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REVIEW

SUBJECT COLLECTION: MICROTUBULE DYNAMICS

Force-generating mechanisms of anaphase in human cells

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ABSTRACT

What forces drive chromosome segregation remains one of the most challenging questions in cell division. Even though the duration of anaphase is short, it is of utmost importance for genome fidelity that no mistakes are made. Seminal studies in model organisms have revealed different mechanisms operating during chromosome segregation in anaphase, but the translation of these mechanisms to human cells is not straightforward. Recent work has shown that kinetochore fiber depolymerization during anaphase A is largely motor independent, whereas spindle elongation during anaphase B is coupled to sliding of interpolar microtubules in human cells. In this Review, we discuss the current knowledge on the mechanisms of force generation by kinetochore, interpolar and astral microtubules. By combining results from numerous studies, we propose a comprehensive picture of the role of individual force-producing and -regulating proteins. Finally, by linking key concepts of anaphase to most recent data, we summarize the contribution of all proposed mechanisms to chromosome segregation and argue that sliding of interpolar microtubules and depolymerization at the kinetochore are the main drivers of chromosome segregation during early anaphase in human cells.

KEY WORDS: Anaphase, Astral microtubules, Bridging microtubules, Interpolar microtubules, Kinetochore microtubules, Mitosis

Introduction

Anaphase as part of the M-phase is one of the most spectacular moments of the cell cycle, when coordinated splitting of replicated chromosomes and segregation of sister chromatids occurs. During anaphase, lagging chromosomes appear only rarely (Worrall et al., 2018), indicating the robustness of mechanisms acting during this phase. Chromosome separation is accomplished by the spindle, a structure composed of microtubules (MTs) and accompanying proteins, which self-assembles at the onset of M-phase (Pavin and Tolić, 2016). During spindle formation, chromosomes attach to MTs through kinetochores – protein complexes at the centromere of each chromosome (Musacchio and Desai, 2017) – and align at the equatorial plate of the spindle to form a tight metaphase plate (Maiato et al., 2017). Anaphase starts when all chromosomes are bi-oriented, with sister kinetochores attached to MTs extending from opposite spindle poles and the spindle assembly checkpoint being satisfied (Joglekar, 2016). Subsequently, chromosome segregation initiates with proteolytic severing of cohesion links between sister chromatids (Hauf et al., 2001), accompanied by global protein dephosphorylation (Wurzenberger and Gerlich, 2011). This is accomplished by inactivation of cyclin-dependent kinase 1 (CDK1) and increased activity of phosphatases reducing metaphase anti-poleward forces, which allows chromosomes to display net motion towards the spindle poles (Su et al., 2016).

The anaphase spindle is composed of different populations of MTs, defined according to their structure, dynamics and function: kinetochore MTs, which connect kinetochores with the spindle poles, and non-kinetochore MTs (Alberts et al., 2014). Kinetochore MTs form parallel bundles known as kinetochore fibers (k-fibers). The main non-kinetochore MTs are interpolar MTs, which extend from the opposite sides to the center of the spindle forming an antiparallel bundle, and astral MTs, which grow from the spindle poles towards the cell cortex (Tolić, 2018). In human cell lines, interpolar MT bundles interact laterally with sister k-fibers acting as a bridge between them and are thus termed bridging fibers (Kajtez et al., 2016; Polak et al., 2017) (Fig. 1A). The accompanying proteins that are associated with spindle MTs or chromatin can be divided into several classes – molecular motors from the kinesin and dynein families that convert chemical energy stored into ATP to do mechanical work, non-motor MT-associated proteins that crosslink, bundle or focus spindle MTs, and various protein regulators, such as kinases (Civelekoglu-Scholey and Scholey, 2010; Cross and McAinsh, 2014; Raaijmakers and Medema, 2014; Verhey and Hammond, 2009).

Chromosome segregation is commonly described in two overlapping phases: anaphase A, where chromosomes move poleward and anaphase B, where the spindle elongates (Asbury, 2017; Scholey et al., 2016). Anaphase A and B were first distinguished in pioneering experiments by Hans Ris on grasshopper spermatocytes where the addition of chloral hydrate stopped only anaphase B (Ris, 1949), and later on, human somatic cells where inhibition of Polo-like kinase 1 (Plk1) also blocked only anaphase B (Brennan et al., 2007), suggesting that these processes have distinct molecular mechanisms. Interestingly, the relative contribution of these two processes to chromosome segregation varies among organisms (Roostalu et al., 2010). For example, one-cell stage *Caenorhabditis elegans* embryos display no anaphase A (Oegema et al., 2001) and, in yeast spindles, anaphase A does not significantly contribute to chromosome segregation (Mallavarapu et al., 1999; Straight et al., 1998), whereas *Xenopus* meiotic extract spindles rely entirely on anaphase A and lack anaphase B (Desai et al., 1998). In human cells, both phases seem to be equally important contributors to chromosome segregation (Vukušić et al., 2017). Although the process of anaphase has been well studied for decades, the mechanisms driving anaphase are still under debate, especially in human cells (Asbury, 2017; Maiato and Lince-Faria, 2010; McIntosh et al., 2012; Scholey et al., 2016).

In this Review, we discuss the spindle structures and dynamics during early anaphase in human somatic cells by reviewing recent data on the mechanisms of force production that drive anaphase chromosome motion. We focus on early anaphase because this is the period where chromosomes segregate the most. The later stages of

