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COMMENTARY

Building an integrated model of chromosome congression

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ABSTRACT

A universal feature of mitosis is that all chromosomes become aligned at the spindle equator – the halfway point between the two spindle poles – prior to anaphase onset. This migratory event is called congression, and is powered by centromere-bound protein machines called kinetochores. This Commentary aims to document recent advances concerning the two kinetochore-based force-generating mechanisms that drive mitotic chromosome congression in vertebrate cells: depolymerisation-coupled pulling (DCP) and lateral sliding. We aim to explore how kinetochores can 'read-out' their spatial position within the spindle, and adjust these force-generating mechanisms to ensure chromosomes reach, and then remain, at the equator. Finally, we will describe the 'life history' of a chromosome, and provide a working model for how individual mechanisms are integrated to ensure efficient and successful congression.

KEY WORDS: Congression, Kinetochore, Mitosis, Spindle

Introduction

One key problem faced by cells during mitosis is how to relocate chromosomes to the spindle equator and how to ensure this position is maintained until anaphase onset (Fig. 1). This question has captivated mitosis researchers for over half a century because the alignment of chromosomes and the formation of a metaphase plate is a universal feature of animal cells (Pereira and Maiato, 2012). To think about the problem of congression, it is important to consider what the end point of this process looks like: all sister chromatids (duplicated chromosomes) are positioned halfway between the two spindle poles, with sister kinetochores bound to microtubule bundles, the so-called kinetochore (K)-fibres that emanate from opposite spindle poles. This attachment state is termed 'amphitelic' or 'bi-orientated', and has all kinetochores on the equatorial plane of the spindle, termed the 'metaphase plate' (Fig. 1). In contrast, the start point appears chaotic, with the chromosomes distributed throughout the cell following breakdown of the nuclear envelope (Fig. 1). This Commentary aims to document recent advances with regard to the force-generating mechanisms that are used to physically move chromosomes to the spindle equator. These forces are largely dependent on kinetochores – multiprotein complexes that assemble on each sister chromatid and form both end-on and lateral attachments to spindle microtubules. The subunit composition of kinetochores, how they attach to microtubules and how the process of bi-orientation is regulated will not be a focus here, and the reader is referred to recent reviews on the subject (Foley and Kapoor, 2013; Godek et al., 2015; Cheeseman, 2014; Cheerambathur and Desai, 2014; Westhorpe and Straight, 2013).

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Kinetochores as the force generator

The original idea of how kinetochore pairs found the spindle equator was the so-called 'traction fibre' model, which hypothesised that chromosomes are pulled towards their associated pole with a force that is proportional to the length of the K-fibre (Östergren, 1951). The resulting 'tug-of-war' between sister kinetochores would autonomously mediate congression to the equator, as this is where forces are balanced. Despite some initial experimental evidence (Hays and Salmon, 1990; Hays et al., 1982; Östergren, 1945), experiments in the 1990s showed that this hypothesis is not correct (Czaban et al., 1993; Spurck et al., 1990; Khodjakov et al.,1997), and as a result, the kinetochore became the prime force-generating candidate. In this regard, microtubule labelling studies demonstrated that kinetochores are bound to the dynamic plus-ends of relatively static K-fibres, and changes in microtubule polymerisation and depolymerisation at the kinetochore underpin away-from-the-pole (AP, where a kinetochore moves away from its attached pole) and poleward (P, where a kinetochore moves towards its attached pole) motion (Cassimeris and Salmon, 1991; Centonze and Borisy, 1991; Mitchison et al., 1986; Mitchison and Salmon, 1992; Shelden and Wadsworth, 1992; Wise et al., 1991).

Direct evidence that kinetochore-generated pulling forces mediate congression came from laser ablation studies. Destruction of the P-moving kinetochore on a bi-orientated kinetochore-pair caused the chromosome to stop and pause, followed by the AP kinetochore switching to P motion. Additionally, severing of a congressing chromosome between the two kinetochores resulted in the P kinetochore to continue P migration, whereas the AP kinetochore halted and switched to P motion (Khodjakov and Rieder, 1996). More recently, studies have investigated intra-kinetochore dynamics during directed motion in Ptk1 cells by labelling inner- and outer-kinetochore components, and found that the P kinetochore is compressed relative to the AP kinetochore, further supporting the idea that the AP kinetochore forms a passive attachment to microtubules, whereas force generation originates at the P kinetochore (Dumont et al., 2012).

Two mechanisms for force generation by the P kinetochore were originally proposed: (1) that kinetochores induce depolymerisation of the plus-ends of K-fibre microtubules, which allows the pulling of chromatids to the pole through maintaining attachment to the shortening microtubule – termed 'Pac-man' (Gorbsky et al., 1987; Mitchison et al., 1986), and (2) that microtubule minus-end depolymerisation plus poleward motor-driven sliding of microtubules pulls the attached kinetochores poleward – termed 'poleward flux' (Mitchison, 1989). Work in *Drosophila* embryos concluded that 'Pac-Man' is the principal generator of pulling force during anaphase A (Brust-Mascher and Scholey, 2002; Rogers et al., 2004). In addition, an 80% reduction in poleward flux in human cells had no effect on congression (Ganem et al., 2005), and kinetochore velocity during congression is faster than flux in both grasshopper spermatocytes and HeLa cells (Pereira and Maiato, 2012; Skibbens et al., 1993; and our unpublished data). From here on, we use the term

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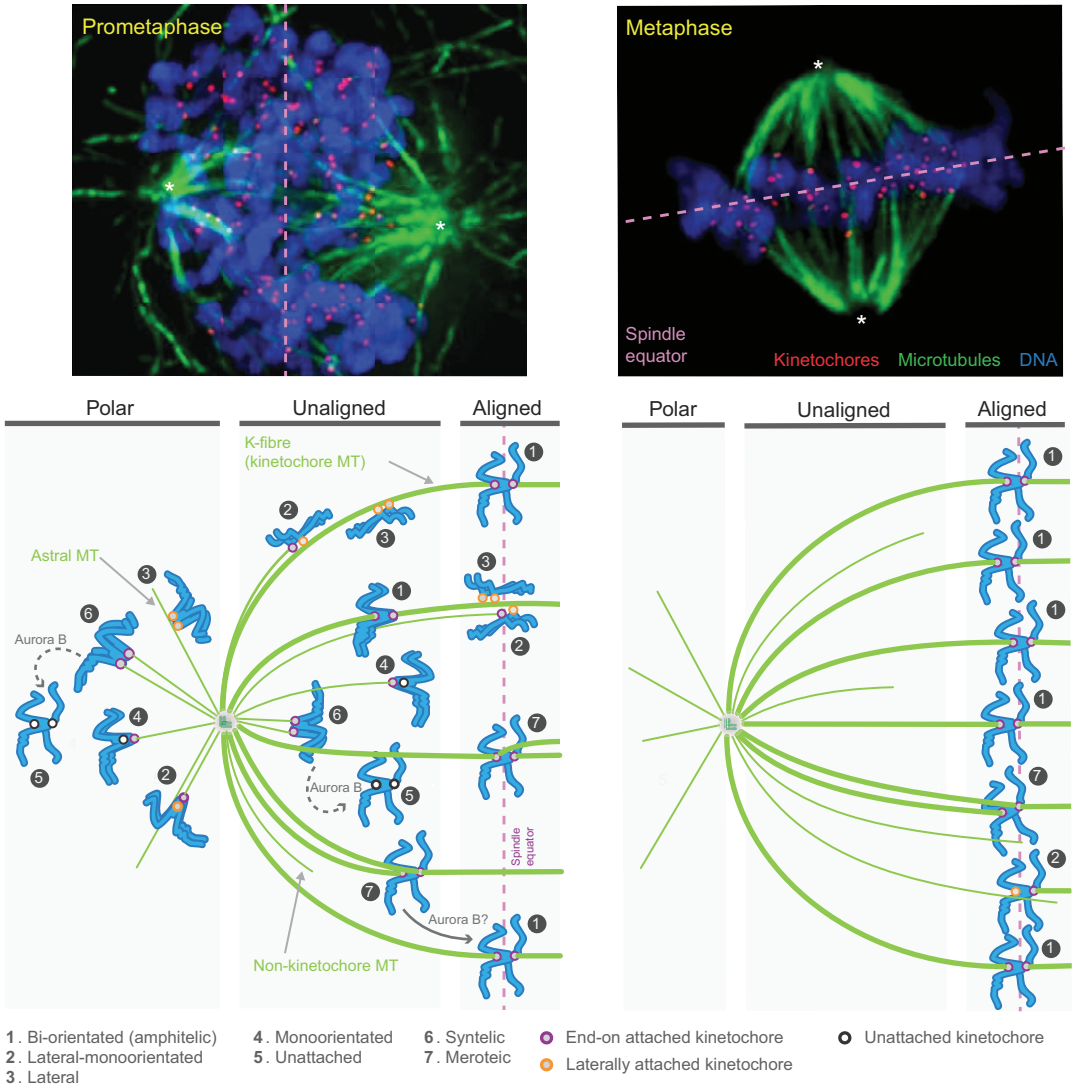

