Dynein, Lis1 and CLIP-170 counteract Eg5-dependent centrosome separation during bipolar spindle assembly

Marvin E Tanenbaum¹, Libor Macůrek¹,
Niels Galjart² and René H Medema^{1,*}

¹Department of Medical Oncology, University Medical Center Utrecht, Utrecht, The Netherlands and ²Department of Cell Biology and Genetics, Erasmus Medical Centre, Rotterdam, The Netherlands

Bipolar spindle assembly critically depends on the microtubule plus-end-directed motor Eg5 that binds antiparallel microtubules and slides them in opposite directions. As such, Eg5 can produce the necessary outward force within the spindle that drives centrosome separation and inhibition of this antiparallel sliding activity results in the formation of monopolar spindles. Here, we show that upon depletion of the minus-end-directed motor dynein, or the dynein-binding protein Lis1, bipolar spindles can form in human cells with substantially less Eg5 activity, suggesting that dynein and Lis1 produce an inward force that counteracts the Eg5-dependent outward force. Interestingly, we also observe restoration of spindle bipolarity upon depletion of the microtubule plus-end-tracking protein CLIP-170. This function of CLIP-170 in spindle bipolarity seems to be mediated through its interaction with dynein, as loss of CLIP-115, a highly homologous protein that lacks the dynein–dynactin interaction domain, does not restore spindle bipolarity. Taken together, these results suggest that complexes of dynein, Lis1 and CLIP-170 crosslink and slide microtubules within the spindle, thereby producing an inward force that pulls centrosomes together.

The EMBO Journal (2008) **27**, 3235–3245. doi:10.1038/emboj.2008.242; Published online 20 November 2008
Subject Categories: cell & tissue architecture; cell cycle
Keywords: bipolar; CLIP-170; dynein; eg5; spindle

Introduction

During mitosis, paired sister chromatids must attach to opposite poles of a bipolar spindle to ensure proper segregation into the two daughter cells. Despite the importance of spindle bipolarity for correct chromosome segregation, the mechanisms that control bipolar spindle assembly are still largely unclear. At the onset of mitosis, the duplicated centrosomes migrate around the nucleus, so that the DNA

is positioned between the two centrosomes at the time of nuclear envelope breakdown (NEB). Studies in worms and flies have implicated the microtubule minus-end-directed motor dynein in the initial separation of the centrosomes (Gonczy *et al*, 1999; Robinson *et al*, 1999; Sharp *et al*, 2000), but it is unclear whether this also applies to mammalian cells. Furthermore, it has been well established that the plus-end-directed microtubule motor Eg5 (kinesin-5) is important for centrosome separation after NEB (Hoyt *et al*, 1992; Roof *et al*, 1992; Sawin *et al*, 1992; Heck *et al*, 1993; Blangy *et al*, 1995), probably by sliding antiparallel microtubules apart (Kapitein *et al*, 2005). In addition, the kinesin-13 family members Kif2a and Kif2b have also been shown to be required for spindle bipolarity, but rather than sliding microtubules apart, they appear to modulate kinetochore–microtubule dynamics, thereby preventing spindle collapse (Ganem and Compton, 2004; Manning *et al*, 2007). Interestingly, neither Eg5 nor Kif2a activity is absolutely essential for bipolar spindle formation, as bipolar spindles can form after simultaneous inhibition of the minus-end-directed kinesin NCD/HSET and Eg5 in both *Drosophila* and mammalian cells (Mountain *et al*, 1999; Sharp *et al*, 1999b) or after double depletion of Kif2a and the microtubule depolymerase MCAK in mammalian cells (Ganem and Compton, 2004). These results suggest that a correct balance of forces is more important for bipolar spindle assembly than the function of any individual protein.

The minus-end-directed motor complex dynein–dynactin has several important functions during mitosis, including the poleward transport of spindle checkpoint components, spindle pole focusing and fast poleward movement of chromosomes (Merdes *et al*, 1996; Howell *et al*, 2001; Wojcik *et al*, 2001; Maiato *et al*, 2004; Yang *et al*, 2007). Furthermore, experiments carried out in *Xenopus* egg extracts have shown that the dynein–dynactin complex functionally antagonizes Eg5 during spindle assembly (Mitchison *et al*, 2005). However, the molecular mechanism of this antagonism is unknown. Furthermore, it is unclear whether such an antagonism exists in intact cells as well, as in *Drosophila* embryos or S2 cells, dynein does not antagonize Eg5 (Goshima and Vale, 2003), but rather dynein appears to cooperate with Eg5 to promote centrosome separation (Sharp *et al*, 2000).

