Peer-reviewed version available at *Cells* **2020**, *9*, 1154; doi:10.3390/cells9051154

Review

How Essential Kinesin-5 Becomes Non-Essential: Force Balance and Microtubule Dynamics Matter

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Abstract: The bipolar mitotic spindle drives accurate chromosome segregation by capturing the kinetochore and pulling each set of sister chromatids to the opposite poles. In this review, we describe recent findings on the multiple pathways leading to bipolar spindle formation in fission yeast and discuss these results from a broader perspective. Roles of four mitotic kinesins (Kinesin-5, Kinesin-6, Kinesin-12 and Kinesin-14) in spindle assembly are depicted, and how a group of microtubule-associated proteins, sister chromatid cohesion and the kinetochore collaborates with these motors is shown. We have paid special attention to the molecular pathways that render otherwise essential Kinesin-5 to become non-essential: how cells build bipolar mitotic spindles without the need for Kinesin-5 and where the alternate forces come from are considered. We highlight the force balance for bipolar spindle assembly and explain how outward and inward forces are generated by various ways, in which the proper fine-tuning of microtubule dynamics plays a crucial role. Overall, these new pathways have illuminated remarkable plasticity and adaptability of spindle mechanics. Kinesin molecules are regarded as prospective targets for cancer chemotherapy and many specific inhibitors have been developed. However, several hurdles have arisen against their clinical implementation. This review provides insight into possible strategies to overcome these challenges.

Keywords: bipolar mitotic spindle; fission yeast; kinesin; kinetochore; microtubule dynamics; microtubule polymerase; microtubule–associated proteins (MAPs); spindle pole body (SPB); sister chromatid cohesion

1. Introduction

1.1. Bipolar mitotic spindles and kinesin motor proteins

The bipolar mitotic spindle is a dynamic ensemble of core microtubule polymers and a cohort of microtubule-associated proteins (MAPs). It attaches to the kinetochore on sister chromatids to align chromosomes at the spindle equator in metaphase, and pulls each pair of sister chromatids towards the opposite poles in anaphase A. During anaphase B, spindles further elongate to ensure equal partition of each set of chromosomes, which is followed by cytokinesis [1, 2]. MAPs are required for spindle assembly and coordinate the multiple events of mitosis in a spatiotemporal manner. Kinesin motors comprise one of the major MAPs and couple the energy of ATP hydrolysis to force generation [3]. Kinesin superfamilies comprise at least 15 families, which are further structurally categorised into three groups, called N-kinesin (N-terminal), M-kinesin (middle) and C-kinesin (C-terminal), depending on the location of the motor domain within each molecule [4-6]. Mitotic kinesins include

10 kinesin families that are functionally designated as they are localised to the spindle microtubule and regulate structure and function of the spindle and mitotic progression [7]. This review is based upon recent work using fission yeast as a model, but includes the comparison with other systems, in which evolutionary conservation and diversification are discussed.

1.2. Kinesin-5 plays an essential role in bipolar spindle assembly and cell survival

The type 5 kinesin (Kinesin-5) belongs to the N-kinesin that moves on microtubules towards their plus ends. This motor forms homotetramers, thereby crosslinking and sliding apart antiparallel microtubules [8, 9]. During early mitosis, this process generates an outward pushing force towards two duplicated spindle poles (centrosomes in animal cells and the spindle pole bodies (SPBs) in fungi), which promotes centrosome/SPB separation, thereby establishing spindle bipolarity [10, 11]. In most, if not all, eukaryotes, Kinesin-5 (budding yeast Cin8 and Kip1, fission yeast Cut7, *Aspergillus* BimC, *C. elegans* BMK-1, *Drosophila* Klp61F, *Xenopus* Eg5 and human KIF11) is essential for mitosis, in which any means of its inactivation, e.g. chemical inhibition, genetic deletion or RNAi-mediated depletion, leads to the emergence of monopolar spindles, the failure of chromosome segregation and viability loss [12-19].

2. How essential Kinesin-5 becomes non-essential

Surprising findings came to light that cells can divide in the absence of Kinesin-5 function under certain conditions across a wide range of species including human beings, frogs, flies, filamentous fungi and the budding and fission yeasts. This is because bipolar spindle assembly is driven by the finely tuned, antagonistic force balance exerted by opposing motors. More precisely, an outward force generated by plus end-directed Kinesin-5 is antagonised by an inward force produced by minus end-directed Kinesin-14 that belongs to the C-kinesin (budding yeast Kar3, fission yeast Pkl1 and Klp2, *Aspergillus* KlpA, *Drosophila* Ncd, *Xenopus* XCTK2 and human HSET/KIFC1) or Dynein [20]. Accordingly, inactivation of minus end-directed motors could neutralise loss of Kinesin-5 activity. In other words, cells without Kinesin-5 and Kinesin-14 or Dynein are now capable of forming bipolar spindles and will continue to divide.

3. Conditions under which cells do not need Kinesin-5 for survival

As aforementioned, the main means in which Kinesin-5 becomes dispensable is by simultaneous inactivation of opposing Kinesin-14 or Dynein. In order to explore the genetic network that plays a role in conferring the non-essentiality of Kinesin-5, we conducted systematic screening for suppressors against *cut7* temperature sensitive (ts) mutants in fission yeast. Spontaneous survivors of *cut7* mutant strains grown at the restrictive temperature (36°C) were isolated. After standard genetic analyses and nucleotide sequencing, suppressor genes (designated *skf*=suppressor of *k*inesin *f*ive) were identified (Table 1) [21]. Suppressors can be classified into three main groups: Kinesin-14s, non-motor MAPs and tubulins.

3.1. Suppression by mutations in Kinesin-14s or their cofactors

In fission yeast, two Kinesin-14s, Pkl1 and Klp2, form distinct complexes with specific cofactors and are localised to different sites on the microtubule to play non-redundant roles in spindle assembly and mitotic progression [22-25]. Pkl1 forms a ternary complex with Msd1 and Wdr8 (referred to as the MWP complex) and is localised predominantly to the mitotic SPB, thereby anchoring the minus end of the spindle microtubule to the SPB [26-28]. During early mitosis, when the duplicated SPBs start to separate that is driven by the Cut7-mediated outward force, the SPB-tethered MWP complex is loaded onto the spindle microtubule that nucleates from the other SPB, where this complex exerts an antagonistic inward force. Thus, the reason for suppression of *cut7* mutants by *pkl1Δ*, *msd1Δ* or *wdr8Δ* is that the two SPBs can separate because the overwhelming inward force exerted by the MWP Kinesin-14 complex disappears (Figure 1A).

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Table 1. A list of inward force generators

Gene	*Synonym*	*Protein*	*Homologue*	*Function*
pkl1[1]	*skf1*	Kinesin-6	HSET/KIFC1	Minus end-directed motor
wdr8	*skf2*	MAP	WDRB/WRAP73	A component of the MWP complex
msd1[1]	*skf3*	MAP	hMsd1/SSX2IP	A component of the MWP complex
klp2		MAP	HSET/KIFC1	Minus end-directed motor
nda3	*skf4*	MAP	β-tubulin	Microtubule
atb2	*skf5*	MAP	α2-tubulin	Microtubule
mal3	*skf6*	Coiled coil	EB1	A plus-end tracking protein
alp16		SPB localising	GCP6	A component of the γTuC
alp7	*mia1*	MAP	TACC	Complex formation with Alp14/chTOG
alp14	*mtc1*	MAP	chTOG/XMAP215	Microtubule polymerase
dis1		MAP	chTOG/XMAP215	Microtubule polymerase
pka1		Protein kinase	PKA	cAMP-dependent protein kinase

[1] Only *pkl1* or *msd1* deletion bypasses a complete deletion of *cut7*. Mutations in the remaining genes suppress only the *cut7* ts mutant, but not *cut7Δ*. The other condition that renders *cut7Δ* viable is treatment with microtubule-destabilising drugs [30].

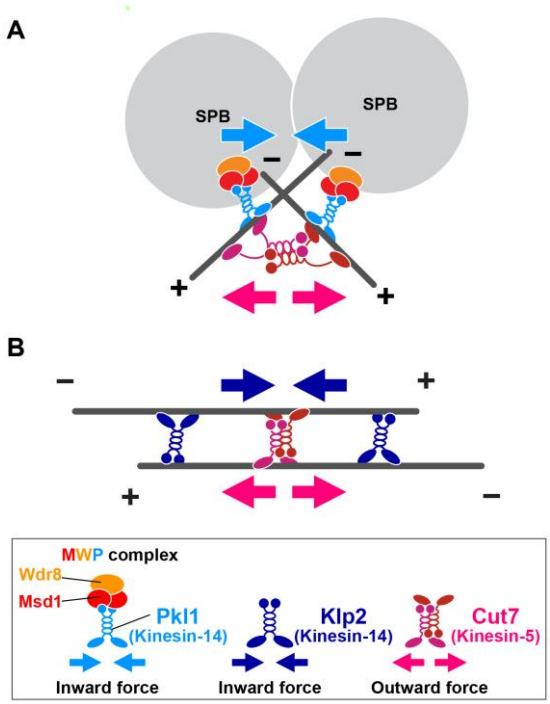

Figure 1. Generation of collaborative inward forces by two Kinesin-14s Pkl1 and Klp2. (**A**) When spindle bipolarity starts to be established upon, SPB-tethered Pkl1 (the MWP complex) engages with the spindle microtubule that emanates from the opposite SPB. Minus end-directed motility of Pkl1 generates an inward force (blue arrows). This pulling force antagonises an outward force exerted by Cut7 (red arrows) that is also localised in the vicinity of the SPB and promotes interdigitation of microtubules emanating from the two SPBs. In addition to inward force generation, the MWP complex plays a crucial role in anchoring the minus end of the spindle microtubule to the mitotic SPB as a barrier [28]. (**B**) Once bipolar spindles are formed, Klp2 and Cut7 are localised on antiparallel microtubules. These two kinesins antagonistically generate an inward force (blue arrows) and an outward force (red arrows), respectively. + and – stand for the microtubule plus and minus ends, respectively. Note that Cut7 is reportedly localised to the two other sites. One is a medial microtubule contact site. This localisation is seen when bipolar spindles are depolymerised first and then allowed for repolymerisation. Under this condition, Cut7 is required for interpolar bundle formation [116]. The other site is the kinetochore, to which Cut7 is recruited when chromosomes are misaligned. Under this condition, Cut7 is required for chromosome gliding towards the spindle equator [117].