Fig. 1. Chromosome states during prometaphase and metaphase. Top left, immunofluorescence image of a prometaphase HeLa cell stained for kinetochores (CENP-A, red), microtubules (α-tubulin, green) and DNA (DAPI, blue). Chromosomes are distributed throughout the early spindle, and a subpopulation is already positioned at the spindle equator (dotted line), which is located halfway between the two spindle poles (asterisks). Bottom left, cartoon representation of the various spindle positions and attachment states occupied by prometaphase chromosomes. Chromosomes are categorised as polar if they are located behind the pole within the astral region, unaligned if they are located between the pole and equator, and aligned if they are positioned at the equator. Within these regions, kinetochores attach to spindle microtubules in numerous orientations; (1) bi-orientated (amphitelic), where sister kinetochores are bound to opposite spindle poles, (2) lateral-monoorientated, where one kinetochore in the sister pair is attached end-on and the other is bound to the wall of a pre-existing microtubule bundle, (3) lateral, where both sister kinetochores are bound to the wall of a pre-existing microtubule bundle, (4) monoorientated, where one sister kinetochore is attached end-on and the other unattached, (5) unattached, where both sister kinetochores are unbound, (6) syntelic, where both sister kinetochores are attached end-on to a single pole, (7) merotelic, where one sister in a bi-orientated pair forms a second attachment to its distal pole. These erroneous attachments (6 and 7) are actively destabilised by Aurora B, enabling reattachment in a conformation that permits congression. Top right, immunofluorescence image of a metaphase HeLa cell. Bottom right, cartoon representation of the position and attachment states of metaphase chromosomes. Kinetochore pairs are located at the spindle equator and are predominantly bi-orientated (1); however, merotelic (7) and lateral-monoorientated (2) attachments can persist within this region at a low frequency (Cimini et al., 2003; Magidson et al., 2011). Asterisks in the images denote the position of the spindle poles.

depolymerisation-coupled pulling (DCP) to describe the dominant ‘Pac-Man’ mechanism.

Molecular mechanisms of DCP

Three criteria must be satisfied for a pair of sister kinetochores to move by DCP: (1) kinetochores need to form stable end-on attachments to K-fibres, (2) kinetochores have to maintain physical coupling to depolymerising microtubules, and (3) sister kinetochores must ensure that the K-fibres attached to the P and AP kinetochores are in a depolymerising and polymerising state, respectively (polymerisation bias) (Fig. 2A–C).

Physically coupling to depolymerising microtubules

Force generation by the physical coupling of a kinetochore to a depolymerising microtubule is thought to depend on structural changes at the microtubule plus-end. When the GTP cap of a polymerising microtubule is lost and the microtubule undergoes catastrophe, individual protofilaments peel away from the lattice in a ‘rams-horn’ like conformation (Simon and Salmon, 1990). This bending can generate a predicted force of 65 pN (Desai and Mitchison, 1997; Grishchuk et al., 2005); however, early observations from grasshopper spermatocytes suggested that the force that can be generated per kinetochore microtubule during

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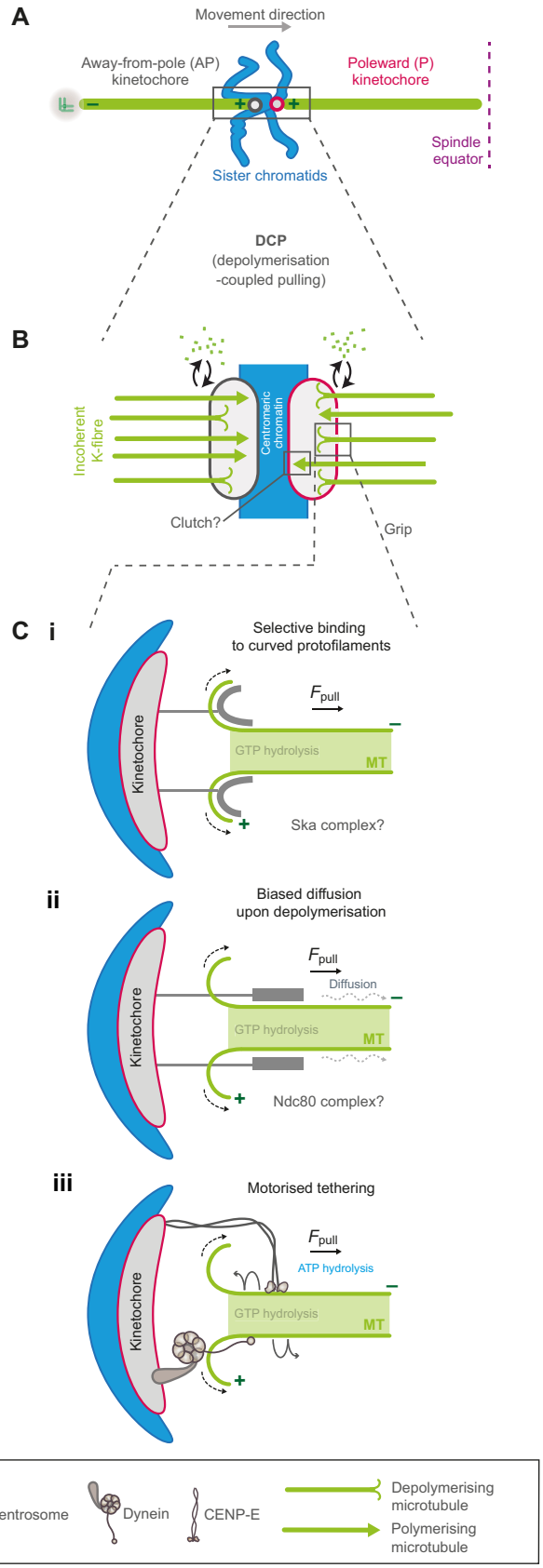