CLIP-170 is an evolutionarily conserved microtubule-binding protein and belongs to a large family of proteins that specifically binds to the plus-ends of growing microtubules (Akhmanova and Hoogenraad, 2005; Galjart, 2005). The N terminus of CLIP-170, which is highly homologous to the N terminus of CLIP-115, contains two microtubule-binding domains called CAP-Gly domains. In interphase, CLIP-115 and CLIP-170 control microtubule dynamics by promoting the transition of microtubule shrinkage to growth (called a ‘rescue’) and it was shown that the CAP-Gly-containing N terminus of CLIP-115 and CLIP-170 was necessary and sufficient for this function (Komarova *et al*, 2002). The

*Corresponding author. Department of Medical Oncology, University Medical Center Utrecht, Universiteitsweg 100, Stratum 2.118, 3584 CG Utrecht, The Netherlands. Tel.: +31 88 75 68066; Fax: +31 88 75 68479; E-mail: r.h.medema@umcutrecht.nl

Received: 4 July 2008; accepted: 23 October 2008; published online: 20 November 2008

C terminus of CLIP-170 contains two zinc-finger domains that interact with the dynein–dynactin complex components, p150glued and Lis1 (Coquelle *et al*, 2002; Tai *et al*, 2002; Lansbergen *et al*, 2004) and it is thought that CLIP-170 can function as a physical linker between the microtubule plus-end and the dynein–dynactin complex due to its ability to bind microtubules and the dynein/dynactin complex simultaneously (Komarova *et al*, 2002; Lansbergen *et al*, 2004). Indeed, it was shown that CLIP-170 targets p150glued to the microtubule plus-end (Valetti *et al*, 1999; Komarova *et al*, 2002; Lansbergen *et al*, 2004). Similarly, the yeast CLIP-170 homologue (Bik1) was shown to target dynein to microtubules through its C-terminal domain (Sheeman *et al*, 2003). CLIP-115, on the other hand, lacks the C-terminal zinc-fingers and therefore cannot interact with p150glued and Lis1. In fact, it is thought that CLIP-115 functions as a negative regulator of the CLIP-170-dynein–dynactin complex through two distinct activities. First, CLIP-115 competes with CLIP-170 for microtubule plus-end binding, thereby limiting the amount of dynactin that is recruited to the microtubule plus-ends (Hoogenraad *et al*, 2002). Second, the microtubule-binding domain of CLIP-115 competes with p150glued and Lis1 for binding to the zinc-fingers in the C terminus of CLIP-170 (Lansbergen *et al*, 2004). Thus, CLIP-115 cooperates with CLIP-170 to promote microtubule rescue, but antagonizes CLIP-170 in its control of the dynein–dynactin complex.

Results

Dynein antagonizes Eg5 during bipolar spindle assembly

To investigate the function of dynein in spindle bipolarity in intact human cells, dynein was depleted using an siRNA targeting the dynein heavy chain (DHC) in human osteosarcoma cells (U2OS) (for all siRNA information, see Supplementary Table 1). DHC knockdown resulted in a severe dispersal of the Golgi apparatus in interphase (Supplementary Figure S1A) and a prominent mitotic arrest (data not shown). Spindle poles were less focused in DHC-depleted cells as compared with control cells (Supplementary Figure S1B), consistent with earlier observations (Corthesy-Theulaz *et al*, 1992; Maiato *et al*, 2004; Draviam *et al*, 2006). Furthermore, knockdown of DHC also resulted in loss of the dynein intermediate chain (DIC) (Supplementary Figure S1C), as was reported previously (Grigoriev *et al*, 2007), further validating the efficiency of the siRNA.

To analyse a possible antagonism between dynein and Eg5, DHC-depleted cells were treated with the Eg5 inhibitor S-trityl-L-cysteine (STLC) (DeBonis *et al*, 2004) (for IC₅₀ values of the inhibitors used in this study, see Supplementary Table S2) for 18 h at a final concentration of 2 μ M and the number of bipolar spindles with separated centrosomes was quantified. In untransfected cells or cells transfected with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) siRNA as a control, the percentage of STLC-treated mitotic cells with bipolar spindles was 5 ± 1 and $4 \pm 1\%$, respectively. In contrast, in DHC-depleted cells treated with STLC, $37 \pm 9\%$ of spindles were bipolar with separated centrosomes (Figure 1A and B) (three independent experiments). Similar to U2OS cells, DHC depletion in HeLa cells also resulted in a strong restoration of spindle bipolarity in STLC-treated cells (1 ± 1 versus $54 \pm 10\%$ bipolar spindles in GAPDH-depleted cells