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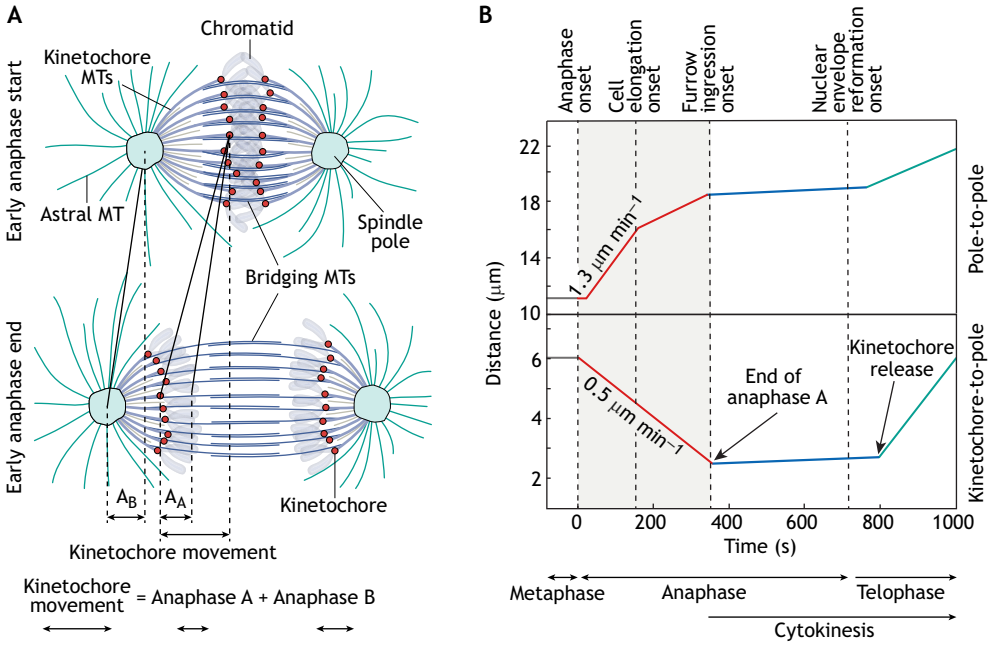

Fig. 1. An overview of anaphase spindle structures and dynamics. (A) Anaphase A and B (A_A and A_B) contribute roughly equally to kinetochore movement; A_A and A_B represent kinetochore movement owing to its poleward motion and spindle elongation, respectively. Different spindle structures and MT populations are depicted. (B) Spindle elongation (top graph) and kinetochore poleward movement (bottom graph) are characterized by phases with different velocity regimes (color coded). Chromosomes segregate the most during early anaphase (0 to 360 s, shaded area).

anaphase, after the onset of cytokinesis, including formation of the central spindle, midbody and the contractile ring, have been reviewed elsewhere (D'Avino et al., 2015). We further highlight the role of different MT populations in early anaphase and the role of MT-associated motor and non-motor proteins in anaphase dynamics, and propose a comprehensive model for early anaphase in human cells.

Anaphase in human cells by the numbers

Anaphase is marked by distinct events regarding poleward movement and spindle elongation (Fig. 1B). Poleward movement of kinetochores (anaphase A) in HeLa and U2OS cells starts at an average kinetochore-to-pole distance of ∼6 µm (Su et al., 2016; Vukušić et al., 2017). The average poleward velocity is ∼0.5 µm/min during the first phase, which is almost linear and lasts for 6 min (Su et al., 2016; Vukušić et al., 2017). Although net chromosome motion in anaphase is poleward, neighboring chromosomes can move both poleward and anti-poleward and individual chromosomes switch direction at different rates (Stumpff et al., 2008; Su et al., 2016), arguing that kinetochores in anaphase move individually, not as a group. Afterwards, anaphase A movement stops, and the poleward motion enters the stationary phase when there is a distance of ∼3 µm between kinetochores and spindle poles. At the end of anaphase, ∼13 min after anaphase onset, the distance between kinetochores and poles increases suddenly and synchronously, indicating detachment of kinetochores from their MTs (Su et al., 2016) (Fig. 1B).

Spindle elongation (anaphase B) initiates shortly after anaphase onset (Su et al., 2016) and occurs in four distinct phases: an initial fast and linear spindle pole separation phase that lasts until 3 min after anaphase onset and occurs at a velocity of ∼1.3 µm/min (Vukušić et al., 2017), a second, somewhat slower phase, that coincides with cell elongation, a third, almost stationary phase that lasts ∼13 min from anaphase onset, and a fourth phase with an increase in velocity that coincides with kinetochore release from MTs (Afonso et al., 2014) (Fig. 1B). In parallel, the onset of cytokinesis in human cells is marked by the initiation of a furrow, which takes place ∼6 min after anaphase onset (Janisch et al., 2018) (Fig. 1B). The first three phases of spindle elongation are commonly considered as anaphase B, whereas the fourth phase happens after nuclear reformation in telophase and is sometimes referred to as anaphase C (McIntosh, 1994). At the end of anaphase, tubulin is excluded from the newborn nuclei and lamin B accumulates around the same structure, which marks nuclear envelope reformation and defines the onset of telophase (Afonso et al., 2014). Since the chromosomes segregate the most during the first, almost linear, phase of anaphase A that coincides with first two phases of anaphase B, we will mainly focus on the events taking place during these time intervals and refer to them as early anaphase (Fig. 1B, shaded area).

The role of k-fibers in force generation in anaphase

General principles derived from model organisms

Depolymerizing MTs as primary source of force generation

The most important structures for anaphase A movement are k-fibers, whose kinetochore-attached MTs drive poleward movement of chromosomes (Maiato and Lince-Faria, 2010) (Fig. 1A). Early groundbreaking experiments in different organisms demonstrated that when the kinetochore of a single chromosome is damaged and its connection to k-fibers is severed, the movement of the rest of the chromosome stops; conversely, if chromosome arms are ablated, the movement is not affected (McNeill and Berns, 1981; Uretz et al., 1954). These experiments showed that chromosome connection to k-fibers is crucial for poleward movement whereas chromosome arms are mainly passive during this process. Movement of chromosomes is coupled to either growth or shortening of the fibers to which they are attached (Cimini et al., 2004); the shortening of k-fibers seems to drag chromosomes poleward, one of the most conserved and fundamental features of mitosis (Asbury, 2017). In accordance, MT depolymerization induced by exposure to low temperatures, addition of colchicine or high pressure *in vivo* can generate the force able to induce or accelerate the rate of poleward movement of chromosomes (Inoue, 1952; Inoue and Ritter, 1975; Salmon et al., 1976). Further confirming these observations, the same studies reported that the rate of poleward movement is dependent on the rate of MT depolymerization, suggesting that depolymerization is a direct force producer or a main rate-limiting step in anaphase A.