By contrast, Klp2 mainly is localised along spindles in a punctate manner [29]. Spindle-localising Klp2 crosslinks the antiparallel microtubule bundles, which produces an inward force by exploiting minus-end motility, and this force acts antagonistically with the Cut7-driven outward force on the spindle microtubule [30]. The reason for suppression of *cut7* mutants by *klp2Δ* is that antiparallel microtubules can elongate in the absence of Cut7, as Klp2-driven inhibitory inward force is lost (Figure 1B). Collectively, Pkl1 acts mainly during the early stages of bipolar spindle assembly, while Klp2 plays a role in spindle elongation at later stages of mitosis. These distinct modes of the spatiotemporal regulation between Pkl1 and Klp2 underlie the collaborative actions of these two Kinesin-14s.

The deletion of either *pkl1* or *klp2* suppresses the temperature sensitivity caused by the *cut7* mutations [23, 25, 31]. Intriguingly, gene deletion of *pkl1*, but not *klp2*, is capable of rescuing a complete deletion of *cut7* [30, 32-34]. Despite apparent ordinary growth, *cut7Δpkl1Δ* cells display mitotic delay in which cells spend a longer period of time with short spindles. This implies that in the absence of Cut7 and Pkl1, an excessive inward force is imposed during early mitosis. This inward force at least in part is generated by Klp2, as slower spindle elongation rate is significantly ameliorated in the *cut7Δpkl1Δklp2Δ* triple mutant [30].

3.2. Suppression by compromised microtubule nucleation, polymerisation and stability

We have found that mutations in the genes encoding tubulins and 5 non-motor MAPs are also capable of rescuing *cut7* ts mutants. In fission yeast, tubulin molecules are encoded by *nda2* (α1-tubulin), *atb2* (α2-tubulin) and *nda3* (β-tubulin) [35, 36] (Table 1). Mutations in tubulin genes would compromise microtubule integrity. One of the 5 MAPs is Mal3/EB1, a conserved MAP that tracks on the microtubule plus end [37]. Its mutation leads to microtubule destabilisation and defects in kinetochore-microtubule attachment [38, 39]. Alp16 is a homologue of GCP6 and a component of the microtubule-nucleator γ-tubulin complex (γ-TuC) [40-42]. The other three MAP-encoding suppressors are *alp7* (encoding an orthologue of the transforming acidic coiled-coil protein TACC) [43, 44], *alp14* and *dis1* (two genes encoding XMAP215/TOG microtubule polymerases) [45-49]. Alp7 and Alp14 form a stable complex in the cell and promotes microtubule polymerisation [43, 48, 50]. The Alp7-Alp14 complex also is required for efficient nucleation of the microtubule from the SPB [51].

Apart from its role in microtubule stabilisation, Mal3/EB1 is known to interact with Klp2, which is a prerequisite for this motor to be loaded on the spindle microtubule [29]. Thus, suppression of the *cut7* ts mutation by the *mal3* mutation could be ascribable to the loss of Klp2 function [21]. Overall, the common features of suppressor genes encoding tubulins and MAPs are that all these mutations lead to the destabilisation of the spindle microtubules. It is worth noting that in *cut7* mutant cells, intensities of spindle microtubules are augmented [21]. Importantly, in these mutant cells, intensities of Klp2 on the spindle microtubule are also substantially increased. Notably, in the double mutant containing *cut7* ts and any of the suppressor mutations, the intensities of Klp2 are reduced. Given these observations, we propose that the rescue of *cut7* mutants by these suppressors is derived from quantitative downregulation of Klp2 activity. In fact, the overproduction of Klp2 in the double mutants between *cut7* and suppressor mutations restored a ts phenotype similar to a single *cut7* mutant [21], corroborating the notion that the reduced localisation/activity of Klp2 is the primary, if not the sole, reason for the rescue of the *cut7* mutation. In summary, suppressor analyses have uncovered that multiple factors that regulate microtubule structures are involved by which Kinesin-5/Cut7 becomes dispensable and importantly, inactivation of Kinesin-14s, Pkl1 and Klp2, is the main means for the rescue of the *cut7* mutation by these suppressors.

3.3. Suppression by microtubule-destabilising drugs

As previously mentioned, *cut7* mutants exhibit increased intensities of the spindle microtubule accompanied by more Klp2 proteins on the spindle microtubule. In line with this observation, these cells display hyper-resistance against microtubule-depolymerising drugs, such as thiabendazole (TBZ) or methyl 2-benzimidazolecarbamate (MBC), and interestingly, treatment of *cut7* ts mutants with TBZ or MBC rescues temperature sensitivity [21]. Under this condition, Klp2 levels are lessened

as in the other suppressor mutations. Remarkably, drug treatment rescues even a complete deletion of *cut7*. Collectively, the impairment of microtubule stability and/or dynamics by either suppressor mutations or treatment with microtubule-destabilising drugs renders fission yeast cells viable in the absence of Kinesin-5 (Figure 2).

It is worth pointing out that in cultured human cells (HeLa or U2OS), microtubule destabilisation can also effectively rescue monopolar spindle phenotypes induced by Kinesin-5/KIF11 inhibition [52, 53]. This underscores the evolutionary conservation of Kinesin-5 function and its regulation from fission yeast to human beings.

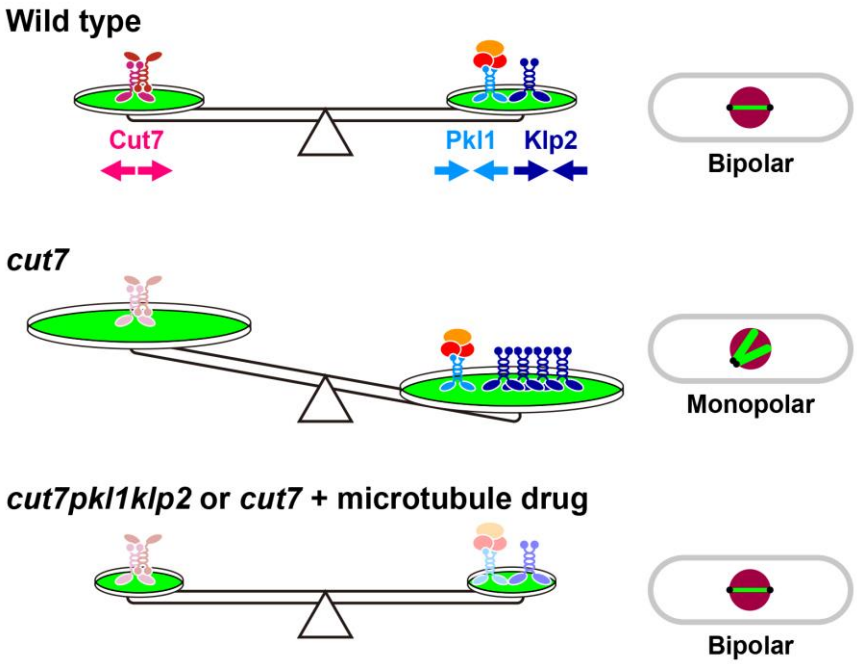

Figure 2. Bipolar spindle formation requires a collaborative force balance exerted by mitotic kinesins, MAPs and microtubule dynamics. In wild type cells (top), Kinesin-5/Cut7 generates an outward force (red arrows), while Kinesin-14s/Pkl1 and Klp2 generate an opposing inward force (blue arrows). Microtubule stability and dynamics promoted by a cohort of MAPs play positive roles in Klp2 activity by enhancing its localisation to the spindle microtubule, which Kinesin-5/Cut7 opposes. In *cut7* ts or *cut7Δ* cells (middle), Kinesin-14-mediated inward forces dominate, leading to the formation of monopolar spindles. In double mutants between *cut7* and *pkl1/klp2* or the *cut7* mutant treated with microtubule-depolymerising drugs (bottom), loss of inward forces or the compromised microtubule dynamics, respectively, renders Cut7 to become dispensable for bipolar spindle assembly and therefore the survival.