versus DHC-depleted cells, respectively) (Supplementary Figure S2A). These results were not due to aspecific effects of STLC, as similar results were obtained with another Eg5 inhibitor, monastrol (Mayer *et al*, 1999) (Supplementary Figure S2B) (four independent experiments). In addition, DHC depletion was also able to restore spindle bipolarity after Eg5 RNAi (Supplementary Figure S3), demonstrating that dynein and Eg5 indeed function antagonistically during spindle assembly. Finally, to exclude that the effects of DHC siRNA were due to off-target effects, a second independent DHC siRNA was tested. Also using this siRNA, a large increase in the percentage of bipolar spindle was observed after STLC treatment (Supplementary Figure S2C). Surprisingly, depletion of HSET did not restore spindle bipolarity in STLC-treated HeLa or U2OS cells (Supplementary Figure S4 and data not shown), even though HSET protein levels were very strongly reduced (Supplementary Figure S4). Taken together, these results show for the first time that in intact mammalian cells Eg5 and dynein function antagonistically during spindle assembly and suggest that dynein produces an inward force within the mitotic spindle that counteracts the outward force produced by Eg5.

Lis1 antagonizes Eg5 during bipolar spindle assembly

Lis1 is a highly conserved protein that associates with the dynein–dynactin complex and was shown to stimulate the ATPase activity of dynein (Mesngon *et al*, 2006). Furthermore, Lis1 was suggested to link CLIP-170 to the dynein complex (Coquelle *et al*, 2002; Tai *et al*, 2002; Lansbergen *et al*, 2004). We therefore tested whether Lis1, similar to dynein, antagonizes Eg5 during bipolar spindle assembly. Like depletion of DHC depletion of Lis1 resulted in dispersal of the Golgi apparatus and an increase in the mitotic index (3 versus 8% mitotic cells) (Supplementary Figure S5). Lis1-depleted cells treated with 2 μ M STLC for 18 h showed a significant increase in the percentage of bipolar spindles, from $4 \pm 1\%$ in control cells to $19 \pm 12\%$ in Lis1 RNAi cells (Figure 1C) (three independent experiments). These results further substantiate the fact that the dynein complex and Eg5 function antagonistically and indicate that Lis1 is required for this function of dynein.

Dynein depletion can partially overcome the mitotic arrest after Eg5 inhibition

Inhibition of Eg5 results in monopolar spindle formation and as a result cells fail to progress to anaphase. Indeed, while $41 \pm 7\%$ of mitotic cells transfected with GAPDH siRNA were in anaphase or telophase at the time of fixation (Figure 1D), the vast majority of monastrol-treated cells were arrested in prometaphase, with only $1 \pm 1\%$ of cells in anaphase/telophase. In contrast to GAPDH-depleted cells, only $11 \pm 3\%$ of DHC-depleted mitotic cells were in anaphase or telophase (Figure 1D), likely due to a combination of defects in chromosome alignment and spindle checkpoint inactivation (Howell *et al*, 2001; Draviam *et al*, 2006). Thus, inhibition of either dynein or Eg5 alone results in a strong mitotic arrest. The observation that simultaneous inhibition of dynein and Eg5 partially restores bipolar spindle assembly (Figure 1A and B) would predict that simultaneous inhibition might also increase the fraction of cells in anaphase/telophase. Indeed, we found that $6 \pm 2\%$ of mitotic DHC-depleted cells treated with monastrol were in anaphase or telophase (Figure 1D) (three independent experiments), in contrast to only 1% in

Figure 1 Dynein and Lis1 antagonize Eg5 during bipolar spindle assembly. U2OS (A–D, F) or HeLa (G) cells were transfected by reverse transfection with indicated siRNA and 48 h after transfection 2 μM STLC (A–C) or 200 μM monastrol (D, F) was added for 18 h. Cells were then fixed and spindles (α-tubulin), centrosomes (γ-tubulin) and DNA (DAPI) were stained. Either the percentage of mitotic cells with bipolar spindles was determined (B, C) or the percentage of mitotic cells in anaphase/telophase was determined (D). (E) HeLa cells were treated as in (G), but 48 h after transfection cells were treated with indicated concentrations of STLC for 18 h and the percentage of mitotic cells with bipolar spindles was determined. (H) HeLa cells were transfected as in (G), but 72 h after transfection cells were treated with 20 μM STLC and MG132 for 30 min before fixation. All scale bars indicate 5 μm.

control-transfected cells treated with monastrol. These results suggest that bipolar spindles formed in the absence of both dynein and Eg5 activity are capable of aligning chromosomes on the metaphase plate and entering anaphase. However, as a large fraction of DHC-depleted cells treated with monastrol still arrest before anaphase, it is likely that dynein has additional functions during spindle assembly, consistent with earlier reports (Merdes *et al*, 2000; Howell *et al*, 2001).