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Depolymerization at both ends is coupled to chromosome movement

Shortening of the k-fiber could occur through MT depolymerization at their plus- and/or minus-ends. Classic electron microscopy and photo-bleaching experiments in mammalian fibroblasts (BSC1 cells) showed that depolymerization occurs at the kinetochore – and thus MT plus-ends – concomitantly with chromosome poleward movement in anaphase (Mitchison et al., 1986) and micromanipulation studies on grasshopper spindles demonstrated that chromosomes continue to move poleward in anaphase as long as the k-fiber stub is longer than 1 µm (Nicklas, 1989). Therefore, the primary site of force generation in anaphase A is on or near kinetochores. In addition, the energy for poleward movement of isolated chromosomes and MTs *in vitro* is derived solely from MT depolymerization at kinetochores (Koshland et al., 1988). This coupling of chromosome movement and MT disassembly at kinetochores is referred to as plus 'tip-coupling' (Asbury, 2017), previously known as the 'Pac-man' model (Cassimeris et al., 1987) (Fig. 2A). On the contrary, the contribution of depolymerization at the k-fiber minus-ends was revealed during metaphase of mammalian fibroblasts upon incorporation of labeled tubulin at k-fiber plus-ends and subsequent extension towards the pole (Mitchison et al., 1986) as well as poleward movement of tubulin in newt lung cells, which slowed down as anaphase progressed (Mitchison and Salmon, 1992); this indicated a poleward flux of MT subunits caused by depolymerization at the spindle pole (Fig. 2A). These observations define the modern version of the classic traction fiber model (Inoue and Ritter, 1975; Maiato and Lince-Faria, 2010), in which the polymerization at the kinetochore decreases after anaphase onset and the depolymerization at the pole pulls the k-fiber, along with the kinetochore, polewards.

Relevance of general principles to human cells

Plus tip-coupling as a major mechanism of anaphase A in human cells

The fraction of the observed k-fiber shortening at both MT ends varies greatly between different model organisms (Asbury, 2017). Poleward MT flux during anaphase in human cells accounts for ∼30–40% of anaphase A chromosome-to-pole movement when measured directly by photoactivation of tubulin (Vukušić et al.,