3.4. Suppression through downregulation of the cAMP/PKA pathway

In many organisms, the extracellular environment such as nutritional cues regulates microtubule dynamics through the intracellular signal transduction pathways. In yeasts, glucose in the media activates the cAMP/PKA pathway, by which it controls a diverse set of downstream events [54-56]. Deletion of the *pka1* gene rescues the *cut7* ts mutant [57]. Pka1 reportedly fine-tunes microtubule dynamics at least in part through downregulating the Cls1/Peg1/CLASP MAP [58-60], and consistent with this notion, overproduction of Cls1/Peg1 is capable of rescuing the *cut7* ts mutant like *pka1Δ* [57]. Cls1/Peg1 is shown to promote microtubule bundling [61]. It is possible that enhanced bundling activity by overproduced Cls1/Peg1 would help convert the monopolar spindle in the *cut7* mutation to a bipolar spindle, leading to rescue of this mutant.

4. Outward force generators in the absence of Kinesin-5

Cells without Kinesin-5 become viable if Kinesin-14 is defective (e.g. *cut7Δpkl1Δ*) or if microtubules are destabilised (e.g. *cut7Δ* treated with microtubule-destabilising drugs). This finding poses the following important question: how do bipolar spindles assemble in the absence of Kinesin-5-mediated outward force? Detailed genetic and cell biological analyses have unravelled this puzzle; at least 11 gene products are capable of generating outward forces instead of Kinesin-5 (Table 2).

Table 2. A list of outward force generators

Gene	*Synonym*	*Protein*	*Homologue*	*Function*
klp9		Kinesin-6	MKLP1, MKLP2	Plus end-directed motor
ase1		MAP	PRC1	Microtubule crosslinker
cls1	*peg1*	MAP	CLASP	Microtubule stabiliser/crosslinker
alp7	*mia1*	MAP	TACC	Complex formation with Alp14/chTOG
alp14	*mtc1*	MAP	chTOG/XMAP215	Microtubule polymerase
dis1		MAP	chTOG/XMAP215	Microtubule polymerase
csi1		Coiled coil		Targeting Alp7 to the mitotic SPB
csi2		SPB localising		Targeting Csi1 to the mitotic SPB
swi6		Chromodomain	HP1	Heterochromatin
rad21		Kleisin	hRad21/Scc1/Mcd1	Cohesin
nuf2		Coiled coil	Nuf2	Kinetochore

4.1. Outward forces exerted by Kinesin-6

One possibility of the survival of *cut7Δpkl1Δ* cells is that the other kinesin motors exert an outward force in place of Kinesin-5, thereby promoting spindle bipolarity. The fission yeast genome contains in total 9 genes encoding kinesin motors. Genetic crosses indicate that only one kinesin, Klp9, becomes essential when combined with *cut7Δpkl1Δ*; *cut7Δpkl1Δklp9Δ* triple mutants are inviable. Klp9 belongs to the N-kinesin Kinesin-6. Interestingly, like Cut7, it moves on the microtubule towards the plus end and forms homotetramers, thereby crosslinking antiparallel microtubules [62, 63]. Previous work showed that this kinesin accumulates at the spindle midzone upon onset of anaphase B and promotes spindle elongation during late mitosis [62], though it appears to also play additional roles during earlier stages of mitosis [64-66]. Detailed analysis shows that Klp9 accelerates spindle elongation only during anaphase B in both wild type and *cut7Δpkl1Δ* cells and that inviable *cut7Δpkl1Δklp9Δ* cells are in fact capable of assembling bipolar spindles. However, these spindles are shorter comparted to those in wild type or *cut7Δpkl1Δ* cells, and upon mitotic exit, the nucleus and chromosomes are intersected by the cytokinetic actomyosin contractile ring and the septum, resulting in cell death imposed by a catastrophic "cut" (cell untimely torn) phenotype [63, 67].

How do the two N-kinesins, Kinesin-5/Cut7 and Kinesin-6/Klp9, act in concert to drive spindle elongation in wild type cells? Differential localisation patterns of these two kinesins on the spindle microtubule may give us a clue. While Cut7 mainly accumulates near the SPB, Klp9 is localised exclusively to the spindle midzone where antiparallel microtubules interdigitate (Figure 3A). Given these different localisations, we posit that during anaphase B, Cut7 crosslinks mainly parallel microtubules near the SPBs, while Klp9 bundles antiparallel microtubules at the spindle midzone (Figure 3B).

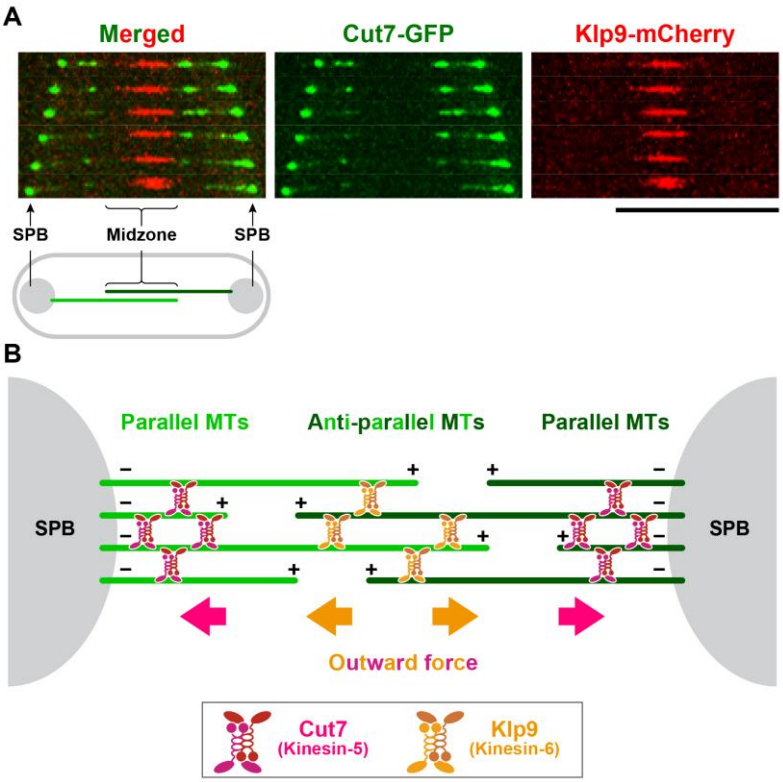

Figure 3. Spatially distinct localisations between Kinesin-5/Cut7 and Kinesin-6/Klp6 on anaphase B spindles. (**A**) Localisation of Cut7 and Klp9 on the spindle microtubule during anaphase B. The fission yeast strain containing *cut7-GFP* and *klp9-mCherry* (expressed from individual native promoters) were observed under fluorescence microscopy and a late mitotic cell imaged. Images were obtained using a DeltaVision microscope system (DeltaVision Elite; GE Healthcare, Chicago, IL, U. S. A.) comprising of a wide-field inverted epifluorescence microscope (IX71; Olympus, Tokyo, Japan) and a Plan Apochromat 60×, NA 1.42, oil immersion objective (PLAPON 60×O; Olympus Tokyo, Japan). DeltaVision image acquisition software (softWoRx 6.5.2; GE Healthcare, Chicago, IL) equipped with a charge-coupled device camera (CoolSNAP HQ2; Photometrics, Tucson, AZ) was used. Time-lapse live imaging was performed, in which pictures were taken at 1 min intervals after incubation of cultures at 27°C. Images were taken as 16 sections along the z-axis at 0.2 μm intervals. The sections of images acquired at each time point were compressed into a 2D projection using the DeltaVision maximum intensity algorithm. Deconvolution was applied before the 2D projection. Captured images were processed with Photoshop CS6 (version 13.0; Adobe, San Jose, CA). Scale bar, 10 μm. (**B**) A schematic showing localisations of Cut7 and Klp9 on anaphase B spindles. Cut7 bundles parallel microtubules in the vicinity of the SPB, while Klp9 bundles antiparallel microtubules at the spindle midzone. Note that Klp9 bundles antiparallel microtubules at the spindle midzone independent of its motor activity [63]. + and – stand for the microtubule plus and minus ends, respectively.

4.2. Outward forces exerted by the microtubule crosslinker and stabiliser

Fission yeast cells are capable of forming nearly normal bipolar spindles in the presence of only Kinesin-6 Klp9, which acts in spindle elongation later in mitosis [57]. How then could spindle bipolarity be established in the first place under this condition? It transpires that two conserved MAPs, Ase1/PRC1 [68, 69] and Cls1/Peg1/CLASP, in concert, play an indispensable role in this process. Ase1 and Cls1/Peg1 bundle and stabilise antiparallel spindle microtubules (Figure 4). Notably, theoretical modelling supports bipolar spindle assembly by these two factors; Brownian dynamics-kinetic Monte Carlo simulations show that Ase1 and Cls1/Peg1 activity are sufficient for initial bipolar spindle formation [57, 70, 71].