Fig. 2. Mechanics of depolymerisation-coupled pulling. (A) Chromosomes that form amphitelic attachments away from the spindle equator utilise depolymerisation-coupled pulling (DCP) to congress. During this movement, kinetochores are described as moving poleward (P) if they move towards their attached pole (shown in red), and away-from-the-pole (AP) if moving away from their attached pole (shown in dark grey). (B) The force required for DCP is generated by microtubule depolymerisation at the P kinetochore, which pulls the chromosome towards the equator. This implies that there is a polymerisation or depolymerisation bias between K-fibres that are attached to the P and AP kinetochores, with P K-fibres in a net depolymerising state and AP K-fibres in a net polymerising state. Owing to the incoherent nature of the K-fibre, the P kinetochore might selectively disengage from polymerising microtubules using a clutch-like mechanism. (C) Three potential mechanisms have been proposed for how kinetochores grip depolymerising microtubules (see main text for details): (i) selective binding to curved protofilaments, a unique structural feature of depolymerising microtubules; (ii) biased diffusion along the microtubule lattice upon depolymerisation; and (iii) motorised tethering in which kinetochore-tethered kinesin (top) and/or dynein (bottom) step along the microtubule. MT, microtubule.

congression is only ~7 pN (Nicklas, 1988), which is similar to the calculated value for centromere stretching in budding yeast (Powers et al., 2009). Regardless of its magnitude, this force acts in the P direction and could be utilised by kinetochores to mediate the pulling of chromosomes (Fig. 2A–C). Evidence that kinetochores might indeed directly bind to curved protofilaments in order to harness energy from microtubule depolymerisation to generate a pulling force (Fig. 2C) came from electron microscopy studies of kinetochores in cells, which revealed the existence of fibrils that connect curved protofilaments with the inner kinetochore (McIntosh et al., 2008). A major current effort of kinetochore research is to identify the proteins that mediate this coupling. In this regard, work in fungi has described the Dam1 complex, which forms force coupling oligomeric rings around microtubules *in vitro* (Grishchuk et al., 2008; Miranda et al., 2005; Umbreit et al., 2014; Westermann et al., 2005) and is required for kinetochore–microtubule attachment *in vivo* (Cheeseman et al., 2001; Umbreit et al., 2014). In vertebrates, it has been suggested that the spindle- and kinetochore-associated (Ska) complex, consisting of Ska1, Ska2 and Ska3 (also known as RAMA1), might be a functional homologue of budding yeast Dam1. This view has emerged because Ska can autonomously track depolymerising microtubule plus-ends *in vitro* and transduce this force to a polystyrene bead (Fig. 2C) (Schmidt et al., 2012; Welburn et al., 2009). Moreover, the Ska complex demonstrates an equal binding preference for both curved and straight protofilaments, unlike the Ndc80 complex, which almost exclusively binds straight lattice configurations (Schmidt et al., 2012). In agreement, structural work has demonstrated that the Ska complex binds unique tubulin domains that do not alter their accessibility in response to depolymerisation-induced conformational changes, whereas the Ndc80-complex-binding site is obscured during depolymerisation (Abad et al., 2014; Alushin et al., 2010). Thus, the Ska complex represents the current best *in vitro* candidate for a molecule that directly binds protofilaments as they dissemble. Nevertheless, the *in vivo* functions of the Ska complex are somewhat unclear: initial studies reported either a prolonged mitotic delay with a few misaligned kinetochores (Abad et al., 2014; Hanisch et al., 2006; Jeyaprakash et al., 2012; Theis et al., 2009) or a more severe congression defect and associated changes in the stability of microtubule–kinetochore attachment (Chan et al., 2012; Gaitanos et al., 2009; Schmidt et al., 2012; Welburn et al., 2009). The latter phenotype is most likely due to cohesion fatigue (the asynchronous separation of sister chromatids), which occurs downstream of a prolonged metaphase delay (Daum et al., 2009; Sivakumar et al., 2014). Nevertheless, all studies agree that the Ska complex is required for normal and efficient chromosome congression, albeit to varying degrees. These

studies also point to the need to move away from describing generic phenotypes such as 'congression' problems and instead defining the precise alterations in chromosome dynamics that are associated with the depletion of a particular protein to a specific extent. Such rationale was successfully used for the investigation of CENP-Q and CENP-E, where analysis of prometaphase chromosome subgroups, identified by spindle position and orientation, highlighted key roles for these proteins in distinct steps of congression (Bancroft et al., 2015; Barisic et al., 2014). In addition to Ska, CENP-F has now emerged as a second potential candidate that can directly bind to curved protofilaments and generate force (Volkov et al., 2015). *In vitro*, CENP-F displays a preference for curved microtubule configurations, can track the plus-ends of depolymerising microtubules and transduce filament disassembly to the movement of beads coated in a truncated version of the protein (Volkov et al., 2015). Its function *in vivo*, however, is again less clear. CENP-F has been implicated in the recruitment of the key congression factors CENP-E and dynein (Bomont et al., 2005; Vergnolle and Taylor, 2007; Yang et al., 2005), although the former is disputed (Feng et al., 2006). Nevertheless, CENP-F depletion leads to a severe congression defect (Bomont et al., 2005; Feng et al., 2006; Holt et al., 2005; Yang et al., 2005) and is suggested to perturb K-fibre stability and the establishment of tension across aligned kinetochore pairs (Bomont et al., 2005; Feng et al., 2006; Holt et al., 2005). Thus, CENP-F potentially represents another attachment and/or force-coupling factor that specifically associates with curved microtubule structures.

Microtubule disassembly also generates an energy gradient from the depolymerising tip to the remaining lattice, which could be utilised by molecules that can diffuse along the microtubule lattice to maintain attachment during depolymerisation, and thus generate a pulling force (Hill, 1985; Vladimirou et al., 2011) (Fig. 2C). For instance, the Ndc80 complex (Ndc80–Nuf2–Spc24–Spc25) directly binds to microtubules *in vitro* through multiple interaction surfaces (Alushin et al., 2012, 2010; Miller et al., 2008; Tooley et al., 2011). Loss of this complex in all systems tested results in a complete failure of end-on microtubule attachment at kinetochores (Cheeseman and Desai, 2008). However, this does not implicate Ndc80 in DCP per se, as it might function exclusively as an attachment factor. Nevertheless, *in vitro* reconstitution of the complete Ndc80 complex has revealed that it can persistently attach to dynamic microtubules and transduce filament disassembly to the movement of beads coated with physiological concentrations of the complex (Powers et al., 2009). Moreover, individual Ndc80 complexes undergo one-dimensional diffusion along taxol-stabilised microtubules (which prevents depolymerisation) as a result of transient electrostatic interaction (Powers et al., 2009). Upon encountering microtubule-bound Ndc80 complexes, the energy gradient generated by the depolymerising tip is predicted to favour diffusion of the complex along the lattice as opposed to their detachment (biased lattice diffusion). This is equivalent to the behaviours predicted for a 'Hill's sleeve', a theoretical model for how a kinetochore could maintain attachment to depolymerising microtubules (Hill, 1985). In accordance with this prediction, Ndc80 complex ensembles display tip tracking *in vitro,* and individual complexes have been observed to bounce off the depolymerising tip back onto the lattice without dissociating (Powers et al., 2009). However, it has been shown that a truncated monomeric Ndc80 complex (consisting of the head domains and short adjacent coiled-coil of Nuf2 and Ndc80) cannot track the depolymerising end of microtubules (Schmidt et al., 2012). As vertebrate kinetochores contain at least nine copies of Ndc80 complex per kinetochore–microtubule (Johnston et al., 2010), which can self-assemble into oligomeric arrays (Alushin et al., 2010), the Ndc80 complex potentially functions as a higher-order structure that utilises biased lattice diffusion to maintain attachment upon microtubule depolymerisation.