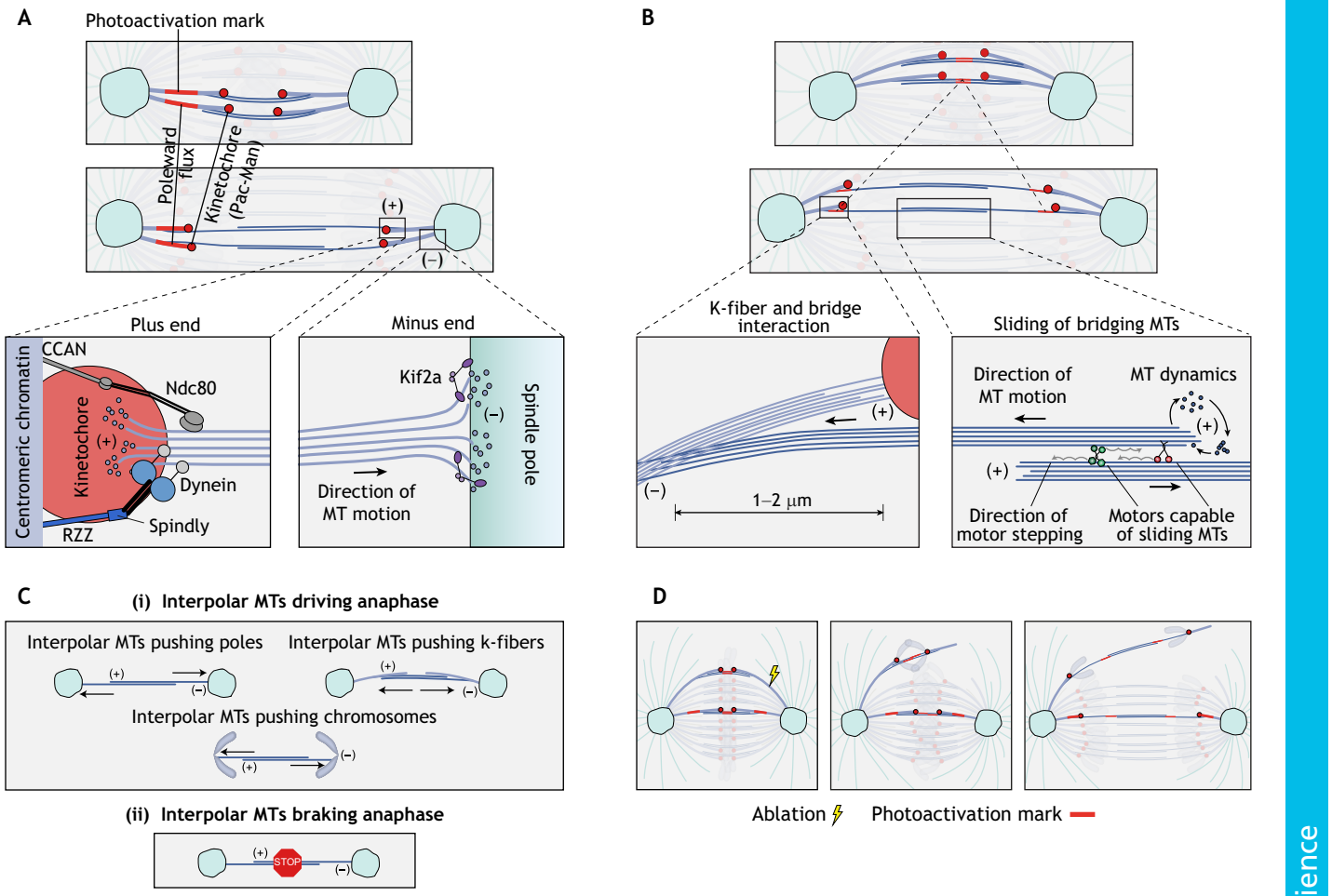

Fig. 2. K-fibers and interpolar microtubules in force generation in anaphase. (A) Mechanisms of k-fiber depolymerization during anaphase. Top, plus tip-coupling (i.e. the Pac-Man mechanism, which occurs at the kinetochore) contributes more than the MT poleward flux (as can be visualized through a photoactivation mark) to the poleward chromosome movement. Bottom left, the Ndc80 complex is a main coupler and dynein–dynactin is a main regulator during k-fiber plus-end depolymerization (bottom left). CCAN, constitutive centromere associated network; RZZ, Rod-Zw10-Zwilch. Bottom right, k-fiber minus-end depolymerization is facilitated by Kif2a. (B) Bridging MTs slide apart (red lines, top) during anaphase. Bridging MTs interact with k-fibers (bottom left) and push them apart by sliding of plus-end directed motors, coupled with regulation of MT plus-end dynamics (bottom right). (C) Interpolar MTs can (i) drive anaphase by different means or (ii) act as a brake (stop sign). Black arrows indicate where interpolar MTs exert pushing forces. (D) Schemes of experimental results on human cells showing that after laser ablation (yellow) of one k-fiber, kinetochores segregate independently of the spindle pole, driven by bridging MT sliding and k-fiber depolymerization. At the same time, intact k-fibers and their bridging MTs slide apart at a similar velocity. Spindle parts are color-coded as in Fig. 1.

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2017), whereas the remaining 60–70% is due to plus-end disassembly (Fig. 2A). A member of the kinesin-13 family of molecular motors, namely Kif2a, is required for flux of k-fibers during metaphase in human cells (Ganem et al., 2005), similar to what is seen for its fly homolog kinesin-like protein A (Klp10A) (Rogers et al., 2004). However, the anaphase A chromosome movement rates in cells with inhibited flux during metaphase, following kinesin-13 depletion, decreased by only 20% (Ganem et al., 2005), demonstrating that, in human cells, kinetochore-led depolymerization is sufficient and predominant in powering chromosome poleward movements during anaphase. Similarly, in mammalian oocytes, the disassembly of MTs at the plus-end makes a major contribution to anaphase A (FitzHarris, 2012). Confirming the predominance of the plus-end force generation, it was recently shown that a reduction of the number of MTs that end at kinetochores diminishes some of the forces acting on them and that these forces are important for anaphase A movement (Dudka et al., 2018): a reduction of MT occupancy at kinetochores by 30% led to a reduction of anaphase A movement by 40%, without affecting spindle elongation (anaphase B) (Dudka et al., 2018). Thus, in human cells, the ∼17 MTs typically contacting a kinetochore per fiber (McEwen et al., 2001) are an important part of the anaphase A segregation machinery. Moreover, recent studies have pointed to additional anaphase mechanisms as fast poleward movement of short k-fibers generated by laser ablation during anaphase is observed (Elting et al., 2014; Sikirzhytski et al., 2014) but less than 10% of kinetochores exhibit frequent fast poleward motions mediated by dynein in unperturbed anaphase (Sikirzhytski et al., 2014). In conclusion, chromosome-to-pole motion in human cells is a superposition of two mechanisms: the predominant MT plus-end disassembly at kinetochores and MT poleward flux that is linked with minus-end disassembly (Fig. 2A).

Coupling of depolymerizing MTs to spindle poles and kinetochores

An important question that is still under debate is how kinetochores or spindle poles can maintain a persistent connection with MT tips that are depolymerizing (Asbury, 2017). Evidence against simple models in which tip-coupling is based primarily on a single type of conventional molecular motor was gathered by depleting all kinetochore-localized proteins in fission yeast (Grishchuk and McIntosh, 2006) or all minus-end-directed kinetochore motors in budding yeast (Tanaka et al., 2007) and poleward movement of chromosomes coupled with depolymerization still continued.

In agreement with this, perturbation of various kinetochore or spindle pole motors and regulators in human cells (Auckland and McAinsh, 2019 preprint; Ganem et al., 2005; Maffini et al., 2009; Mukherjee et al., 2012; Stumpff et al., 2008; Su et al., 2016; Wandke et al., 2012; Zhu et al., 2005) did not completely abolish anaphase chromosome-to-pole movements. However, there are studies that have provided evidence for some motor-involvement during this process in human cells (see Box 1). The current view is that motor proteins play a passive role in regulating tip-coupling in anaphase of human cells or actively generate forces through redundant pathways where there is no single dominant motor involved (Fig. 2A).

As opposed to motor proteins, it appears that non-motor proteins that bind to kinetochores are central to plus-tip coupling. The highly conserved Ndc80 complex (Powers et al., 2009; Volkov et al., 2018), a hetero-tetramer with a fibrillary structure with one end that binds MTs and the other end stably anchored into the constitutive centromere-associated network (CCAN) of the inner kinetochore (Varma and Salmon, 2012) (Fig. 2A). Interestingly, inhibition of parts of this complex results in complete stop of poleward chromosome motion in *Xenopus* S3 cells (McCleland et al., 2003; Vorozhko et al., 2008) whereas, in human cells, its depletion results in disrupted kinetochore–MT attachments, failure in chromosome alignment, abnormally long spindles and prometaphase block (DeLuca et al., 2003, 2002). Despite evidence that forcing the anaphase in the blocked cells results in anaphase completion with misaligned chromosomes (Meraldi et al., 2004) and fragmented nuclei (Martin-Lluesma et al., 2002), it is still not known if the Ndc80 complex is indispensable for anaphase poleward movement (Kops et al., 2010). Additionally, the replacement of endogenous Ska1 protein (Schmidt et al., 2012; Welburn et al., 2009), that might bridge neighboring Ndc80 complexes and serve as an attachment-stabilizer, with mutants lacking the MT-binding domain does not induce a significant change in chromosome dynamics during anaphase A in human cells (Su et al., 2016). Finally, coupling is most probably mediated by fibrillary supracomplexes connecting the bending protofilaments of MTs to the outer kinetochore, visualized by electron microscopy (McIntosh et al., 2008), possibly including Ndc80 with contributions from long kinetochore-associated motors (McIntosh et al., 2010). In conclusion, the k-fiber depolymerization driving chromosome movement in human cells is largely independent of molecular motors and instead