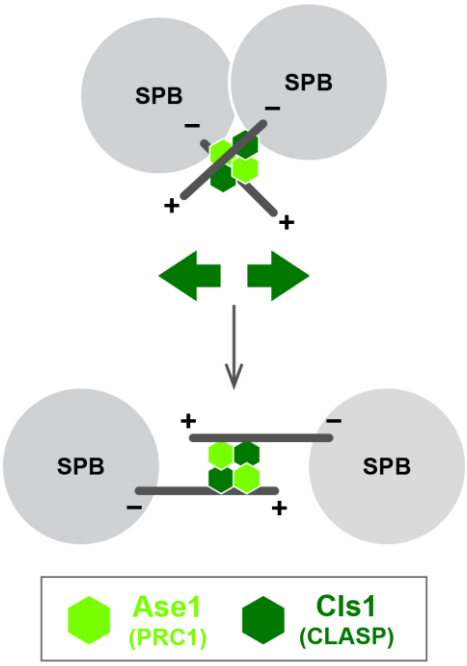

Figure 4. Outward force generation through the spindle midzone. Short microtubules nucleating from the two SPBs are crosslinked in an antiparallel manner and stabilised by Ase1/PRC1 and Cls1/Peg1/CLASP. This provides an outward force (green arrows) towards the SPB that is sufficient to form short bipolar spindles in the absence of Kinesin-5 and Kinesin-14 [57]. + and – stand for the microtubule plus and minus ends, respectively.

Unlike in other species, *C. elegans* does not require Kinesin-5/BMK-1 for bipolar spindle formation [72]. During embryonic division of this organism, the spindle midzone could produce outward forces in concert with cortical pulling forces, thereby promoting chromosome segregation [18]. Interestingly, in this process, the midzone components including SPD-1/Ase1 and CLASP play vital roles in force generation, though SPD-1 seems to antagonise CLASP-mediated spindle elongation [18, 73]. Taken together, the spindle midzone could produce outward forces in which the microtubule crosslinking and stabilising MAPs are key players.

4.3. Outward forces exerted by microtubule polymerases

Alp14 and Dis1 belong to a conserved MAP family of XMAP215/TOG that catalyses microtubule polymerisation [45-49, 74, 75]. As aforementioned, Alp7 forms a stable complex with Alp14 and targets the Alp14 microtubule polymerase to the SPB upon mitotic onset [43, 50]. Genetic analysis indicates that any of triple deletion mutants, *cut7Δpkl1Δalp14Δ*, *cut7Δpkl1Δdis1Δ*, or *cut7Δpkl1Δalp7Δ*, are inviable. SPB-localising Csi1 and Csi2 are required for Alp7 localisation to the mitotic SPBs [76-78]. Consistent with this, deletion of either *csi1* or *csi2* is lethal in combination with *cut7Δpkl1Δ*. Temperature sensitive *cut7Δpkl1Δalp7* cells display monopolar spindles or very short spindles (<0.5 μm) that fail to elongate [79]. These results suggest that in *cut7Δpkl1Δ* cells outward forces are generated through microtubule polymerisation, in which the growing plus ends of the microtubule push the SPB, leading to separation of the SPBs (Figure 5A)

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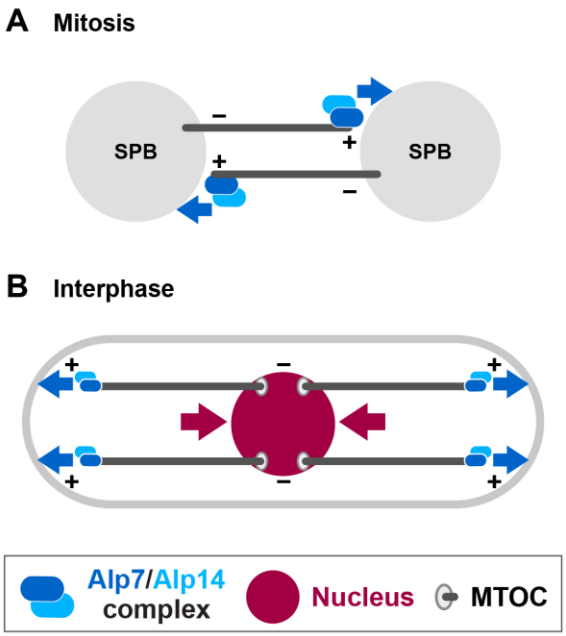

Figure 5. Force generation through microtubule polymerisation during interphase and mitosis. (**A**) During early mitosis, the Alp7/TACC-Alp14/TOG microtubule polymerase complex (blue ovals) is localised to the SPB (not shown) and the polymerising plus ends. Interaction of growing microtubule plus ends with the other SPB generates an outward force (blue arrows). This force is sufficient to separate the duplicated SPBs in the absence of Kinesin-5 and Kinesin-14, thereby promoting short bipolar spindle formation. (**B**) During interphase, the plus ends of the polymerising cytoplasmic microtubules reach and push the cell tip at either end (blue arrows), thereby pushing the nucleus through the opposite minus ends (deep red arrows). This allows positioning of the nucleus at the geometrical centre of the cell [80, 81]. MTOC stands for microtubule organising centre, which is localised to multiple positions on the nuclear membrane during interphase [118, 119]. + and – stand for the microtubule plus and minus ends, respectively.

Mutations in *dis1*, *alp7* or *alp14* rescue the *cut7* ts mutation, while rather contradictorily, the same mutations become indispensable in the *cut7Δpkl1Δ* background; defects in the microtubule polymerisation confer both positive and negative impacts on the *cut7* mutation. In a single *cut7* mutation, microtubule polymerisation is negative for cell survival, while in *cut7Δpkl1Δ*, it plays a positive role. This illuminates a remarkable mechanistic plasticity of bipolar spindle assembly; cells could generate either inward or outward forces using microtubule polymerases in a context-dependent manner.

Intriguingly, in interphase fission yeast cells, the nucleus is centred by pushing forces that are generated as growing cytoplasmic microtubules hit the cell tip at each end [80, 81] (Figure 5B). Hence, pushing forces generated through the polymerising microtubule plus ends play important roles in both interphase and mitosis: nuclear positioning during interphase and SPB separation/bipolar spindle assembly during mitosis. The generation of an outward pushing force by the polymerising microtubule plus end is widely observed in other systems. For instance, during embryonic divisions of animal cells, the plus ends of astral microtubules physically interact with and push the cell cortex, and this force ensures proper spindle positioning [82, 83].

4.4. Outward forces exerted by the kinetochore and sister chromatid cohesion

Recent work has identified a fourth class of outward force generators [84]. This force is elicited through the kinetochore, a several-MDa–sized proteinaceous structure assembled on a specialised region of the chromosome called the centromere [85]. The spindle microtubule attaches to the kinetochore for accurate sister chromatid segregation. Mutations in genes encoding the conserved

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kinetochore component (Nuf2) [86] or those required for centromere-mediated sister chromatid cohesion (Rad21/Scc1/Mcd1 and Swi6/HP1) [87-90] confer a severe synthetic growth defect to *cut7Δpkl1Δ*. These triple mutant cells impair proper spindle assembly and display largely monopolar spindles. These results show that the kinetochore is captured by the plus end of the spindle microtubule, thereby producing outward forces that support bipolar spindle assembly (Figure 6) [84].

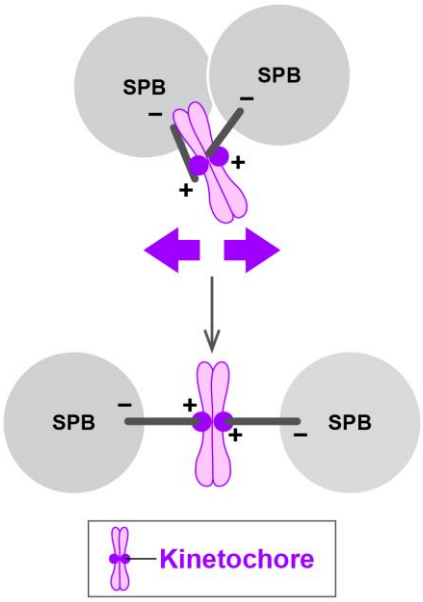

Figure 6. Outward force generation through the kinetochore and sister chromatid cohesion. The kinetochore and sister chromatid cohesion contribute to the generation of outward forces for SPB separation in mitosis. In the absence of Kinesin-5 and Kinesin-14, outward forces (purple arrows) derived from the kinetochore and/or sister chromatid cohesion are capable of separating the SPBs, thereby promoting bipolar spindle assembly [84]. + and – stand for the microtubule plus and minus ends, respectively.

It is noteworthy that a similar result was reported in human cells, in which stable kinetochore-microtubule attachment plays a crucial role in centrosome separation [91] and becomes essential for maintenance of the bipolar spindle in the absence of Kinesin-5 [92]. Taking all these findings together, the balance between inward and outward forces generated by opposing motor proteins, multiple MAPs, the kinetochore and sister chromatid cohesion underlies mechanisms by which bipolar spindles are formed and maintained.