In summary, the Ndc80 complex and Ska complex or CENP-F likely contribute to DCP in differing ways. The Ndc80 complex forms an initial K-fibre attachment and, once it is bound, both Ndc80 and Ska complexes act in concert to track the depolymerising microtubule plus-end through lateral diffusion and direct protofilament binding, respectively (Fig. 2C). CENP-F potentially contributes to this pathway through a mechanism reminiscent of that used by the Ska complex; however, more work is required to demonstrate this *in vivo*. Moreover, these pathways are partially interconnected, as Ndc80 mediates recruitment of the Ska complex to kinetochores (Hanisch et al., 2006) and the Ska complex confers plus-end tracking properties on monomeric Ndc80 complex *in vitro* (Schmidt et al., 2012). This is functionally similar to how the Dam1 complex acts as a processivity factor for the Ndc80 complex by enhancing its ability to form load-bearing attachments to dynamic microtubule plus-ends *in vitro* (Lampert et al., 2010; Tien et al., 2010). Nevertheless, thus far, there is no direct *in vivo* evidence that both the Ska and Ndc80 complex drive DPC on congressing chromosomes, and specific Ndc80 mutants will be required to determine whether the complex contributes to DCP independently from its function in attachment.

Coordinating microtubule dynamics

Chromosome movement by DCP also requires a bias in microtubule polymerisation and depolymerisation between the K-fibres that are attached to AP and P kinetochores (Fig. 2A,B). Experiments using fluorescent EB1 (also known as MAPRE1, a microtubule polymerisation marker) provided evidence that this bias is indeed strong, with high levels of polymerisation at the AP kinetochore relative to the P kinetochore (Tirnauer et al., 2002). However, recent live-cell imaging in human cells (Armond et al., 2015) and electron microscopy studies (VandenBeldt et al., 2006) has demonstrated that both P and AP K-fibres are incoherent, i.e. contain both polymerising and depolymerising microtubules, with a small polymerisation bias towards the AP K-fibre (Armond et al., 2015) (Fig. 2B). In agreement, earlier microtubule labelling studies have shown that both metaphase kinetochores incorporate tubulin (Mitchison et al., 1986). Therefore, the P kinetochore must be able to prevent polymerising microtubules from generating a pushing and/or resistive force, which could impede movement. To achieve this, kinetochores could selectively disengage from polymerising microtubules (Fig. 2B), and/or trigger the switching of polymerising microtubules into a depolymerising state. As this could lead to the entire fibre switching to depolymerisation, the kinetochore must tightly control microtubule dynamics to maintain the observed incoherent state. In this regard, we know of several plus-end-tracking proteins and molecular motors that function as regulators of kinetochore–microtubule dynamics (Cross and McAinsh, 2014; Ferreira et al., 2014), but so far only the kinesin-8 Kif18A and kinesin-13 mitotic centromere-associated kinesin (MCAK, also known as Kif2C) have been shown to directly affect the balance of microtubule dynamics within the K-fibre (Armond et al., 2015). Kif18A is present in a comet-like gradient along kinetochore microtubules that is concentrated at the outer plate and proposed to be dependent on K-fibre length (Stumpff et al., 2008, 2012). Its depletion in mammalian cells leads to a severe

chromosome alignment defect (Mayr et al., 2007), although the misaligned chromosomes are able to establish bi-orientated attachments that are under tension (Stumpff et al., 2008, 2012). Kinetochore-tracking experiments have shown that Kif18A depletion causes an increase in metaphase sister-kinetochore oscillation velocity (Jaqaman et al., 2010; Stumpff et al., 2011, 2008, 2012), a decrease in switching frequency (Stumpff et al. 2008; although that observation is disputed, Jaqaman 2010), and loss of spatially controlled directional switching (Stumpff et al., 2012). These aberrant chromosome movements might reflect the reported increase in the microtubule polymerisation bias between AP and P kinetochores (Armond et al., 2015), a finding that is consistent with the idea that Kif18A normally acts to suppress microtubule dynamics (at the AP kinetochore) and stimulate directional switching *in vivo* (Du et al., 2010; Stumpff et al., 2008, 2012).

MCAK, unlike Kif18A, is a pure microtubule catastrophe factor (Hunter et al., 2003). *In vitro* investigation of MCAK has suggested that it also acts as a force coupler, as MCAK-coated beads can produce tension at both microtubule ends (Oguchi et al., 2011). *In vivo*, MCAK localises to both the centromere and outer kinetochoral plate, where microtubule plus-ends terminate, and demonstrates a bias to the P (leading) kinetochore on a congressing chromosome (Honnappa et al., 2009; Kline-Smith et al., 2004). Selective perturbation of MCAK at the kinetochore, achieved by using a dominant-negative fusion construct as well as small interfering RNA (siRNA)-mediated depletion, gives rise to congression defects (Kline-Smith et al., 2004; Shrestha and Draviam, 2013). Moreover, kinetochore tracking has revealed that the oscillation dynamics of aligned kinetochores are slightly dampened, with sisters displaying a decrease in directional coordination and a reduction in kinetochore velocity (Jaqaman et al., 2010; Wordeman et al., 2007). These MCAK-depleted kinetochores have a reduced AP polymerisation bias, implying that the K-fibre that is bound to the P kinetochore (which is mediating DCP) has fewer depolymerising microtubules. Thus, MCAK is likely to function at the P kinetochore to promote depolymerisation and increase DCP drive forces (Armond et al., 2015)

Some caution is necessary here because much work on the dynamics of AP and P kinetochore movements is derived from analysis of aligned kinetochores within the metaphase plate and applying this to congressing chromosomes might not be appropriate. Indeed, the duration of AP movements are considerably longer during congression [~2 min compared to 10–60 s in metaphase (Jaqaman et al., 2010; Khodjakov et al., 1999)], and the molecular composition of unaligned and aligned kinetochores is known to differ (Gudimchuk et al., 2013; Kline-Smith et al., 2004; Schmidt et al., 2010; Magidson et al., 2015). Investigation into the dynamics and composition of congressing kinetochores will therefore be necessary if we are to fully understand the mechanics and mechanisms that underpin DCP. For example, by tracking the fates of unaligned kinetochores, CENP-Q, a component of the constitutive centromere associated network (CCAN), has been shown to be specifically required for DCP, but not for attachment (Bancroft et al., 2015). CENP-Q is a subunit of the CENP-O complex [CENP-O, CENP-P, CENP-Q, CENP-U; the orthologue of the budding yeast COMA complex (De Wulf et al., 2003)] (Hori et al., 2008), which can directly bind microtubules *in vitro* (Amaro et al., 2010) and mediates K-fibre turnover *in vivo* (Bancroft et al., 2015). Bi-orientated kinetochores stall in the absence of CENP-Q, failing to migrate towards the metaphase plate, highlighting that end-on microtubule attachment does not guarantee movement and implicating the CENP-O complex in a force-generating step of DCP.