Box 1. Molecular players in anaphase A

Is chromosome poleward movement actively powered by molecular motors? Seminal works inhibiting ATP to test its requirement for anaphase A (Cande, 1982; Spurck et al., 1986) revealed that ATP is required for MT disassembly but not for a kinetochore motor actively pulling the kinetochore polewards (Spurck and Pickett-Heaps, 1987). Interestingly, a significant reduction of poleward velocity was seen upon minus-end-directed motor cytoplasmic dynein perturbations (Sikirzhytski et al., 2014; Yang et al., 2007). However, because dynein is barely detectable at kinetochores in anaphase (Pfarr et al., 1990), its effects are most likely indirect, either by regulating MT dynamics, stability of k-fibers or kinetochore orientation (Bader and Vaughan, 2010). Further exciting candidates for powering poleward movement are CENP-F (Auckland and McAinsh, 2019 preprint), which is an efficient coupler to depolymerizing MTs (Volkov et al., 2015), and CENP-E, found to be required for depolymerization-dependent movement of chromosomes *in vitro* (Lombillo et al., 1995). Interestingly, the kinesin-13 members KLP59C and KLP10A, can form rings around MTs (Tan et al., 2006) and contribute to anaphase A in *Drosophila* cells (Rogers et al., 2004), but the depletion of orthologs in human cells, known to localize to kinetochores or spindle poles and facilitate MT depolymerization *in vitro* (Howard and Hyman, 2007), did not affect anaphase A movement globally (Bakhoum et al., 2009; Ganem et al., 2005; Gupta et al., 2006), indicating that they are not crucial for chromosome segregation in anaphase. Kif18a (a kinesin-8 member) promotes MT disassembly at plus-ends and couples it to the movement of beads *in vitro* (Grissom et al., 2009). Interestingly, *in vivo*, depletion of Kif18a accelerates poleward velocity rates, whereas its overexpression lowers this rate (Stumpff et al., 2008), similar to what is seen in *Drosophila* somatic cells (Buster and Sharp, 2007), suggesting that Kif18a affects kinetochore velocity through reduction of kinetochore-MT plus-end dynamics. Regarding non-motor regulators, poleward velocity rates during anaphase are significantly lower in *Clasp2*-knockout mouse embryonic fibroblasts, implying that this protein has a role in the regulation of kinetochore MT depolymerization in anaphase (Pereira et al., 2006). Furthermore, depletion of the MT-severing protein fidgetin decreased metaphase poleward flux rates and anaphase poleward chromosome velocity, albeit to a minor extent (Mukherjee et al., 2012). An overall picture is that individual motors are dispensable for anaphase A movements and, together with non-motor proteins, have a regulatory role during depolymerization-driven poleward chromosome movement.

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requires force exerted by depolymerizing MTs coupled to kinetochores.

The role of interpolar MTs in force generation in anaphase

Architecture and dynamics of interpolar bundles in anaphase

Interpolar MT bundles in human cells are formed by two sets of MTs that emanate from opposite poles and form an antiparallel overlap region in the spindle midzone (McIntosh and Landis, 1971) (Fig. 2B). The arrangement of plus-ends of interpolar MTs changes slightly from metaphase to mid-anaphase, with a decrease in the overlap length at anaphase-to-telophase transition in long-nosed potoroo epithelial kidney cells (PtK1) (Mastronarde et al., 1993). Tubulin subunits are added to the plus-ends of these antiparallel MTs that slide apart during late anaphase (Saxton and McIntosh, 1987), whereas the overall dynamics of polymerization vary at different anaphase stages in PtK1 cells (Shelden and Wadsworth, 1990). Although k-fibers are the most stable MT subpopulation in metaphase, being more resistant to depolymerization-inducing cold treatment than interpolar or astral MTs in PtK1 cells (Brinkley and Cartwright, 1975), late anaphase central spindle MTs are also very stable and can resist high-pressure forces in HeLa cells (Salmon et al., 1976). However, the details of interpolar MT dynamics, temporal regulation of the overlap length, stability and their impact on anaphase movements in human cells remain to be elucidated (see Box 2).

A similar structural organization of interpolar MTs has been found in most species studied (Ding et al., 1993; Mastronarde et al., 1993; McDonald et al., 1977; McIntosh and Landis, 1971; Winey et al., 1995), although MT numbers vary from less than ten in yeasts (Ding et al., 1993; Winey et al., 1995) to hundreds in human WI-38 and HeLa cells (McIntosh and Landis, 1971). In budding yeast (Winey et al., 1995), fission yeast (Ding et al., 1993) and diatom (McDonald et al., 1977) spindles, the minus-ends of interpolar MTs appear to interact with spindle poles; this could enable the outward pushing force of sliding interpolar MTs to be exerted on spindle poles in order to drive spindle elongation (McIntosh et al., 1969). On the other hand, the minus-ends of the most interpolar MTs in PtK1 spindles do not reach the poles (Mastronarde et al., 1993), suggesting that direct pushing of interpolar MTs on spindle poles is not likely to occur in all organisms.

It has been previously reported that non-kinetochore MTs could extend along the k-fiber to the region between sister kinetochores in metaphase spindles of human cells (McIntosh and Landis, 1971; Nixon et al., 2017). We found that almost all of these non-kinetochore MT bundles act as a bridge between sister k-fibers (Kajtez et al., 2016; Polak et al., 2017) (Fig. 2B). By using a laser ablation assay (Buđa et al., 2017), we observed that these bridging fibers moved together with sister kinetochores and their k-fibers, indicating strong crosslinking between these structures during metaphase (Kajtez et al., 2016; Milas and Tolic, 2016; Tolić, 2018; Tolić and Pavin, 2016) and anaphase (Vukušić et al., 2017). In agreement with the observation of bridging MTs in anaphase (Vukušić et al., 2017), recent large-scale electron tomography reconstructions of spindles in human cells reported different classes of interpolar MTs that were often tightly associated into bundles contacting k-fibers (Yu et al., 2019). It will be interesting to identify the crosslinkers that keep k-fibers and bridging fibers laterally linked, with the kinesins Eg5 (also known as Kif11) and Kif15 being good candidates as they are involved in the regulation of neighboring k-fiber coupling during metaphase (Vladimirou et al., 2013). Moreover, the mechanisms maintaining the connections between bridging and k-fiber as the k-fiber depolymerizes at both ends during anaphase remain an open question.