4.5. Outward force generation by Kinesin-12 in human cells

In fission yeast, Kinesin-6/Klp9 collaborates with Kinesin-5/Cut7 to generate an outward force. However, Klp9 acts in spindle elongation only during late mitosis irrespective of the presence or absence of Cut7, and furthermore, Klp9 cannot be substituted for Cut7 [57, 79]. By contrast, in human cells, a similar role appears to be executed by the N-kinesin Kinesin-12/KIF15/HKLP2, which does not exist in yeasts. It has been shown that Kinesin-12 functions redundantly with Kinesin-5 to promote spindle bipolarity [52, 93-95], and curiously, the overproduction of Kinesin-12 can drive bipolar spindle assembly even when Kinesin-5 activity is fully inhibited. This indicates that Kinesin-12 has the potential to execute all essential functions of Kinesin-5. Therefore, functions of fission yeast Kinesin-6/Klp9 and human Kinesin-12/KIF15/HKLP2 appear similar but mechanistically different.

5. Force generation in human prophase cells

Human cells undergo centrosome separation through two temporally distinct pathways, the prophase pathway and the prometaphase pathway [96-98]. By contrast, yeasts have adopted only the prometaphase pathway. Recent analysis has uncovered key players acting in the prophase pathway

and their individual roles [97]. Duplicated centrosomes in human cells remain closely linked during interphase, in a process called centrosome cohesion. Centrosome cohesion is maintained through dual mechanisms. The first mechanism depends upon a structural linker composed of two proteins, Rootletin and C-NAP1. This linker physically joins the two centrosomes in a side-by-side configuration. The second mechanism involves the Kinesin-14/KIFC3-mediated inward force. KIFC3 forms homotetramers and interconnects a special centrosome-associated microtubule network, thereby producing pulling forces towards the centrosomes. Upon mitotic entry, KIFC3 is inactivated by the NEK2 protein kinase, which also promotes the dissolution of the linker [97]. Antagonising outward forces are produced by Kineisn-5/KIF11 in both prophase and prometaphase pathways.

We contemplate that the difference between human beings and yeasts, in which human cells have developed more complex regulatory mechanisms, stem from different modes of mitosis: an open mitosis in higher eukaryotes vs a closed mitosis in yeasts. As the nuclear membrane disassembles upon mitotic onset, human cells have acquired an additional regulatory process (the prophase pathway), which ensures the temporal order of bipolar spindle assembly to be synchronised with mitotic onset. Implementation of dual, redundant pathways might also be beneficial for the robustness of the system; disrupting one pathway would not result in a catastrophic impact on spindle formation and therefore the fidelity of chromosome segregation.

6. Force generation in the acentrosomal cells

In higher eukaryotes and plants, the bipolar spindle is formed independent of the centrosome through the pathway referred to as the acentrosomal pathway [99-101]. Historically, the acentrosomal pathway is extensively characterised in vitro using extracts prepared from *Xenopus* oocytes, where the Ran GTPase acts as a master regulator [102, 103]. This pathway is functional in vivo in several cell types which are naturally devoid of centrosomes (eg. vertebrate oocytes and plants) or animal somatic cells which are experimentally (chemically, genetically or physically) manipulated to eliminate their centrosomes [104-106]. Interestingly, in this acentrosomal pathway, both in vitro and in vivo, Kinesin-5 also plays a major role in the formation of antiparallel microtubule bundles and the generation of an outward force. However, in contrast with the centrosome-dependent pathway, Kinesin-14 and Dynein appear to act collaboratively, rather than antagonistically, with Kinesin-5 [105, 107]. Therefore, the importance of the force balance for bipolar spindle formation and its underlying mechanism have not been addressed explicitly in the acentrosomal cells and awaits further investigation.

7. Towards cancer therapeutics

Kinesin-5 is required for successive cell division. Furthermore, in actively growing cancer cell lines its activity is tightly, and sometimes causally, linked to tumour progression and malignancy [108]. Accordingly, this kinesin has been deemed to be an attractive target of cancer chemotherapeutics. Indeed, several Kinesin-5 inhibitors were developed and their clinical trials conducted [109]. However, drug-resistant cell lines often appeared to hamper the clinical usage of these inhibitors [110-112]. To tackle this conundrum, a comprehensive understanding of in vivo Kinesin-5 functions and regulations in addition to structural information on the interaction between Kinesin-5 and specific inhibitors [109, 113] are necessary. As Kinesin-12 is essential for the survival of HeLa cells that become resistant to Kinein-5 inhibitors, the development of specific Kinesin-12 inhibitors would be important [95]. Given that destabilisation and/or the reduced dynamics of the microtubule rescues the lethality derived from Kinesin-5 inactivation, the combined treatment of Kinesin-5 inhibitors and microtubule stabilising reagents such as Paclitaxel (Taxol, on its own used widely for chemotherapy) [114, 115] might provide a more effective treatment for cancer therapeutics.

Author Contributions: T.T. wrote the manuscript with input from M.Y. and Y.T. M.Y. and Y.T. performed experiments and analysed the data with T.T.

Funding: This work was supported by the Japan Society for the Promotion of Science (JSPS) [KAKENHI Scientific Research (A) (16H02503 to T.T.) and Scientific Research (C) (19K05813 to M.Y.)].

Acknowledgments: We thank all the laboratory members, past and present, and collaborators for their contributions to our work described in this review. We are grateful to Risa Mori for critical reading of the manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

γ-TuC	γ-tubulin complex
MBC	methyl 2-benzimidazolecarbamate
MAPs	microtubule-associated proteins
SPB	spindle pole body
TBZ	thiabendazole
ts	temperature-sensitive

References

1. Mitchison, T. J.; Salmon, E. D., Mitosis: a history of division. *Nat Cell Biol* **2001**, *3*, (1), E17-E21.

2. Woodruff, J. B.; Wueseke, O.; Hyman, A. A., Pericentriolar material structure and dynamics. *Philos Trans R Soc Lond B Biol Sci* **2014**, *369*, (1650).

3. Vale, R. D.; Reese, T. S.; Sheetz, M. P., Identification of a novel force-generating protein, kinesin, involved in microtubule-based motility. *Cell* **1985**, *42*, (1), 39-50.

4. Hirokawa, N.; Noda, Y.; Tanaka, Y.; Niwa, S., Kinesin superfamily motor proteins and intracellular transport. *Nat Rev Mol Cell Biol* **2009**, *10*, (10), 682-96.

5. Wickstead, B.; Gull, K.; Richards, T. A., Patterns of kinesin evolution reveal a complex ancestral eukaryote with a multifunctional cytoskeleton. *BMC Evol Biol* **2010**, *10*, 110.

6. Lawrence, C. J.; Dawe, R. K.; Christie, K. R.; Cleveland, D. W.; Dawson, S. C.; Endow, S. A.; Goldstein, L. S.; Goodson, H. V.; Hirokawa, N.; Howard, J.; Malmberg, R. L.; McIntosh, J. R.; Miki, H.; Mitchison, T. J.; Okada, Y.; Reddy, A. S.; Saxton, W. M.; Schliwa, M.; Scholey, J. M.; Vale, R. D.; Walczak, C. E.; Wordeman, L., A standardized kinesin nomenclature. *J Cell Biol* **2004**, *167*, (1), 19-22.

7. Yount, A. L.; Zong, H.; Walczak, C. E., Regulatory mechanisms that control mitotic kinesins. *Exp Cell Res* **2015**, *334*, (1), 70-77.

8. Kashina, A. S.; Baskin, R. J.; Cole, D. G.; Wedaman, K. P.; Saxton, W. M.; Scholey, J. M., A bipolar kinesin. *Nature* **1996**, *379*, (6562), 270-272.

9. Kapitein, L. C.; Peterman, E. J.; Kwok, B. H.; Kim, J. H.; Kapoor, T. M.; Schmidt, C. F., The bipolar mitotic kinesin Eg5 moves on both microtubules that it crosslinks. *Nature* **2005**, *435*, (7038), 114-118.

10. Hagan, I.; Yanagida, M., Kinesin-related cut7 protein associates with mitotic and meiotic spindles in fission yeast. *Nature* **1992**, *356*, (6364), 74-76.

11. Sawin, K. E.; LeGuellec, K.; Philippe, M.; Mitchison, T. J., Mitotic spindle organization by a plus-end-directed microtubule motor. *Nature* **1992**, *359*, (6395), 540-543.

12. Blangy, A.; Lane, H. A.; d'Herin, P.; Harper, M.; Kress, M.; Nigg, E. A., Phosphorylation by $p34^{cdc2}$ regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation in vivo. *Cell* **1995**, *83*, (7), 1159-1169.

13. Enos, A. P.; Morris, N. R., Mutation of a gene that encodes a kinesin-like protein blocks nuclear division in *A. nidulans*. *Cell* **1990**, *60*, (6), 1019-1027.

14. Hagan, I.; Yanagida, M., Novel potential mitotic motor protein encoded by the fission yeast *cut7*$^{+}$ gene. *Nature* **1990**, *347*, (6293), 563-566.

15. Heck, M. M.; Pereira, A.; Pesavento, P.; Yannoni, Y.; Spradling, A. C.; Goldstein, L. S., The kinesin-like protein KLP61F is essential for mitosis in *Drosophila*. *J Cell Biol* **1993**, *123*, (3), 665-679.

16. Le Guellec, R.; Paris, J.; Couturier, A.; Roghi, C.; Philippe, M., Cloning by differential screening of a *Xenopus* cDNA that encodes a kinesin-related protein. *Mol Cell Biol* **1991**, *11*, (6), 3395-3398.