Residual Eg5 activity drives bipolar spindle assembly in dynein-depleted cells treated with STLC

How do spindles become bipolar in dynein- or Lis1-depleted cells treated with Eg5 inhibitors? Even if a minus-end-directed force is absent, it is likely that a plus-end-directed force is still required to separate the centrosomes. One possibility is that inhibition of Eg5 is not complete upon treatment with indicated Eg5 inhibitors and that residual Eg5 activity is sufficient to drive centrosome separation when the opposing inward force is severely diminished. Indeed, increasing the concentration of STLC to 5 μ M strongly decreased the percentage of bipolar spindles in DHC-depleted HeLa cells from $56 \pm 12\%$ bipolar spindles at 2 μ M STLC to $19 \pm 7\%$ at 5 μ M (three independent experiments) (Figure 1E). However, increasing the concentration of STLC to 40 μ M did not further reduce the percentage of cells with a bipolar spindle (Figure 1E). Similar results were obtained in U2OS cells (Supplementary Figure S6A). To ensure that the reduction of the fraction of cells that form a bipolar spindle at 5 μ M STLC is not due to aspecific effects of a high dose of this drug, 2 μ M STLC treatment was also combined with Eg5 siRNA to further reduce Eg5 activity. Indeed, the percentage of bipolar spindles was reduced from $65 \pm 7\%$ in DHC/GAPDH-depleted cells treated with 2 μ M STLC to $35 \pm 2\%$ in DHC/Eg5-depleted cells (Supplementary Figure S6B). Thus, assembly of bipolar spindles in DHC-depleted cells treated with 2 μ M STLC is largely dependent on residual Eg5 activity. On the basis of these data, we conclude that reduction of a dynein-dependent inward force decreases the amount of Eg5 activity required to push centrosomes apart.

Interestingly, even at the highest concentration of STLC many of the DHC-depleted cells that failed to separate centrosomes contained kinetochore-microtubules facing away from the centrosomes (Supplementary Figure S7), similar to what has been shown for monopolar spindles after NuMA inhibition (Khodjakov *et al*, 2003). In a few cells, these kinetochore-microtubules had become sufficiently long so that the spindle appeared bipolar even though centrosomes remained unseparated (Figure 1F). Such monoastrol bipolar spindles were never observed in GAPDH-depleted cells. This suggests that two types of bipolar spindles can form when dynein and Eg5 are inhibited simultaneously: centrosomal and acentrosomal bipolar spindles.

Depletion of dynein does not alter Eg5 localization within the spindle

Eg5 is a plus-end-directed motor, but surprisingly in spindles Eg5 was shown to localize mainly in the vicinity of the spindle poles, near the microtubule minus-ends in *Xenopus* extracts (Sawin *et al*, 1992). Interestingly, Eg5 binds to the p150glued subunit of dynactin (Blangy *et al*, 1997; Kapoor and Mitchison, 2001) and the localization of Eg5 to the microtubule minus-ends in *Xenopus* egg extracts is dependent

on dynein activity (Kapoor and Mitchison, 2001; Uteng *et al*, 2008). Furthermore, disruption of the dynein complex reduces the amount of Eg5 that binds to the spindle in *Xenopus* extracts (Uteng *et al*, 2008). We therefore wondered whether the rescue of spindle bipolarity in DHC-depleted cells treated with STLC was due to mislocalization of Eg5. Consistent with earlier data, we found strong Eg5 staining near spindle poles (Figure 1G, upper panel); however, when Eg5 staining was compared with α -tubulin staining, both stainings completely overlapped and no preference for the spindle poles could be observed (Figure 1G). Importantly, the staining for Eg5 was specific, as staining completely disappeared upon Eg5 RNAi (Supplementary Figure S8). Furthermore, Eg5 staining on the spindle was not reduced, nor was the localization detectably altered after DHC RNAi (Figure 2A, lower panel), suggesting that in mammalian cells dynein does not have a major role in regulating steady-state Eg5 localization.

Dynein promotes spindle collapse when Eg5 is inhibited after centrosome separation

To test whether dynein is producing a minus-end-directed force that can pull centrosomes together within an established bipolar spindle, cells were treated with the proteasome inhibitor MG132 to block them in mitosis and simultaneously with a very high dose of STLC (20 μ M) for 30 min to fully inhibit Eg5. Cells were then fixed and the percentage of monopolar spindles was determined. In control cells, $55 \pm 10\%$ of mitotic cells contained a monopolar spindle after this treatment. Interestingly, this means that 45% of spindles do not collapse upon full Eg5 inhibition, which is consistent with the fact that Eg5 is not essential for maintaining spindle bipolarity during metaphase (our unpublished observation). Strikingly, only $9 \pm 7\%$ of DHC-depleted cells contained a monopolar spindle after the same treatment (Figure 1H). Monopolar spindles observed in control cells are not due to cells entering mitosis during this brief STLC treatment, as at this concentration of STLC both control cells and DHC-depleted cells form almost exclusively monopolar spindles (100 and 81%, respectively) and only very few monopolar spindles are observed in the DHC-depleted population. Thus, we conclude that full inhibition of Eg5 during mitosis results in spindle collapse in approximately half of the cells and that this is completely dependent on dynein.