Box 2. Regulators of interpolar MTs in anaphase B

How do the dynamics and stability of midzone MTs influence spindle elongation? The kinesin-4 member Kif4a stops the midzone from growing continually by inhibiting plus-end polymerization of interpolar MTs (Hu et al., 2011). The region of interpolar bridging MTs also contains protein regulator of cytokinesis 1 (PRC1), which cross-links anti-parallel MTs (Jiang et al., 1998) and increases their stability (Hu et al., 2011). PRC1 depletion did not affect chromatid segregation in early anaphase (Mollinari et al., 2005), but spindle elongation continued after furrowing (Uehara et al., 2016), implying that PRC1 is crucial for restricting spindle elongation in late anaphase, as confirmed by a recent study (Pamula et al., 2019). However, since PRC1 removal delocalizes multiple components of the central spindle (Kurasawa et al., 2004; Liu et al., 2009), the discrimination between different contributions remains to be described. Silencing of transforming acidic coiled-coil-containing protein 3 (TACC3) results in the destabilization of MTs in the central spindle and reduced rates of midzone elongation (Lioutas and Vernos, 2013), similar to what occurs upon inhibition of Aurora A kinase (Lioutas and Vernos, 2013; Reboutier et al., 2013). Moreover, inhibition of Aurora B reduces the rates of spindle elongation in a dose-dependent manner (Afonso et al., 2014) and depletion of both Aurora kinases leads to anaphase failure through increased stability of spindle MTs (Hégarat et al., 2011), implicating them as regulators of anaphase movements through regulation of MT stability (Afonso et al., 2017). Taken together, the stability of midzone MTs is important for later stages of anaphase but less so for early stages (Hu et al., 2012). What about the origin of central spindle MTs? In addition to nucleation from centrosomes by MT-nucleating γ-tubulin ring complex (γTuRC), MTs can be also nucleated along pre-existing MTs (Petry and Vale, 2015) by action of the γTuRC-recruiting human augmin complex (HAUS) (Lawo et al., 2009). In accordance, the decreased density of MTs in the central spindle observed in HAUS6-depleted cells during late anaphase (Uehara et al., 2009) indicates that the HAUS complex participates in the generation of new interpolar MTs during anaphase B. However, perturbation of this pathway only affects spindle elongation in late anaphase (Uehara et al., 2016), suggesting that most MTs in the antiparallel region during early anaphase are bridging MTs originating from the metaphase spindle.

Powering versus braking chromosome segregation

In principle, interpolar MTs can contribute to anaphase dynamics in different ways: in the first scenario, antiparallel MTs slide apart powered by motor proteins that walk towards their plus-end (Fig. 2B,C). As they slide, the interpolar MTs could push apart different parts of the spindle attached at or near their minus-ends, such as spindle poles (Brust-Mascher et al., 2004), neighboring k-fibers (Vukušić et al., 2017) or the chromosomes (Dumont et al., 2010) (Fig. 2C). In the second scenario, antiparallel MTs could act as negative regulators of anaphase dynamics through a braking mechanism produced by molecular friction or crowding effects (Collins et al., 2014; Saunders et al., 2007) (Fig. 2C). In addition, the two mechanisms could work together with a temporally displaced activity where interpolar MTs may actively generate forces for chromosome movement in one phase, whereas in the other, they may act as a brake, possibly mediated by concentration of antiparallel crosslinkers in the midzone.

To distinguish between these scenarios, laser ablation of spindle MTs has proven to be a useful tool. The sufficiency and necessity of pushing from spindle midzone for spindle elongation has been confirmed in diatom (Leslie and Pickett-Heaps, 1983) and fission yeast (Khodjakov et al., 2004; Tolić-Nørrelykke et al., 2004) spindles. Interestingly, in other models such as PtK2 cells

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(Aist et al., 1993), *C. elegans* (Grill et al., 2001) and *Nectria haematococca* (Aist et al., 1991), the same ablation experiments on interpolar MTs accelerated pole separation during anaphase B, revealing that midzone MTs are dispensable and instead act as a brake on spindle elongation.

In human cells, displacement of a pair of sister kinetochores from the rest of the spindle just before anaphase onset showed that kinetochores can segregate independently of attachment to one spindle pole, with antiparallel MTs sliding apart between them (Vukušić et al., 2017) (Fig. 2D). We found bridging fibers to be essential for the separation of displaced kinetochores, and continuous cutting of most interpolar MTs in the central spindle also decreased spindle elongation rates (Vukušić et al., 2017). In agreement with the latter result, a single-hit laser ablation of all interpolar MTs in human cells immediately stopped anaphase chromosome motion for a short period of time (Yu et al., 2019). This is reminiscent of the pioneering ablation experiments showing that chromosomes continue to move poleward after centrosome removal as long as the k-fiber stub is longer than 1 µm (Nicklas, 1989; Nicklas et al., 1982), as previously mentioned. We confirmed that ablation close to the kinetochore (less than a micron away) renders kinetochores incapable of separating (Vukušić et al., 2017), possibly due to disruption of the connection between the bridging and k-fiber given that they merge 1–2 µm away from the kinetochores (Kajtez et al., 2016) (Fig. 2B), suggesting that bridging MTs are sites of active force generation essential for proper chromosome segregation.

How is this force transmitted to spindle poles? Together, data from human cells revoke old ideas that interpolar MTs can slide apart and generate forces that can be transmitted along the k-fibers (Bělař, 1929; McIntosh et al., 1969; Östergren, 1951) consequentially exerting forces on poles to push them apart and elongate the spindle (Fig. 2B,C). Accordingly, interpolar MTs originate mostly away from the pole in human cells (Yu et al., 2019), arguing for pushing through k-fibers. The velocity of interpolar MT sliding is greater than the velocity of spindle elongation (2.1 µm/min and 1.3 µm/min, respectively; Fig. 2B) (Vukušić et al., 2017). Such dynamics are expected in a system with MT depolymerization at the minus-end, dissipating some of the force of sliding. Interestingly, in *Drosophila*, reduction of interpolar MT minus-end dynamics at anaphase onset is sufficient to induce spindle elongation (Wang et al., 2013), but the existence of this mechanism in human cells remains to be explored.