In summary, the antagonistic regulation of microtubule dynamics is key to controlling the movement of bi-orientated kinetochore pairs, and these processes form the basis for imparting directional bias during congression (discussed below).

Congression in the absence of end-on pulling

Lateral sliding

Congression through DCP assumes that all kinetochore pairs form bi-orientated attachments prior to migration towards the spindle equator. By contrast, congression can also take place before bi-orientation, with kinetochores sliding along the lattice of spindle microtubules powered by molecular motors (Fig. 3). Early in mitosis, chromosomes that are located behind the pole (or outside the spindle) initially move poleward before migrating to the spindle equator. This movement is driven by dynein motors that step towards microtubule minus-ends located at the pole (Barisic et al., 2014; Li et al., 2007; Savoian et al., 2000; Yang et al., 2007) (Fig. 3, steps 1 and 2). Subsequent AP-directed sliding movements (and congression) are driven by CENP-E, a highly processive plus-end-

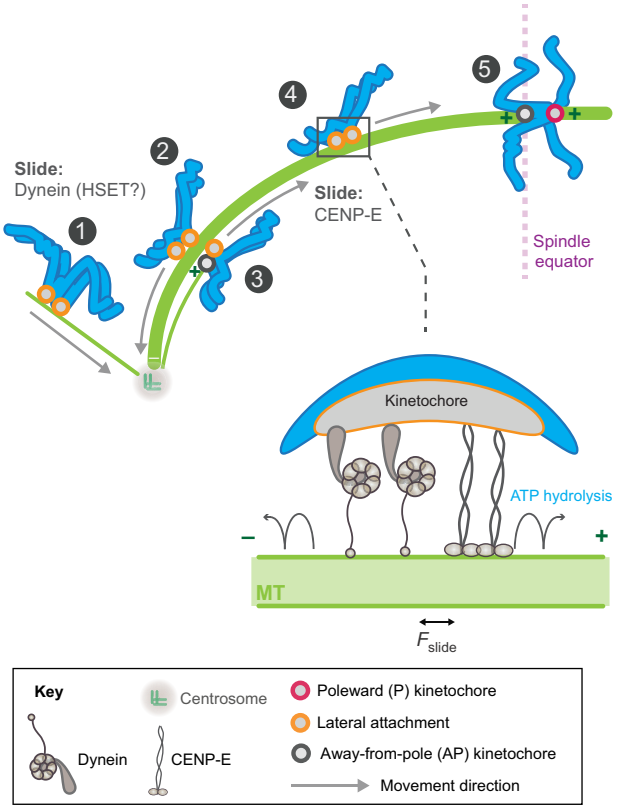

Fig. 3. Congression of laterally attached kinetochores. Congression can take place prior to bi-orientation, mediated by the motor proteins dynein and CENP-E. These motors form lateral attachments with the microtubule lattice, and walk along the microtubule toward the minus-end (dynein, steps 1 and 2) or plus-end (CENP-E, steps 3 and 4). HSET has also been implicated in minus-end-directed polewards movement, however the mechanism remains unknown. These migratory events contribute to distinct steps of congression, with chromosomes first being moved polewards by dynein (steps 1 and 2), before being ejected towards the equator by CENP-E (steps 3 and 4). Motor-driven congression often fails to maintain chromosomes at the spindle equator, and as such, these lateral attachments are converted into the default end-on attachment state during the formation of the metaphase plate (step 5). MT, microtubule.

directed motor (Gudimchuk et al., 2013; Kim et al., 2008; Wood et al., 1997) (Fig. 3, steps 3 and 4). Direct evidence that CENP-E facilitates lateral sliding of chromosomes to the spindle equator came from a study that combined kinetochore tracking with electron microscopy (Kapoor et al., 2006). There, it was demonstrated that monoorientated kinetochore pairs could laterally attach to the mature K-fiber of an aligned chromosome and that this laterally attached kinetochore could generate a plus-end-directed pulling force that facilitated congression (Kapoor et al., 2006) (Fig. 3, step 3). This behaviour was abolished by CENP-E depletion, establishing that CENP-E powers the congression of chromosomes by a mechanism that is independent of bi-orientation (Kapoor et al., 2006). Further validation of this model came from the analysis of cells in which K-fibres were removed by abolishing kinetochore–microtubule attachment through depletion of Nuf2 (a component of the Ndc80 complex), and therefore preventing DCP (Cai et al., 2009). Remarkably, when the minus-end-directed kinesin-14 HSET (also known as KifC1) was co-depleted with Nuf2, half the chromosomes were able to align through CENP-E-mediated lateral sliding (Cai et al., 2009). Similar CENP-E-dependent congression was observed when the microtubule depolymerase MCAK was co-depleted with Ndc80, an essential subunit of the microtubule-binding Ndc80 complex (Iemura and Tanaka, 2015). Thus, both MCAK and HSET depletion were able to suppress an unknown P force, and allow for CENP-E-mediated congression in the absence of end-on attachment, but how? The current idea is that HSET and MCAK do not generate a P force at non-bi-orientated kinetochores per se, but indirectly prevent congression by decreasing the stability of spindle microtubules that are required for CENP-E stepping towards the spindle equator (Iemura and Tanaka, 2015).

Surprisingly, CENP-E has also been implicated in the DCP mechanism itself. In an assay designed to simulate DCP *in vivo*, transport of end-on attached chromosomes to a monopole was perturbed by small-molecule-mediated inhibition of CENP-E (Gudimchuk et al., 2013), suggesting that CENP-E is required for DCP (Fig. 2C). *In vitro* experiments have demonstrated that CENP-E can track with both the polymerising and depolymerising ends of microtubules and can couple depolymerisation with bead motion (Gudimchuk et al., 2013). However, 80% of unaligned bi-orientated kinetochore pairs successfully congressed by DCP when they were depleted of CENP-E (Bancroft et al., 2015). The remaining 20% might require CENP-E for DCP, although this motor is clearly not required for the vast majority of DCP events. It has also been reported that CENP-E depletion does not affect the movement of aligned sister kinetochores (Jaqaman et al., 2010). However, the dynamics of kinetochore microtubules (Maffini et al., 2009) are affected when CENP-E is inhibited or depleted after kinetochores have aligned at the spindle equator – a time when CENP-E levels are reduced. Clearly, more work is needed to tease out how exactly CENP-E motors operate at end-on attached kinetochores.