Dynein does not antagonize Eg5-dependent centrosome separation during prophase

In *Drosophila* cells, Eg5 is not involved in centrosome separation until after NEB (Sharp *et al*, 1999a), although there is evidence suggesting that in mammalian cells Eg5 might contribute to centrosome separation already during prophase (Whitehead and Rattner, 1998). Therefore, we first examined the role of Eg5 during centrosome separation in prophase. For this, we determined the distance between centrosomes in late prophase cells. In untreated control cells, the average distance between centrosomes was $11.7 \pm 2.3 \mu$ m, whereas treatment with 2 μ M STLC decreased this distance to $4.6 \pm 0.9 \mu$ m (Figure 2A and B; see Supplementary Figure S9) (three independent experiments). Increasing the dose of STLC to 20 μ M lead to an even stronger decrease in the inter-centrosomal distance to only $3.1 \pm 0.5 \mu$ m (Figure 2B). Next, centrosome separation was followed using time-lapse microscopy of cells expressing YFP- α -tubulin. In the majority

Figure 2 Dynein does not antagonize Eg5-dependent centrosome separation during prophase. **(A, B)** U2OS cells were treated with indicated concentrations of STLC for 4 h, fixed and microtubules (α -tubulin), centrosomes (γ -tubulin) and DNA (DAPI) were stained. Inter-centrosomal distances were calculated using Zeiss LSM 510 confocal software **(B)**. **(C)** U2OS cells stably expressing YFP- α -tubulin were either untreated or treated with 2 μ M STLC and analysed by time-lapse microscopy. Images were acquired every 3 min. **(D)** Cells were transfected with either GAPDH or DHC siRNA and were treated and analysed as in **(B)**. Scale bars indicate 5 μ m.

of control cells (82%, $n = 50$), centrosomes could clearly be seen moving apart along the nucleus before NEB (Figure 2C, upper panel). In contrast, in only 14% of STLC-treated cells ($n = 49$), centrosome separation was detected in prophase and most cells entered mitosis with unseparated centrosomes (Figure 2C, lower panel). These results show that in mammalian cells, in contrast to *Drosophila* cells, Eg5 is required for centrosome separation during prophase.

Next, we wondered whether inhibition of dynein would affect centrosome separation during prophase. Although centrosomes often detached from the nuclear envelope in DHC-depleted cells (unpublished observation), centrosomes still separated in these cells, but the average inter-centrosomal distance was slightly decreased ($11.6 \pm 1.6 \mu\text{m}$ versus $8.5 \pm 2.3 \mu\text{m}$, for GAPDH- and DHC-depleted cells, respectively) (Figure 2D and Supplementary Figure S9, four independent experiments). To determine whether DHC depletion could restore centrosome separation in STLC-treated cells, either GAPDH- or DHC-depleted cells were treated with 2 μ M

STLC for 4 h and the average inter-centrosomal distance in late prophase cells was calculated. Surprisingly, although the average inter-centrosomal distance in GAPDH-depleted cells treated with STLC was $4.1 \pm 2.3 \mu\text{m}$, this distance was reduced to only $1.3 \pm 0.8 \mu\text{m}$ in DHC-depleted cells treated with STLC. In addition, in 15% of control cells centrosomes were completely unseparated in late prophase, whereas this was the case in 62% of DHC-depleted cells (Supplementary Figure S9). Thus, it appears that during prophase dynein does not antagonize Eg5, but rather dynein might cooperate with Eg5 to promote initial centrosome separation, consistent with a role for dynein in centrosome separation in prophase in *Drosophila* and *Caenorhabditis elegans* embryos as well (Gonczy *et al*, 1999; Sharp *et al*, 2000).

CLIP-170 antagonizes Eg5 during bipolar spindle assembly

Eg5 can slide microtubules apart by forming a homotetramer and walking towards the plus-end of two antiparallel

Figure 3 CLIP-170 antagonizes Eg5 during bipolar spindle assembly. (A–E) U2OS cells were transfected with indicated siRNAs and 36 h after the first transfection, cells were re-transfected. Monastrol (200 μ M) was added 56 h after the first round of transfection and 18 h later cells were lysed and processed for western blot analysis (A) or cells were fixed and stained for α -tubulin and γ -tubulin and DNA (B–E, H). The percentage of mitotic cells that formed a bipolar spindle was quantified based on the spindle and centrosome staining (C, H). Spindle and DNA staining was used to quantify the percentage of anaphase/telophase cells (D). (E) Representative image of monastrol-treated, CLIP-170-depleted cell in anaphase. (F, G) U2OS cells stably expressing YFP- α -tubulin were transfected as in (A–E). At 72 h after transfection, cells were treated with 2 μ M STLC and filmed. Images were acquired every 5 min. Spindles were scored as ‘bipolarizing’ if the spindle was monopolar 20 min after mitotic entry and subsequently became bipolar. (H) Cells were treated as in (A–E), but were treated with varying concentrations of STLC 56 h after transfection. Scale bars indicate 5 μ m.