17. Kapoor, T. M.; Mayer, T. U.; Coughlin, M. L.; Mitchison, T. J., Probing spindle assembly mechanisms with monastrol, a small molecule inhibitor of the mitotic kinesin, Eg5. *J Cell Biol* **2000**, *150*, (5), 975-988.

18. Nahaboo, W.; Zouak, M.; Askjaer, P.; Delattre, M., Chromatids segregate without centrosomes during *Caenorhabditis elegans* mitosis in a Ran- and CLASP-dependent manner. *Mol Biol Cell* **2015**, *26*, (11), 2020-2029.

19. Mann, B. J.; Wadsworth, P., Kinesin-5 regulation and function in mitosis. *Trends Cell Biol* **2019**, *29*, (1), 66-79.

20. She, Z. Y.; Yang, W. X., Molecular mechanisms of kinesin-14 motors in spindle assembly and chromosome segregation. *J Cell Sci* **2017**, *130*, (13), 2097-2110.

21. Yukawa, M.; Yamada, Y.; Toda, T., Suppressor analysis uncovers that MAPs and microtubule dynamics balance with the Cut7/Kinesin-5 motor for mitotic spindle assembly in *Schizosaccharomyces pombe*. *G3 (Bethesda)* **2019**, *9*, (1), 269-280.

22. Braun, M.; Drummond, D. R.; Cross, R. A.; McAinsh, A. D., The kinesin-14 Klp2 organizes microtubules into parallel bundles by an ATP-dependent sorting mechanism. *Nat Cell Biol* **2009**, *11*, 724-730.

23. Pidoux, A. L.; LeDizet, M.; Cande, W. Z., Fission yeast pkl1 is a kinesin-related protein involved in mitotic spindle function. *Mol Biol Cell* **1996**, *7*, (10), 1639-1655.

24. Furuta, K.; Edamatsu, M.; Maeda, Y.; Toyoshima, Y. Y., Diffusion and directed movement: in vitro motile properties of fission yeast kinesin-14 Pkl1. *J Biol Chem* **2008**, *283*, (52), 36465-36473.

25. Troxell, C. L.; Sweezy, M. A.; West, R. R.; Reed, K. D.; Carson, B. D.; Pidoux, A. L.; Cande, W. Z.; McIntosh, J. R., *pkl1*$^{+}$ and *klp2*$^{+}$: two kinesins of the Kar3 subfamily in fission yeast perform different functions in both mitosis and meiosis. *Mol Biol Cell* **2001**, *12*, (11), 3476-3488.

26. Ikebe, C.; Konishi, M.; Hirata, D.; Matsusaka, T.; Toda, T., Systematic localization study on novel proteins encoded by meiotically up-regulated ORFs in fission yeast. *Biosci Biotechnol Biochem* **2011**, *75*, (12), 2364-2370.

27. Toya, M.; Sato, M.; Haselmann, U.; Asakawa, K.; Brunner, D.; Antony, C.; Toda, T., γ-Tubulin complex-mediated anchoring of spindle microtubules to spindle-pole bodies requires Msd1 in fission yeast. *Nat Cell Biol* **2007**, *9*, (6), 646-653.

28. Yukawa, M.; Ikebe, C.; Toda, T., The Msd1-Wdr8-Pkl1 complex anchors microtubule minus ends to fission yeast spindle pole bodies. *J Cell Biol* **2015**, *209*, (4), 549-562.

29. Mana-Capelli, S.; McLean, J. R.; Chen, C. T.; Gould, K. L.; McCollum, D., The kinesin-14 Klp2 is negatively regulated by the SIN for proper spindle elongation and telophase nuclear positioning. *Mol Biol Cell* **2012**, *23*, (23), 4592-4600.

30. Yukawa, M.; Yamada, Y.; Yamauchi, T.; Toda, T., Two spatially distinct kinesin-14 proteins, Pkl1 and Klp2, generate collaborative inward forces against kinesin-5 Cut7 in *S. pombe*. *J Cell Sci* **2018**, *131*, (1), 1-11.

31. Rodriguez, A. S.; Batac, J.; Killilea, A. N.; Filopei, J.; Simeonov, D. R.; Lin, I.; Paluh, J. L., Protein complexes at the microtubule organizing center regulate bipolar spindle assembly. *Cell Cycle* **2008**, *7*, (9), 1246-1253.

32. Syrovatkina, V.; Tran, P. T., Loss of kinesin-14 results in aneuploidy via kinesin-5-dependent microtubule protrusions leading to chromosome cut. *Nat Commun* **2015**, *6*, 7322.

33. Olmsted, Z. T.; Colliver, A. G.; Riehlman, T. D.; Paluh, J. L., Kinesin-14 and kinesin-5 antagonistically regulate microtubule nucleation by γ-TuRC in yeast and human cells. *Nat Commun* **2014**, *5*, 5339.

34. Takeda, A.; Saitoh, S.; Ohkura, H.; Sawin, K. E.; Goshima, G., Identification of 15 new bypassable essential genes of fission yeast. *Cell Struct Funct* **2019**, *44*, (2), 113-119.

35. Toda, T.; Adachi, Y.; Hiraoka, Y.; Yanagida, M., Identification of the pleiotropic cell division cycle gene *NDA2* as one of two different α-tubulin genes in *Schizosaccharomyces pombe*. *Cell* **1984**, *37*, (1), 233-242.

36. Hiraoka, Y.; Toda, T.; Yanagida, M., The *NDA3* gene of fission yeast encodes β-tubulin: a cold-sensitive *nda3* mutation reversibly blocks spindle formation and chromosome movement in mitosis. *Cell* **1984**, *39*, (2 Pt 1), 349-358.

37. Carvalho, P.; Tirnauer, J. S.; Pellman, D., Surfing on microtubule ends. *Trends Cell Biol*. **2003**, *13*, (5), 229-237.

38. Beinhauer, J. D.; Hagan, I. M.; Hegemann, J. H.; Fleig, U., Mal3, the fission yeast homologue of the human APC-interacting protein EB-1 is required for microtubule integrity and the maintenance of cell form. *J Cell Biol* **1997**, *139*, (3), 717-728.

39. Asakawa, K.; Toya, M.; Sato, M.; Kanai, M.; Kume, K.; Goshima, T.; Garcia, M. A.; Hirata, D.; Toda, T., Mal3, the fission yeast EB1 homologue, cooperates with Bub1 spindle checkpoint to prevent monopolar attachment. *EMBO Rep* **2005**, *6*, (12), 1194-1200.

40. Fujita, A.; Vardy, L.; Garcia, M. A.; Toda, T., A fourth component of the fission yeast γ-tubulin complex, Alp16, is required for cytoplasmic microtubule integrity and becomes indispensable when γ-tubulin function is compromised. *Mol Biol Cell* **2002**, *13*, (7), 2360-2373.

41. Anders, A.; Lourenco, P. C.; Sawin, K. E., Noncore components of the fission yeast gamma-tubulin complex. *Mol Biol Cell* **2006**, *17*, (12), 5075-93.

42. Masuda, H.; Toda, T., Synergistic role of fission yeast Alp16^{GCP6} and Mzt1^{MOZART1} in γ-tubulin complex recruitment to mitotic spindle pole bodies and spindle assembly. *Mol Biol Cell* **2016**, *27*, (11), 1753-1763.

43. Sato, M.; Vardy, L.; Angel Garcia, M.; Koonrugsa, N.; Toda, T., Interdependency of fission yeast Alp14/TOG and coiled coil protein Alp7 in microtubule localization and bipolar spindle formation. *Mol Biol Cell* **2004**, *15*, (4), 1609-1622.

44. Peset, I.; Vernos, I., The TACC proteins: TACC-ling microtubule dynamics and centrosome function. *Trends Cell Biol* **2008**, *18*, (8), 379-388.

45. Nabeshima, K.; Kurooka, H.; Takeuchi, M.; Kinoshita, K.; Nakaseko, Y.; Yanagida, M., p93^{dis1}, which is required for sister chromatid separation, is a novel microtubule and spindle pole body-associating protein phosphorylated at the Cdc2 target sites. *Genes Dev* **1995**, *9*, 1572-1585.

46. Garcia, M. A.; Vardy, L.; Koonrugsa, N.; Toda, T., Fission yeast ch-TOG/XMAP215 homologue Alp14 connects mitotic spindles with the kinetochore and is a component of the Mad2-dependent spindle checkpoint. *EMBO J*. **2001**, *20*, (13), 3389-3401.

47. Matsuo, Y.; Maurer, S. P.; Yukawa, M.; Zakian, S.; Singleton, M. R.; Surrey, T.; Toda, T., An unconventional interaction between Dis1/TOG and Mal3/EB1 in fission yeast promotes the fidelity of chromosome segregation. *J Cell Sci* **2016**, *129*, (24), 4592-4606.

48. Al-Bassam, J.; Kim, H.; Flor-Parra, I.; Lal, N.; Velji, H.; Chang, F., Fission yeast Alp14 is a dose dependent plus end tracking microtubule polymerase. *Mol Biol Cell* **2012**, *23*, 2878-2890.