The polar ejection force

This kinetochore-centric view of congression assumes that attachments through the kinetochore (lateral or end-on) are the only drivers of congression. Although DCP and lateral sliding are crucial processes, kinesins that are associated with chromosome arms (chromokinesins) also play a key role in congression. The idea that a half spindle microtubule array could generate an AP force was first proposed by Darlington, who suggested that the arrangement of chromosomes at the metaphase plate was “due to repulsion from the poles acting on the centromeres”, and that the strength of this repulsion was inversely proportional to distance from the pole (Darlington, 1937). At the spindle equator the repulsion from opposite poles is equal, resulting in the positioning of chromosomes in this region. The existence of the polar ejection force (PEF) was later demonstrated by severing chromosomes in living prometaphase cells with a laser microbeam. Once severed, chromosome fragments that lack a kinetochore were actively transported away from their proximal pole (Rieder et al., 1986). This behaviour could be abolished by the addition of nocodazole (that depolymerises microtubules), suggesting that the presence of microtubules is required for the generation of the PEF (Ault et al., 1991). Based on *in vitro* and *in vivo* studies, it was later demonstrated that the plus-end-directed chromokinesins Kid (also known as Kif22) and Kif4a contribute to the PEF (Antonio et al., 2000; Bieling et al., 2010; Brouhard and Hunt, 2005; Funabiki and Murray, 2000; Levesque and Compton, 2001; Yajima et al., 2003). Thus, the PEF is generated by the chromosome-arm-associated kinesins that walk towards the plus-ends of microtubules located at the spindle equator, therefore generating an AP force. As microtubule concentration is proportional to proximity to the pole, the strength of the PEF is inversely proportional to distance from a pole (Cane et al., 2013). Individually, depletion of Kid or Kif4a has only a minor effect on congression; however, co-depletion results in substantial alignment defects (Wandke et al., 2012), suggesting that these motors have independent and/or coordinated functions. In line with this, Kid, the principal PEF generator, functions to position chromosome arms and control oscillation dynamics, but is not required for metaphase plate formation (Levesque and Compton, 2001; Wandke et al., 2012) unless co-depleted with CENP-E (Iemura and Tanaka, 2015). Moreover, Kid operates in concert with Kif18A to position bi-orientated chromosomes near the spindle equator by inducing position-dependent kinetochore tension, which increases the probability of directional switching towards the equator (Fig. 4C) (Stumpff et al., 2012). Interestingly, despite having a synergistic relationship, Kid and Kif18A have opposite effects on kinetochore-movement parameters, with Kid increasing kinetochore velocity and reducing the rate of directional switching (Stumpff et al., 2012; Wandke et al., 2012). In contrast to Kid, the function of Kif4a is less well defined. It has been shown that Kif4a antagonises Kid and controls non-kinetochore microtubule dynamics through suppression of polymerisation during early mitosis (Stumpff et al., 2012; Wandke et al., 2012). This suggests that it has a PEF-independent function, which could involve Kif4a regulation of antiparallel microtubule overlaps – a well-documented function in cytokinesis (Nunes Bastos et al., 2013). Nevertheless, numerous studies have implicated Kif4a and kinesin-4 motors in PEF generation (Antonio et al., 2000; Muzmumdar et al., 2004; Bieling et al., 2010; Brouhard and Hunt, 2005; Funabiki and Murray, 2000; Levesque and Compton, 2001; Yajima et al., 2003). In summary, despite individually being dispensable for mitosis, the coordinated function of Kid and Kif4a is important for the fidelity of chromosome segregation.

Coupling congression with position-sensing in the spindle

How do kinetochores sense their position within the spindle and feedback this information to regulate kinetochore-force-generating mechanisms? Recent advances have begun to illuminate mechanisms that could bias the migration of bi-orientated kinetochores to the spindle equator (Fig. 4). One such mechanism is the antagonistic regulation of microtubule dynamics by Kif18A and MCAK (see above). Here, an unaligned bi-orientated chromosome accumulates MCAK on the P (lead) kinetochore (Kline-Smith et al., 2004), thereby enhancing microtubule

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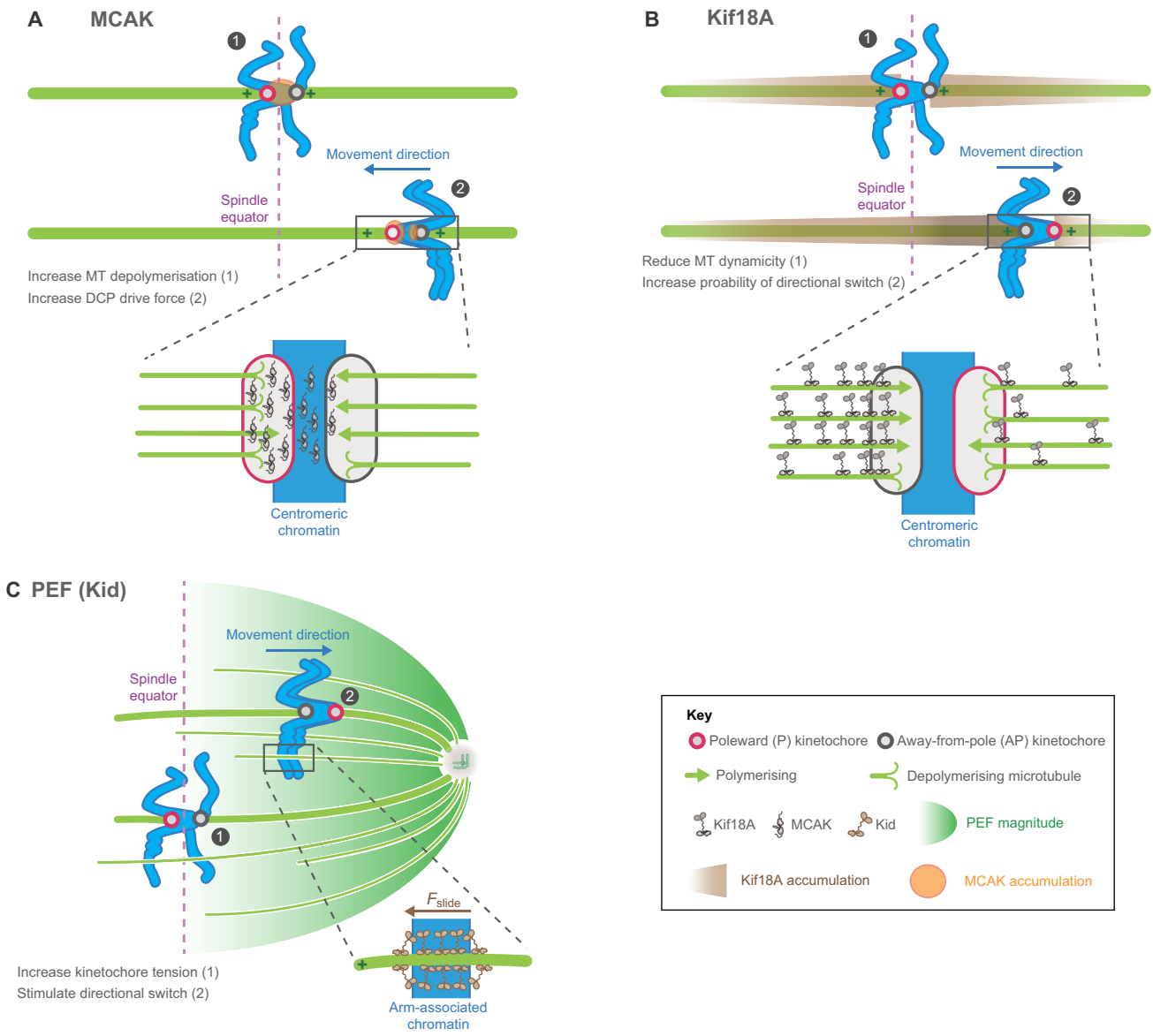