microtubules simultaneously (Kapitein *et al*, 2005). However, dynein is unlikely to form a similar homo-tetrameric configuration. Therefore, other proteins should connect minus-end-directed movement of dynein on one microtubule to neighbouring microtubules to produce a minus-end-directed force within the spindle. One possible candidate is CLIP-170, as CLIP-170 can bind the dynein-dynactin complex on the spindle and through a distinct domain can interact with spindle microtubules (Dzhindzhev *et al*, 2005; Tanenbaum *et al*, 2006). Therefore, we tested whether CLIP-170 was

required for the minus-end-directed force production within the spindle. Indeed, although only $4 \pm 1\%$ of mock-depleted and $3 \pm 2\%$ of GAPDH-depleted monastrol-treated mitotic cells contained bipolar spindles (Figure 3A–C), knockdown of CLIP-170 with two independent siRNAs resulted in a substantial increase in the percentage of bipolar spindles after monastrol treatment (46 ± 21 and $31 \pm 6\%$ for siRNA nos. 1 and 2, respectively) (Figure 3B and C) (five independent experiments). Similar results were obtained after treatment with 2 μ M STLC (data not shown). These results

demonstrate that CLIP-170, similar to dynein and Lis1, antagonizes Eg5 during bipolar spindle assembly.

To determine whether the mitotic arrest after Eg5 inhibition could be overcome by depletion of CLIP-170, the percentage of anaphase/telophase cells was determined in GAPDH- and CLIP-170-depleted cells treated with or without STLC. In untreated GAPDH-depleted cells, $35 \pm 7\%$ of mitotic cells were in anaphase or telophase at the time of fixation, whereas in CLIP-170-depleted cells only $18 \pm 4\%$ of mitotic cells showed an anaphase/telophase configuration, consistent with a role for CLIP-170 in chromosome alignment (Green *et al*, 2005; Draviam *et al*, 2006; Tanenbaum *et al*, 2006). However, whereas only $1 \pm 1\%$ of GAPDH-depleted cells treated with monastrol were in anaphase or telophase, $8 \pm 1\%$ of CLIP-170-depleted cells had entered anaphase/telophase (Figure 3D and E) (four independent experiments). Thus, depletion of CLIP-170 increases the amount of cells that can enter anaphase after Eg5 inhibition, consistent with an increase in the percentage of cells that form a bipolar spindle and can therefore align their chromosomes to the metaphase plate.

To further investigate the mechanism by which bipolar spindles are formed in cells lacking both CLIP-170 and Eg5 activity, time-lapse microscopy was used. Cells transfected with GAPDH siRNA and treated with STLC formed a bipolar spindle in only $26 \pm 7\%$ ($n = 93$) of all cases (three independent experiments), whereas the remaining 74% of cells formed a monopolar spindle. It should be noted that the percentage of bipolar spindles as quantified in live cells is slightly higher than the percentage of bipolar spindles in fixed samples. This is because cells that form a monopolar spindle will arrest in mitosis and accumulate over time relative to cells that formed a bipolar spindle and are thus over-represented in fixed samples. Nonetheless, consistent with analysis of fixed cells, CLIP-170 depletion led to a large increase in the percentage of cells that formed a bipolar spindle after STLC treatment ($84 \pm 13\%$) ($n = 77$) (three independent experiments). CLIP-170 depletion also resulted in a slightly increased inter-centrosomal distance in prophase (3.1 ± 2.0 versus $4.4 \pm 1.1 \mu\text{m}$), but this increase was not responsible for the increase in the percentage of cells that formed a bipolar spindle, as individual CLIP-170-depleted cells with an equal inter-centrosomal distance as GAPDH-depleted cells still formed bipolar spindles at much higher frequencies (Supplementary Figure S10). Importantly, in all live cell imaging experiments, spindles were scored as bipolar if they were bipolar at the moment the film ended or when the cell exited mitosis. Often, CLIP-170-depleted cells initially formed a monopolar spindle, in which centrosomes would subsequently separate to form a bipolar spindle (a representative cell is shown in Figure 3F). In total, 53% of CLIP-170-depleted cells that initially formed a monopolar spindle (as measured 20 min after NEB) had formed a bipolar spindle at the time the film ended or the cell exited mitosis, whereas this was observed only in 2% GAPDH-depleted cells (Figure 3G). CLIP-170-depleted cells that changed from a monopolar to a bipolar spindle would switch suddenly from a monopolar spindle to a bipolar spindle with a normal size. No cells were observed that maintained partially separated spindle poles. These results suggest that monopolar and bipolar spindles with normal lengths are the only two stable spindle configurations and that the absence of CLIP-170 increases the like-