49. Hussmann, F.; Drummond, D. R.; Peet, D. R.; Martin, D. S.; Cross, R. A., Alp7/TACC-Alp14/TOG generates long-lived, fast-growing MTs by an unconventional mechanism. *Scientific reports* **2016**, *6*, 20653.

50. Sato, M.; Toda, T., Alp7/TACC is a crucial target in Ran-GTPase-dependent spindle formation in fission yeast. *Nature* **2007**, *447*, (7142), 334-337.

51. Flor-Parra, I.; Iglesias-Romero, A. B.; Chang, F., The XMAP215 ortholog Alp14 promotes microtubule nucleation in fission yeast. *Curr Biol* **2018**, 28, (11), 1681–1691.e4.

52. Florian, S.; Mayer, T. U., Modulated microtubule dynamics enable Hklp2/Kif15 to assemble bipolar spindles. *Cell Cycle* **2011**, *10*, (20), 3533-3544.

53. Kollu, S.; Bakhoum, S. F.; Compton, D. A., Interplay of microtubule dynamics and sliding during bipolar spindle formation in mammalian cells. *Curr Biol* **2009**, *19*, (24), 2108-2113.

54. Gupta, D. R.; Paul, S. K.; Oowatari, Y.; Matsuo, Y.; Kawamukai, M., Multistep regulation of protein kinase A in its localization, phosphorylation and binding with a regulatory subunit in fission yeast. *Curr Genet* **2011**, *57*, (5), 353-365.

55. Hoffman, C. S., Glucose sensing via the protein kinase A pathway in *Schizosaccharomyces pombe*. *Biochem Soc Trans* **2005**, *33*, (Pt 1), 257-260.

56. Hanyu, Y.; Imai, K. K.; Kawasaki, Y.; Nakamura, T.; Nakaseko, Y.; Nagao, K.; Kokubu, A.; Ebe, M.; Fujisawa, A.; Hayashi, T.; Obuse, C.; Yanagida, M., *Schizosaccharomyces pombe* cell division cycle under limited glucose requires Ssp1 kinase, the putative CaMKK, and Sds23, a PP2A-related phosphatase inhibitor. *Genes Cells* **2009**, *14*, (5), 539-554.

57. Rincon, S. A.; Lamson, A.; Blackwell, R.; Syrovatkina, V.; Fraisier, V.; Paoletti, A.; Betterton, M. D.; Tran, P. T., Kinesin-5-independent mitotic spindle assembly requires the antiparallel microtubule crosslinker Ase1 in fission yeast. *Nat Commun* **2017**, *8*, 15286.

58. Grallert, A.; Beuter, C.; Craven, R. A.; Bagley, S.; Wilks, D.; Fleig, U.; Hagan, I. M., *S. pombe* CLASP needs dynein, not EB1 or CLIP170, to induce microtubule instability and slows polymerization rates at cell tips in a dynein-dependent manner. *Genes Dev* **2006**, *20*, (17), 2421-2436.

59. Bratman, S. V.; Chang, F., Stabilization of overlapping microtubules by fission yeast CLASP. *Dev Cell* **2007**, *13*, (6), 812-827.

60. Kelkar, M.; Martin, S. G., PKA antagonizes CLASP-dependent microtubule stabilization to re-localize Pom1 and buffer cell size upon glucose limitation. *Nat Commun* **2015**, *6*, 8445.

61. Ebina, H.; Ji, L.; Sato, M., CLASP promotes microtubule bundling in metaphase spindle independently of Ase1/PRC1 in fission yeast. *Biology open* **2019**, *8*, (10), bio045716.

62. Fu, C.; Ward, J. J.; Loiodice, I.; Velve-Casquillas, G.; Nedelec, F. J.; Tran, P. T., Phospho-regulated interaction between kinesin-6 Klp9p and microtubule bundler Ase1p promotes spindle elongation. *Dev Cell* **2009**, *17*, (2), 257-267.

63. Yukawa, M.; Okazaki, M.; Teratani, Y.; Furuta, K.; Toda, T., Kinesin-6 Klp9 plays motor-dependent and -independent roles in collaboration with Kinesin-5 Cut7 and the microtubule crosslinker Ase1 in fission yeast. *Scientific reports* **2019**, *9*, (1), 7336.

64. Choi, S. H.; McCollum, D., A role for metaphase spindle elongation forces in correction of merotelic kinetochore attachments. *Curr Biol* **2012**, *22*, 225-230.

65. Meadows, J. C.; Lancaster, T. C.; Buttrick, G. J.; Sochaj, A. M.; Messin, L. J.; Del Mar Mora-Santos, M.; Hardwick, K. G.; Millar, J. B., Identification of a Sgo2-dependent but Mad2-independent pathway controlling anaphase onset in fission yeast. *Cell Rep* **2017**, *18*, (6), 1422-1433.

66. Kruger, L. K.; Sanchez, J. L.; Paoletti, A.; Tran, P. T., Kinesin-6 regulates cell-size-dependent spindle elongation velocity to keep mitosis duration constant in fission yeast. *eLife* **2019**, *8*, e42182.

67. Yanagida, M., Fission yeast cut mutations revisited: control of anaphase. *Trends Cell Biol* **1998**, *8*, 144-149.

68. Loiodice, I.; Staub, J.; Setty, T. G.; Nguyen, N. P.; Paoletti, A.; Tran, P. T., Ase1p organizes antiparallel microtubule arrays during interphase and mitosis in fission yeast. *Mol Biol Cell* **2005**, *16*, (4), 1756-1768.

69. Yamashita, A.; Sato, M.; Fujita, A.; Yamamoto, M.; Toda, T., The roles of fission yeast ase1 in mitotic cell division, meiotic nuclear oscillation, and cytokinesis checkpoint signaling. *Mol Biol Cell* **2005**, *16*, (3), 1378-1395.

70. Blackwell, R.; Edelmaier, C.; Sweezy-Schindler, O.; Lamson, A.; Gergely, Z. R.; O'Toole, E.; Crapo, A.; Hough, L. E.; McIntosh, J. R.; Glaser, M. A.; Betterton, M. D., Physical determinants of bipolar mitotic spindle assembly and stability in fission yeast. *Sci Adv* **2017**, *3*, (1), e1601603.

71. Edelmaier, C.; Lamson, A. R.; Gergely, Z. R.; Ansari, S.; Blackwell, R.; McIntosh, J. R.; Glaser, M. A.; Betterton, M. D., Mechanisms of chromosome biorientation and bipolar spindle assembly analyzed by computational modeling. *eLife* **2020**, *9*, e48787.

72. Bishop, J. D.; Han, Z.; Schumacher, J. M., The *Caenorhabditis elegans* Aurora B kinase AIR-2 phosphorylates and is required for the localization of a BimC kinesin to meiotic and mitotic spindles. *Mol Biol Cell* **2005**, *16*, (2), 742-756.

73. Saunders, A. M.; Powers, J.; Strome, S.; Saxton, W. M., Kinesin-5 acts as a brake in anaphase spindle elongation. *Curr Biol* **2007**, *17*, (12), R453-R454.

74. Yukawa, M.; Kawakami, T.; Pinder, C.; Toda, T., Two XMAP215/TOG microtubule polymerases, Alp14 and Dis1, play non-exchangeable, distinct roles in microtubule organisation in fission yeast. *International journal of molecular sciences* **2019**, *20*, (20), E5108.

75. Al-Bassam, J.; Chang, F., Regulation of microtubule dynamics by TOG-domain proteins XMAP215/Dis1 and CLASP. *Trends Cell Biol* **2011**, *21*, 604-614.

76. Zheng, F.; Li, T.; Jin, D. Y.; Syrovatkina, V.; Scheffler, K.; Tran, P. T.; Fu, C., Csi1p recruits alp7p/TACC to the spindle pole bodies for bipolar spindle formation. *Mol Biol Cell* **2014**, *25*, (18), 2750-2760.

77. Costa, J.; Fu, C.; Khare, V. M.; Tran, P. T., csi2p modulates microtubule dynamics and organizes the bipolar spindle for chromosome segregation. *Mol Biol Cell* **2014**, *25*, (24), 3900-3908.

78. Hou, H.; Zhou, Z.; Wang, Y.; Wang, J.; Kallgren, S. P.; Kurchuk, T.; Miller, E. A.; Chang, F.; Jia, S., Csi1 links centromeres to the nuclear envelope for centromere clustering. *J Cell Biol* **2012**, *199*, 735-744.

79. Yukawa, M.; Kawakami, T.; Okazaki, M.; Kume, K.; Tang, N. H.; Toda, T., A microtubule polymerase cooperates with the kinesin-6 motor and a microtubule cross-linker to promote bipolar spindle assembly in the absence of kinesin-5 and kinesin-14 in fission yeast. *Mol Biol Cell* **2017**, *28*, (25), 3647-3659.

80. Tran, P. T.; Marsh, L.; Doye, V.; Inoue, S.; Chang, F., A mechanism for nuclear positioning in fission yeast based on microtubule pushing. *J Cell Biol* **2001**, *153*, (2), 397-412.

81. Daga, R. R.; Yonetani, A.; Chang, F., Asymmetric microtubule pushing forces in nuclear centering. *Curr Biol* **2006**, *16*, (15), 1544-1550.