Fig. 4. Biased congression towards the spindle equator. (A) Role of MACK in biased congression. At aligned kinetochore pairs (1), the microtubule depolymerase MCAK localises to the inner-centromere. In contrast, at unaligned kinetochore pairs undergoing congression (2) MCAK displays a bias to the P kinetochore, suggesting that it might augment the DCP-mediated drive force and enhance migration towards the spindle equator. (B) Kif18A forms a concentration gradient along the K-fibre (illustrated by the brown 'cloud') and accumulates at the outer-kinetochore. Here, it acts to suppress microtubule dynamics and promote directional switching. At aligned kinetochore pairs, its concentration is comparable between sisters and so it promotes maintenance of this position (1); however, at unaligned kinetochore pairs, Kif18A promotes switching of the AP kinetochore into a P state, resulting in its migration towards the spindle equator (2). (C) The PEF is generated by plus-end-directed kinesins that are associated with chromosome arms (for instance Kid), which interact with spindle microtubules and walk towards the spindle equator (directionality indicated by F_{slide}). This force therefore decays with increasing distance from the pole and reducing microtubule density. Aligned kinetochore pairs are positioned equidistantly from either pole and therefore experience a more symmetrical PEF (1). Unaligned kinetochore pairs, however, experience an increase in PEF as they move towards a pole, which increases kinetochore tension and promotes their directional switching towards the equator (2). MT, microtubule.

depolymerisation and causing the chromosome to move at increased velocity towards the spindle equator, similar to the effect seen at aligned kinetochore pairs (Jaqaman et al., 2010) (Fig. 4A). Once at the equator, MCAK is unloaded from the kinetochores (Kline-Smith et al., 2004), presumably reducing the P force and favouring a positional equilibrium. In parallel, the length-dependent accumulation of Kif18A on the AP-attached K-fibre acts to constrain the amplitude of migratory events by increasing the probability of an AP-to-P switch as the kinetochore moves away from the equator (Fig. 4B) (Stumpff et al., 2012). Here, the increasing Kif18A concentration on the AP-attached K-fibre suppresses microtubule dynamics (Stumpff et al., 2011; Du et al., 2010) such that the GTP cap might eventually be compromised, leading to the observed switch in plus-end dynamics. Once at the spindle equator, the difference in Kif18A on both sister kinetochores is reduced, resulting in a comparable suppression of microtubule dynamics at both kinetochores and the maintenance of their position at the metaphase plate through low-amplitude oscillation. Thus, Kif18A senses K-fibre length, whereas MCAK senses kinetochore position. Recently, research in mice has questioned the importance of this Kif18A-dependent equatorial positioning mechanism (Czechanski et al., 2015). Here, mice

homozygous for a Kif18A motor domain mutation (Kif18A^{gcd2}) were overtly normal with the exception of sterility, in agreement with previous studies of Kif18A$^{-/-}$ mice (Liu et al., 2010). However, unlike HeLa cells expressing the mutant Kif18A, Kif18A^{gcd2} mouse embryonic fibroblasts (MEFs) successfully progressed through mitosis, entering anaphase with unaligned chromosomes (Czechanski et al., 2015). One interpretation of this finding is that the absolute positioning of chromosomes at the spindle equator might not be necessary, although it remains to be tested whether sister kinetochores are in fact correctly bi-orientated and that the metaphase plate is simply broader.

It is also possible that concentration gradients of other factors within the spindle influence chromosome movement. It has been established that a gradient of Plk1 and Ran-GTP concentrations control spindle position (Kiyomitsu and Cheeseman, 2012), and Aurora A forms a spatial gradient that originates at the spindle pole (Ye et al., 2015; Hochegger et al., 2013). Abrogation of the Ran-GTP gradient that is established around aligned chromosomes using a dominant-negative RanT24N mutant has no effect on congression (Barisic et al., 2015). In contrast, the Aurora kinases and Plk1 are known to regulate key microtubule attachment proteins and feedback mechanisms (Chan et al., 2012; Ems-McClung et al., 2013; Godek et al., 2015; Hochegger et al., 2013; Kettenbach et al., 2011; Kim et al., 2010; Lampson and Cheeseman, 2011; Park et al., 2015), thus providing a potential mechanism for the regulation of kinetochore composition and/or activity during congression. So far, the best-documented example is Aurora-A-mediated phosphorylation of CENP-E, which is dependent upon the proximity of the motor to Aurora A at the spindle pole (Kim et al., 2010). Phosphorylation of CENP-E by Aurora A regulates the affinity of the motor for microtubules, and is predicted to bias CENP-E to walk along K-fibres towards the spindle equator. Nevertheless, CENP-E-mediated congression does not absolutely require K-fibres (Cai et al., 2009), and a new model of directionality based on the 'tubulin code' has now been proposed (see below) (Barisic et al., 2015). As already discussed, an alternative gradient is the PEF, which generates a force field that is proportional to microtubule density and therefore the proximity to a pole (Cane et al., 2013). The principal PEF generator Kid has been shown to bias kinetochore directional switching towards the equator by inducing a position-dependent increase in kinetochore tension (Fig. 4C) (Stumpff et al., 2012). The proportion of microtubule-engaged Kid increases as a chromosome comes into the proximity of an attached pole, increasing the probability of its associated kinetochore switching state and therefore promoting migration towards the equator. However, it must be noted that Kid is dispensable for congression (Levesque and Compton, 2001), probably because Kif18A mediates the dominant position-sensing mechanism and can compensate for its loss (Stumpff et al., 2012).

Recently, the 'tubulin code' has been implicated in conveying directional bias to CENP-E lateral sliding (Barisic et al., 2015). This model is based upon the detyrosination of microtubules pointing towards the spindle equator, but not those in the astral region. Barisic and colleagues have elegantly demonstrated that CENP-E is more processive and can bear larger loads when on detyrosinated microtubules *in vitro*, and that aberrant chromosome movements induced by abrogation of detyrosination *in vivo* are dependent on CENP-E (Barisic et al., 2015). As a result, they proposed a model in which CENP-E transports pole-proximal chromosomes preferentially on detyrosinated microtubule tracks, which are normally orientated towards the spindle equator (Barisic et al., 2015).

A working model of chromosome congression

Although we are beginning to understand the mechanistic details of individual congression pathways, our knowledge of how these are integrated during prometaphase is incomplete. Recent work has started to explore this question by focusing on how multiple motor forces are coordinated (Barisic et al., 2014). Here, we lay out a working model of congression that is derived from the study of Barisic et al. and the other recent advances documented above (Fig. 5). We propose that, following nuclear envelope breakdown, some chromosomes rapidly bi-orientate at the spindle equator, as a result of a pre-positioning step that requires labile lateral attachments and PEFs, termed 'instantaneous' bi-orientation by Magison and colleagues (Fig. 5, step 1) (Barisic et al., 2014; Magidson et al., 2011). The remaining chromosomes that are located both within and outside of the spindle (Barisic et al., 2014), form initial attachments to microtubules in various configurations (Fig. 5, steps 2–7). Any syntelic (Fig. 1) attachments, where both sister kinetochores attach end-on to one pole, are rapidly destabilised by Aurora-B, generating unattached kinetochores that require re-capture (Lampson and Cheeseman, 2011). Chromosomes that are positioned outside of the spindle need to be first transported to the spindle pole by either dynein-dependent lateral sliding (Fig. 5, steps 2 and 3) (Barisic et al., 2014; Li et al., 2007; Yang et al., 2007) or DCP, if a monoorientated attachment has formed (Fig. 5, steps 4 and 5) (Bancroft et al., 2015). In both cases, this polewards transport overcomes the chromokinesin-generated PEF and CENP-E-driven AP force (Fig. 5, steps 3 to 5). This highlights that end-on attachment and dynein are dominant over CENP-E when chromosomes are located behind the pole (Bancroft et al., 2015; Barisic et al., 2014). Laterally attached kinetochores are also actively converted into a monoorientated state (Fig. 5, step 3) though a process that depends on CENP-E and MCAK in mammalian cells (Shrestha and Draviam, 2013), and dynein in *C. elegans* (Cheerambathur et al., 2013). In summary, the initial polewards transport yields a population of laterally attached or monoorientated kinetochore pairs in close proximity to the pole.