lihood that cells switch to the bipolar state. A similar model of spindle bistability was previously proposed in *Drosophila* S2 cells (Goshima *et al*, 2005). Furthermore, increasing the concentration of STLC almost completely blocked bipolar spindle formation in CLIP-170-depleted cells (Figure 3H), similar to results obtained after dynein and Eg5 double inhibition. Thus, we conclude that, similar to dynein, loss of CLIP-170 reduces the amount of Eg5 activity required to separate centrosomes.

Spindle bipolarity is not restored by defects in kinetochore-microtubule attachments

Loss of CLIP-170 results in defects in kinetochore-microtubule attachments (Green *et al*, 2005; Draviam *et al*, 2006; Tanenbaum *et al*, 2006). Therefore, we tested whether loss of kinetochore-microtubules by itself was sufficient to restore spindle bipolarity after Eg5 inhibition. Cells were depleted of the kinetochore protein Hec1 by RNAi (Supplementary Figure S11), which results in a very severe kinetochore-microtubule attachment defect (Martin-Lluesma *et al*, 2002). Consistent with earlier findings (Ganem and Compton, 2004), loss of Hec1 was unable to rescue spindle bipolarity after Eg5 inhibition (Figure 4A) (two independent experiments), indicating that the effect of loss of CLIP-170 on spindle bipolarity is unlikely due to defects in kinetochore-microtubule attachments. Furthermore, the rescue of spindle bipolarity in STLC-treated cells after depletion of CLIP-170 is unlikely to be due to defects in dynein recruitment to the spindle or kinetochores, as no differences were observed in the amount of dynein on the spindle or kinetochores after CLIP-170 depletion, nor was the localization to the minus-ends of the spindle microtubules affected (Figure 4B; Supplementary Figure S12). In addition, the fraction of DHC-depleted cells that contained monopolar spindles almost always ($>90\%$) contained kinetochore-microtubules facing away from the pole (for an example, see Figure 1F and Supplementary Figure S7), whereas in CLIP-170-depleted cells that contained monopolar spindles, such kinetochore-microtubules were never observed (data not shown). Finally, in contrast to DHC-depleted cells, the Golgi was positioned normally in CLIP-170-depleted cells (data not shown). Taken together, these results indicate that other functions of dynein are unaffected by CLIP-170 depletion and that therefore CLIP-170 is probably not a general regulator of dynein function, but rather specifically cooperates with dynein to produce a minus-end-directed force within the spindle.

CLIP-115 and CLIP-170 have opposite effects on spindle bipolarity

CLIP-170 binds to microtubules through its N-terminal microtubule-binding domain (Pierre *et al*, 1994) and can bind the dynein-dynactin complex subunits Lis1 and p150glued through its C terminus (Figure 4C) (Coquelle *et al*, 2002; Tai *et al*, 2002; Lansbergen *et al*, 2004). Thus, if CLIP-170 antagonizes Eg5 through its interaction with the dynein-dynactin complex, it is expected that the C-terminal domain of CLIP-170 is essential. To test this hypothesis, we attempted to perform RNAi rescue experiments with either wild-type CLIP-170 or a mutant of CLIP-170 lacking the C-terminal domain. Unfortunately, expression of both wild-type and the C-terminal deletion mutant resulted in the formation of monopolar spindles even in control cells (unpublished

Figure 4 CLIP-170 and CLIP-115 have opposite effects on spindle bipolarity. (A) U2OS cells stably expressing YFP- α -tubulin were transfected with GAPDH or Hec1 siRNA. At 48 h after transfection, cells were treated with 2 μ M STLC and immediately analysed using time-lapse microscopy and the percentage of cells that formed a bipolar spindle was determined. (B) Cells were transfected with either GAPDH siRNA or CLIP-170 siRNA, re-transfected 36 and 72 h after the first round of transfection cells were fixed, and stained for γ -tubulin, dynein intermediate chain and DNA. (C) Schematic representation of the domain structure of CLIP-115 and CLIP-170. In red, the CAP_Gly domains are shown and in green the dynein/dynactin-binding domain. (D, E) Cells were transfected as in (B), but 56 h after the first transfection, cells were treated with 1.5 μ M STLC and 18 h later, cells were fixed and stained for α -tubulin, γ -tubulin and DNA (D) or cells were harvested and lysed for western blot analysis using an antibody that recognizes the N terminus of CLIP-115 and CLIP-170 (E). Scale bars indicate 5 μ m.