What is the importance of motor proteins driving spindle elongation? In permeabilized PtK1 cells, anaphase B requires ATP (Cande, 1982) and we speculate that the motor dependence of anaphase B can be applied to human cells as well. The main candidates involved in the generation of outward forces through sliding of interpolar MTs are plus-end directed motors (Fig. 2B): the kinesin-5 family protein Eg5 (Kapitein et al., 2005; Saunders et al., 1995; Straight et al., 1998) in cooperation with the kinesin-12 protein Kif15 (Tanenbaum et al., 2009; van Heesbeen et al., 2014), and the kinesin-6 protein MKLP1/Kif23 (Fu et al., 2009; Kruger et al., 2019; Nislow et al., 1992). However, inhibition of Eg5 and depletion of Kif15 did not affect sliding velocities in human cells in anaphase or spindle elongation velocities (Vukušić et al., 2017), similar to what is seen in *Drosophila* embryos (Brust-Mascher et al., 2009), although a large fraction of spindles collapsed in metaphase or showed delay in anaphase B onset. MKLP1, on the other hand, is involved in the regulation of midzone length in human cells (Hu et al., 2011), but has a minor effect on spindle elongation velocities, probably through regulation of the midzone stability (Vukušić et al., 2017). In conclusion, the anaphase B motors that drive sliding of antiparallel MTs in human cells are yet to be elucidated, where a promising approach may be testing multiple motors together as well as exploring additional candidates such as the kinesin-7 protein CENP-E (Kurasawa et al., 2004) and the kinesin-8 protein Kif18A (Stumpff et al., 2008; Su et al., 2013).

The role of astral MTs in force generation in anaphase

Spindle positioning

In most vertebrates, MTs emanate from spindle poles and grow towards the cell periphery (Fig. 3A). These astral MTs can be long enough to interact with proteins at the cell cortex, the actin layer

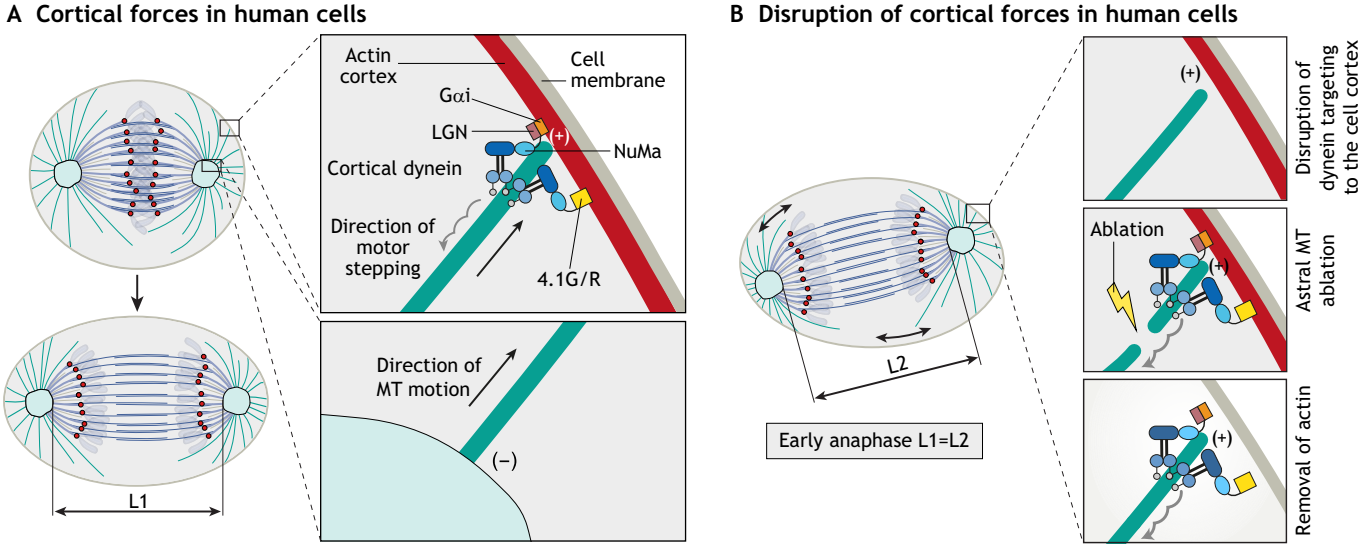

Fig. 3. Astral microtubules in force generation in anaphase. (A) Cortical forces are mediated by cortical dynein and its adaptors that connect astral MTs to the cell cortex (magnified region, top), and are transmitted to the spindle pole (magnified region, bottom). (B) Perturbation of cortical forces impacts spindle positioning and orientation (double-headed arrows indicate increased oscillatory movement) but does not affect spindle length at the end of early anaphase (denoted L1 and L2 in unperturbed and perturbed cells, respectively). Different perturbations of cortical force generation are depicted on the right. Spindle parts are color-coded as in Fig. 1.

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beneath the inner surface of the plasma membrane (Kotak, 2019). Not all cells have astral MTs (Bannigan et al., 2008; Dumont et al., 2010) and they seem to be non-essential for mitosis, but they contribute to spindle positioning in some systems, most notably the *C. elegans* embryo (Grill et al., 2003; Grill and Hyman, 2005). In human cells, at least in part, the position of the mitotic spindle is controlled by dynein-dependent cortical pulling forces exerted on astral MTs (Kiyomitsu and Cheeseman, 2012). Specifically, the dynein–dynactin complex is recruited to the cell cortex in anaphase by parallel pathways mediated by LGN (also known as GPSM2) and 4.1 family proteins, both of which interact with nuclear mitotic apparatus protein (NuMA) (Fig. 3A); disruption of both pathways through protein knockdown results in unequally sized daughter cells after cell division and defects in spindle centering relative to cell boundaries (Kiyomitsu and Cheeseman, 2013) (Fig. 3B).