From the pole, both lateral (Fig. 5, step 2) and monoorientated-lateral (Fig. 5, step 6) sister kinetochore pairs can be transported towards the equator by CENP-E (Barisic et al., 2014; Kapoor et al., 2006). Here, directionality is imposed by both specific detyrosination of tubulin tracks orientated towards the equator, and by Aurora A phosphorylation of CENP-E at the pole (Barisic et al., 2015; Kim et al., 2010). Moreover, as chromosomes move away from Aurora A at the pole and towards the equator, the latter model states that CENP-E is dephosphorylated through recruitment of the phosphatase PP1, which then actively promotes end-on attachment and bi-orientation (see Fig. 5) (Kim et al., 2010). Chromosomes within the spindle, but away from the equator, can also bi-orientate and then congress by DCP (Fig. 5, step 7). Current models suggest that the (spatial) position-dependent loading of Kif18A in conjunction with the PEF gradient is the major mechanism responsible for imposing directionality (Stumpff et al., 2008, 2012).

Taken together, both CENP-E-mediated sliding and DCP move chromosomes from the pole to spindle equator. Here, DCP appears to be the prominent mechanism as clear lateral sliding events have only been observed in a quarter of Ptk1 cells (Kapoor et al., 2006), and only 15–20% of congression events were CENP-E-dependent in U2OS and HeLa cells (Bancroft et al., 2015; Barisic et al., 2014). Nevertheless, both mechanisms act in conjunction with the 'instantaneous' bi-orientation of kinetochores early in mitosis to

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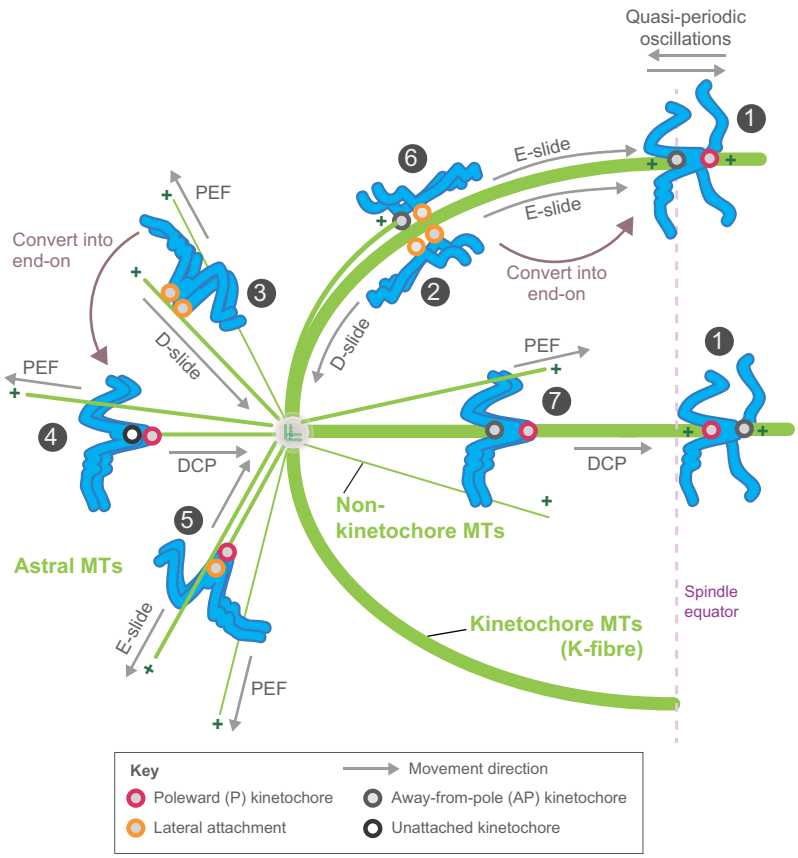

Fig. 5. A working model of chromosome congression. After nuclear envelope breakdown, chromosomes can be positioned throughout the cytoplasm and the nascent spindle, resulting in the formation of various microtubule attachments (1 to 7). Chromosomes at the spindle equator instantaneously bi-orientate, and do not require congression (1). Chromosomes that are positioned behind the pole or in the spindle periphery first move poleward through the action of dynein (D-slide) (2 and 3), or by DCP if a monoorientated attachment has formed (4 and 5). Within the polar region, an MCAK-dependent pathway operates to actively convert lateral attachments into monoorientated ones. Once at the pole, lateral (2) and monoorientated or lateral (6) chromosomes are transported to the spindle equator by CENP-E sliding (E-slide). This pathway often fails to maintain chromosomes at the metaphase plate and is not compatible with anaphase – as such, these chromosomes are converted into the default amphitelic state at the equator by PP1-mediated dephosphorylation of CENP-E (1). Pole-proximal chromosomes within the spindle can also bi-orientate and congress by DCP (7). These kinetochores are subject to the spatial control mechanisms outlined in Fig. 4 and Barisic et al. (2015).

provide a robust, multi-layered system that ensures all chromosomes congress in preparation for anaphase.

Conclusions and perspectives

From its origins in the polar repulsion (Darlington, 1937) and traction fibre models (Östergren, 1951) described well over half a century ago, congression can now be thought of a multi-step process that incorporates numerous spindle positions, kinetochore–microtubule attachment states and mechanistic drivers. The two major pathways, DCP and lateral sliding, have been well described in terms of the major players and over-arching mechanics. A key goal for the field will now be to extract the fine mechanical detail and regulatory elements that underpin these pathways and couple this to investigation of *in vitro* reconstituted mammalian kinetochores. Moreover, analysis of their relative contribution and molecular requirements in different cell types will be required. With these criteria in mind, the understanding of lateral sliding appears to be ahead, with both a description of spindle-position hierarchies and mechanisms that confers a directional bias on CENP-E on a congressing chromosome, advances that seem to reflect the simplicity of the mechanism. Describing similar models for DCP has proven challenging, given that the majority of candidate factors carry out multiple roles and that we lack *in vitro* assays to test mechanisms with reconstituted kinetochores. Nevertheless, we are beginning to illuminate the complexity of attachment, force generation and regulation of microtubule dynamics at the kinetochore, and in some cases, separate these properties. Despite these advances, we still lack a detailed mechanistic understanding of how DCP generates force given the incoherent nature of K-fibres, and how factors drive DCP on congressing chromosomes. Moreover, how congression is biased towards the spindle equator and how sister-kinetochores communicate their state to one another is still poorly understood. Future work will be required to understand these mechanisms, and to shed light on how congression is integrated with the regulation of kinetochore composition, error correction and the spindle assembly checkpoint signalling – all of which need to be tightly coordinated to ensure high fidelity chromosome segregation in mitosis.

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Competing interests

The authors declare no competing or financial interests.

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