82. Wu, H. Y.; Nazockdast, E.; Shelley, M. J.; Needleman, D. J., Forces positioning the mitotic spindle: theories, and now experiments. *Bioessays* **2017**, *39*, (2).

83. Garzon-Coral, C.; Fantana, H. A.; Howard, J., A force-generating machinery maintains the spindle at the cell center during mitosis. *Science* **2016**, *352*, (6289), 1124-1127.

84. Shirasugi, Y.; Sato, M., Kinetochore-mediated outward force promotes spindle pole separation in fission yeast. *Mol Biol Cell* **2019**, *30*, (22), 2802-2813.

85. Hara, M.; Fukagawa, T., Dynamics of kinetochore structure and its regulations during mitotic progression. *Cell Mol Life Sci* **2020**, *77*, s00018-020-03472-4.

86. Nabetani, A.; Koujin, T.; Tsutsumi, C.; Haraguchi, T.; Hiraoka, Y., A conserved protein, Nuf2, is implicated in connecting the centromere to the spindle during chromosome segregation: a link between the kinetochore function and the spindle checkpoint. *Chromosoma* **2001**, *110*, (5), 322-334.

87. Bernard, P.; Maure, J. F.; Partridge, J. F.; Genier, S.; Javerzat, J. P.; Allshire, R. C., Requirement of heterochromatin for cohesion at centromeres. *Science* **2001**, *294*, 2539-2542.

88. Nonaka, N.; Kitajima, T.; Yokobayashi, S.; Xiao, G.; Yamamoto, M.; Grewal, S. I.; Watanabe, Y., Recruitment of cohesin to heterochromatic regions by Swi6/HP1 in fission yeast. *Nat Cell Biol* **2002**, *4*, (1), 89-93.

89. Michaelis, C.; Ciosk, R.; Nasmyth, K., Cohesins: chromosomal proteins that prevent premature separation of sister chromatids. *Cell* **1997**, *91*, (1), 35-45.

90. Tatebayashi, K.; Kato, J.; Ikeda, H., Isolation of a *Schizosaccharomyces pombe rad21*^{ts} mutant that is aberrant in chromosome segregation, microtubule function, DNA repair and sensitive to hydroxyurea: possible involvement of Rad21 in ubiquitin-mediated proteolysis. *Genetics* **1998**, *148*, 49-57.

91. Toso, A.; Winter, J. R.; Garrod, A. J.; Amaro, A. C.; Meraldi, P.; McAinsh, A. D., Kinetochore-generated pushing forces separate centrosomes during bipolar spindle assembly. *J Cell Biol* **2009**, *184*, (3), 365-372.

92. Gayek, A. S.; Ohi, R., Kinetochore-microtubule stability governs the metaphase requirement for Eg5. *Mol Biol Cell* **2014**, *25*, (13), 2051-2060.

93. Tanenbaum, M. E.; Macurek, L.; Janssen, A.; Geers, E. F.; Alvarez-Fernandez, M.; Medema, R. H., Kif15 cooperates with eg5 to promote bipolar spindle assembly. *Curr Biol* **2009**, *19*, (20), 1703-1711.

94. Vanneste, D.; Takagi, M.; Imamoto, N.; Vernos, I., The role of Hklp2 in the stabilization and maintenance of spindle bipolarity. *Curr Biol* **2009**, *19*, (20), 1712-1717.

95. Sturgill, E. G.; Norris, S. R.; Guo, Y.; Ohi, R., Kinesin-5 inhibitor resistance is driven by kinesin-12. *J Cell Biol* **2016**, *213*, (2), 213-227.

96. Kaseda, K.; McAinsh, A. D.; Cross, R. A., Dual pathway spindle assembly increases both the speed and the fidelity of mitosis. *Biology open* **2012**, *1*, (1), 12-18.

97. Hata, S.; Pastor Peidro, A.; Panic, M.; Liu, P.; Atorino, E.; Funaya, C.; Jakle, U.; Pereira, G.; Schiebel, E., The balance between KIFC3 and EG5 tetrameric kinesins controls the onset of mitotic spindle assembly. *Nat Cell Biol* **2019**, *21*, (9), 1138-1151.

98. Tanenbaum, M. E.; Medema, R. H., Mechanisms of centrosome separation and bipolar spindle assembly. *Dev Cell* **2010**, *19*, (6), 797-806.

99. Hashimoto, T., A ring for all: γ-tubulin-containing nucleation complexes in acentrosomal plant microtubule arrays. *Curr Opin Plant Biol* **2013**, *16*, (6), 698-703.

100. Meunier, S.; Vernos, I., Acentrosomal microtubule assembly in mitosis: the where, when, and now. *Trends Cell Biol* **2016**, *26*, (2), 80-87.

101. Takeda, Y.; Kuroki, K.; Chinen, T.; Kitagawa, D., Centrosomal and non-centrosomal functions emerged through eliminating centrosomes. *Cell Struct Funct* **2020**, *45*, csf.20007.

102. Gruss, O. J., Animal female meiosis: the challenges of eliminating centrosomes. *Cells* **2018**, *7*, (7), cells7070073.

103. Karsenti, E.; Vernos, I., The mitotic spindle: a self-made machine. *Science* **2001**, *294*, (5542), 543-547.

104. Khodjakov, A.; Cole, R. W.; Oakley, B. R.; Rieder, C. L., Centrosome-independent mitotic spindle formation in vertebrates. *Curr Biol* **2000**, *10*, 59-67.

105. Chinen, T.; Yamamoto, S.; Takeda, Y.; Watanabe, K.; Kuroki, K.; Hashimoto, K.; Takao, D.; Kitagawa, D., NuMA assemblies organize microtubule asters to establish spindle bipolarity in acentrosomal human cells. *EMBO J* **2020**, *39*, (2), e102378.

106. Basto, R.; Lau, J.; Vinogradova, T.; Gardiol, A.; Woods, C. G.; Khodjakov, A.; Raff, J. W., Flies without centrioles. *Cell* **2006**, *125*, (7), 1375-1386.

107. Walczak, C. E.; Vernos, I.; Mitchison, T. J.; Karsenti, E.; Heald, R., A model for the proposed roles of different microtubule-based motor proteins in establishing spindle bipolarity. *Curr Biol* **1998**, *8*, (16), 903-913.

108. Shu, S.; Iimori, M.; Wakasa, T.; Ando, K.; Saeki, H.; Oda, Y.; Oki, E.; Maehara, Y., The balance of forces generated by kinesins controls spindle polarity and chromosomal heterogeneity in tetraploid cells. *J Cell Sci* **2019**, *132*, (24), 1-14.

109. El-Nassan, H. B., Advances in the discovery of kinesin spindle protein (Eg5) inhibitors as antitumor agents. *Eur J Med Chem* **2013**, *62*, 614-631.

110. Wacker, S. A.; Houghtaling, B. R.; Elemento, O.; Kapoor, T. M., Using transcriptome sequencing to identify mechanisms of drug action and resistance. *Nat Chem Biol* **2012**, *8*, (3), 235-237.

111. Ma, H. T.; Erdal, S.; Huang, S.; Poon, R. Y., Synergism between inhibitors of Aurora A and KIF11 overcomes KIF15-dependent drug resistance. *Molecular oncology* **2014**, *8*, (8), 1404-1418.

112. Dumas, M. E.; Sturgill, E. G.; Ohi, R., Resistance is not futile: Surviving Eg5 inhibition. *Cell Cycle* **2016**, *15*, (21), 2845-2847.

113. Pena, A.; Sweeney, A.; Cook, A. D.; Topf, M.; Moores, C. A., Structure of microtubule-trapped human kinesin-5 and its mechanism of inhibition revealed using cryoelectron microscopy. *Structure* **2020**, *28*, 1–8.

114. Huszar, D.; Theoclitou, M. E.; Skolnik, J.; Herbst, R., Kinesin motor proteins as targets for cancer therapy. *Cancer Metastasis Rev* **2009**, *28*, (1-2), 197-208.

115. Mitchison, T. J., The proliferation rate paradox in antimitotic chemotherapy. *Mol Biol Cell* **2012**, *23*, (1), 1-6.

116. Winters, L.; Ban, I.; Prelogovic, M.; Kalinina, I.; Pavin, N.; Tolic, I. M., Pivoting of microtubules driven by minus-end-directed motors leads to spindle assembly. *BMC Biol* **2019**, *17*, (1), 42.

117. Akera, T.; Goto, Y.; Sato, M.; Yamamoto, M.; Watanabe, Y., Mad1 promotes chromosome congression by anchoring a kinesin motor to the kinetochore. *Nat Cell Biol* **2015**, *17*, 1124–1133.

118. Bao, X. X.; Spanos, C.; Kojidani, T.; Lynch, E. M.; Rappsilber, J.; Hiraoka, Y.; Haraguchi, T.; Sawin, K. E., Exportin Crm1 is repurposed as a docking protein to generate microtubule organizing centers at the nuclear pore. *eLife* **2018**, *7*, e33465.

119. Liu, W.; Zheng, F.; Wang, Y.; Fu, C., Alp7-Mto1 and Alp14 synergize to promote interphase microtubule regrowth from the nuclear envelope. *J Mol Cell Biol* **2019**, *11*, (11), 944-955.