate at the polar cortex, and depletion of either reduces spindle elongation, especially in late anaphase (Collins et al., 2012; Kotak et al., 2013); overexpression of NuMA enhances elongation in late anaphase (Kotak et al., 2013). NuMA is targeted to the cortex by LGN and Gαi, as well as by an anaphase-specific pathway in which 4.1 family proteins, which are important for organization of membrane proteins, recruit dephosphorylated NuMA (Kiyomitsu and Cheeseman, 2013). NuMA has also been shown to interact directly with membrane phosphoinositides (Kotak et al., 2014). The centralspindlin complex negatively regulates NuMA accumulation at the equatorial cortex, thus confining motor complexes to the cell poles (Kotak et al., 2014). Although cortical dynein is thought to play a major role in force generation, cytoplasmic dynein motors have also been shown to contribute to pulling forces, especially in large cells (Telley et al., 2012).

Aster-based forces could be generated as cortically anchored dynein motors pull on microtubules as they walk toward the microtubule minus-end (Grill and Hyman, 2005). However, live-cell imaging during spindle positioning shows that astral microtubules transition to disassembly following cortical contact, suggesting an alternative model in which force is generated by polymer disassembly, and cortical complexes provide a link to the dynamic microtubule (Kozlowski et al., 2007). Consistent with this, *in vitro* systems composed of a centrosomal array of microtubules and dynein motors bound to the edge of a microfabricated chamber show that dynein can regulate both microtubule dynamics and generate pulling forces (Laan et al., 2012).

The distinct outcomes of midzone-disruption experiments – faster motion in some experiments and reduced motion in other cases – is likely related to differences in aster-based force generation in anaphase. One consideration is that an array of astral microtubules and the cortical motors that interact with them might not be present or sufficient in all cells or under all experimental conditions. A second consideration is that the magnitude of astral- and midzone-generated forces likely vary as anaphase progresses. This could explain why midzone cutting in early anaphase (Yu et al., 2019; Vukušić et al., 2017) has a different result to cutting later in anaphase when midzone microtubules are extensively bundled (Aist et al., 1993) and astral microtubules have extended to the cortex (Rusan and Wadsworth, 2005). Finally, force production by the midzone and asters must be coordinated; how force generation at one location impacts other sites remains incompletely understood and is an important topic for future work.

## Perspectives

The midzone plays a key role in cell division – it serves as a platform for the accumulation of signaling factors that specify the location of cytokinesis, physically separates the two daughter cell genomes prior to abscission and impacts chromosome segregation. Recent work has provided insight into how this new array of microtubules forms during anaphase, identifying many of the component parts. Future work is needed to understand how the components are regulated in space and time to form and elongate the midzone, and link the midzone to kinetochore fibers. Another important topic is how forces generated by astral microtubules and those generated within the midzone are coordinated during spindle elongation. A promising approach to help resolve this issue are *in vitro* reconstitutions coupled with biophysical measurements (see Box 2) (Gaska et al., 2019; Nguyen et al., 2014). Agent-based models of spindle formation provide insight into how microtubules and motors drive spindle formation; similarly, modeling of antiparallel microtubule arrays provides new, testable predictions about spindle elongation (Lera-Ramirez and Nédélec, 2019). Quantitative measurements of the distribution and dynamic behavior of midzone components are also needed to provide realistic values of protein abundance, dynamics and interactions for building and refining models. Finally, much of what we know about the midzone formation and behavior during anaphase comes from a handful of well-studied systems. Expanding investigations into cells within intact tissues and/or at various developmental stages and

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looking at a greater diversity of species will provide new insight into many aspects of cell division (Davies et al., 2018; Bourdages et al., 2014).

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**Cell science at a glance**
Individual poster panels are available for downloading at https://journals.biologists.com/jcs/article-lookup/doi/10.1242/jcs.250001

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