Spindle elongation

In some organisms, there is evidence for external pulling forces on the spindle poles acting to elongate the spindle (Aist et al., 1993; Grill et al., 2001). Potential mechanisms could involve depolymerization of the plus-ends of astral MTs (Grishchuk et al., 2005) or pulling by motors that walk towards the minus-end of astral MTs (Ananthanarayanan et al., 2013; Pavin and Tolić-Nørrelykke, 2013), and it has been shown that dynein can participate in both mechanisms (Laan et al., 2012). This suggests a requirement for direct contact between astral MTs and the cell cortex (Kozlowski et al., 2007), although with possible exceptions, including anchoring to the cytoplasmic actin filament network as shown in cell-free *Drosophila* embryo extract (Telley et al., 2012).

In human cells, the amounts of cortical NuMA and dynein increase during transition from metaphase to anaphase, and the final spindle length at the end of anaphase is reduced after NuMA depletion (Kotak et al., 2013). However, whereas it is important for spindle positioning during anaphase, removal of both LGN and a 4.1 family protein does not affect rates of sister chromatid segregation in HeLa cells during early anaphase, but only during later stages (Kiyomitsu and Cheeseman, 2013) (Fig. 3B). In this case, dynein, NuMA and the dynactin subunit p150 (also known as DCTN1), which regulates dynein motility, are barely detectable at the cell cortex from the beginning of anaphase (Kiyomitsu and Cheeseman, 2013). In agreement, our laser ablation of astral MTs in U2OS cells did not slow down chromatid segregation and spindle elongation (Vukušić et al., 2017) (Fig. 3B) and there was no acceleration of spindle pole separation after cutting the spindle midzone region during early anaphase (Vukušić et al., 2017; Yu et al., 2019). Thus, in contrast to what is seen for *C. elegans* and PtK2 cells, where astral MTs drive spindle elongation (Aist et al., 1993; Grill et al., 2001), cortical pulling forces seem dispensable for fast spindle elongation and chromosome segregation during early anaphase in human cells.

In line with the above results, disruption of the actin cytoskeleton by actin-antagonizing drugs, which abolishes the astral MT connection to the cortex and thus forces on the spindle, does not affect spindle pole separation in human cells (Wheatley et al., 1997) (Fig. 3B). However, these treatments have an impact on spindle positioning, cytokinesis and cell elongation (Charnley et al., 2013; Fernandez et al., 2011). These results support the role of astral MTs in spindle positioning and argue against a force-generating role during the phase of fast spindle elongation in early anaphase, leaving the role of astral MTs in late human anaphase and telophase movements to be investigated (Kotak and Gönczy, 2014).

Conclusions and perspectives

Here, we discussed long-standing hypotheses and recent studies on the forces required for chromosome movements during human anaphase. Because the application of concepts derived from various model organisms to human anaphase is not straightforward, and multiple cooperating and opposing mechanisms may exist in different sub-phases, a complex and at times contradictory picture has emerged, even within different cells from the same organism.

Anaphase A poleward chromosome movement and anaphase B spindle elongation seem to be equally important contributors to chromosome segregation in human cells (Fig. 4). The nature of anaphase A is k-fiber depolymerization oriented and largely independent of motor proteins, therefore relying on intrinsic MT force production where MT-associated proteins of the kinetochore are either auxiliary couplers or regulators of this process. Depolymerization at the plus-end coupled to kinetochore currently appears to be the main contributor to anaphase A poleward movement (70%), whereas minus-end depolymerization coupled to the spindle pole contributes up to 30% (Vukušić et al., 2017) (Fig. 4). The details of the precise coupling mechanisms between kinetochores, MTs and spindle poles involving dynamic fibrillary supra-structures during anaphase A await further studies.

Anaphase B, in turn, seems mechanistically distinct from anaphase A and relies on active force generation by interpolar bridging MTs that are strongly crosslinked to k-fibers (Fig. 4). This crosslinking indicates that interpolar MTs could potentially regulate some aspects of anaphase A, but that remains to be tested. The observation of MT sliding in the antiparallel overlap region throughout anaphase suggests the involvement of plus-end-directed motor proteins and growth at the interpolar MT plus-ends. However, despite the knowledge about regulators of interpolar MTs, there is no evidence for motor proteins being directly involved in anaphase B in human cells to date. Finally, astral MTs seem to play a role in spindle positioning, whereas the impact on

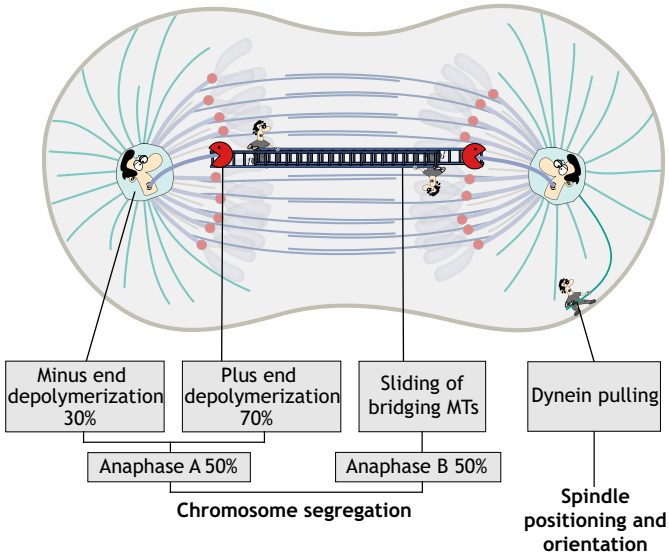

Fig. 4. Integrated model of force generation during early anaphase in human cells. Sliding of bridging MTs driving anaphase B, depicted as an extension ladder, and k-fiber depolymerization, driving anaphase A, contribute roughly equally to movement of chromosomes. K-fiber depolymerization occurs mainly at the plus-end, represented by the Pac-Man, and to a smaller extent at the minus-end, illustrated as a man with a straw. The percentages refer to the contribution to kinetochore movement. Dynein pulling on astral MTs regulates spindle positioning and orientation.

chromosome segregation is limited to later stages of mitosis (Fig. 4). We expect that future studies will reveal the identity of motors generating sliding of interpolar MTs, which are likely to act through redundant pathways as in yeast (Kruger et al., 2019). New experimental data should elucidate the complexity of anaphase by temporally distinguishing the contribution of different mechanisms of anaphase and focusing on potential partnerships between different motors and their regulators.

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Competing interests
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