observation), making interpretation of these experiments very difficult. Therefore, we undertook an alternative approach and examined the role of the highly homologous protein CLIP-115 in spindle bipolarity. CLIP-115 has an N-terminal domain that is very similar to the N terminus of CLIP-170, but completely lacks the dynein–dynactin interaction domain in the C terminus. If CLIP-170 antagonizes Eg5 through its direct control of microtubule dynamics, it is expected that CLIP-115 will have a similar function and that simultaneous depletion of CLIP-115 and CLIP-170 will have a much more dramatic effect in restoring spindle bipolarity than CLIP-170 depletion alone. However, if dynein–dynactin binding is required for CLIP-170 to antagonize Eg5, then CLIP-115 will not have the same function, and might even be expected to have an opposite effect, either by competing with CLIP-170 for binding to the microtubule plus-end or by competing with p150glued or Lis1 for binding to the C terminus of CLIP-170 (Hoogenraad *et al*, 2002; Lansbergen *et al*, 2004). CLIP-115 and CLIP-170 were therefore depleted either in combination with GAPDH or in combination with each other and cells were treated with 1.5 μ M STLC for 18 h (a slightly lower dose was used here, so both an increase and decrease in the number of bipolar spindles could be observed). In control GAPDH-depleted cells, $9 \pm 5\%$ of cells formed a bipolar spindle and this was increased to $37 \pm 11\%$ in cells depleted of CLIP-170 and GAPDH (Figure 4D). In contrast, depletion of GAPDH and CLIP-115 reproducibly decreased the percentage of bipolar spindles compared with control cells to only $5 \pm 4\%$ of cells (four independent experiments). Furthermore, cells doubly depleted of CLIP-170 and

CLIP-115 formed substantially fewer bipolar spindles than cells depleted of GAPDH and CLIP-170 ($18 \pm 9\%$; Figure 4D, four independent experiments) and this was not due to decreased knockdown of CLIP-170 (Figure 4E). Taken together, these results show that depletion of CLIP-115 has an opposite effect on spindle bipolarity as compared with CLIP-170 and strongly suggests that the C-terminal dynein–dynactin-binding domain of CLIP-170 is required for CLIP-170 to antagonize Eg5.

Discussion

Here, we show for the first time in intact mammalian cells that dynein antagonizes Eg5 during bipolar spindle assembly. Our results suggest that dynein produces a minus-end-directed force within the spindle, which pulls centrosomes together when the Eg5-dependent outward pushing force is reduced. Furthermore, we show that cells in which dynein and Eg5 are simultaneously inhibited often enter anaphase and segregate their chromosomes, suggesting that fully functional bipolar spindles are formed in these cells. Finally, depletion of CLIP-170, similar to dynein, restores bipolar spindle assembly after Eg5 inhibition. This function of CLIP-170 is likely dependent on CLIP-170's interaction with the dynein–dynactin complex, as depletion of CLIP-115, which has a similar function as CLIP-170 at the microtubule plus-end, but lacks the dynein–dynactin interaction domain, does not restore spindle bipolarity after Eg5 inhibition.

How does dynein depletion rescue spindle bipolarity after Eg5 inhibition? One possibility is that dynein functions directly through Eg5, as the dynein–dynactin complex

directly interacts with Eg5 (Blangy *et al*, 1997; Kapoor and Mitchison, 2001) and was recently shown to transport Eg5 away from the area of antiparallel microtubule overlap (where Eg5 is likely to be active). Consistent with this, bipolar spindle formation after double inhibition of dynein and Eg5 still depends on residual Eg5 activity. However, it is unlikely that dynein negatively regulates spindle bipolarity solely through control of Eg5 localization in mammalian cells, as (1) Eg5 localization and levels are not detectably altered within the spindle in mammalian cells after dynein RNAi and (2) full inhibition of Eg5 during mitosis by either 40 μ M STLC or a combination of STLC and Eg5 RNAi leads to spindle collapse and this can be rescued by simultaneous inhibition of dynein, indicating that dynein functions in spindle bipolarity in the absence of Eg5 activity. It is therefore more likely that dynein opposes Eg5 by sliding microtubules in opposite directions, thereby pulling centrosomes together. In this model, Eg5 pushes centrosomes apart, but is continuously counteracted by an inward force of dynein. However, in normal cells the Eg5-dependent force is larger than the dynein-dependent force, thus allowing centrosomes to separate. When Eg5 activity is reduced, the balance is tipped the other way and dynein will pull centrosomes together, resulting in monopolar spindles. However, when dynein activity is simultaneously reduced, the balance is restored and centrosomes separate normally. It should be noted, though, that even at the highest concentration of STLC some bipolar spindles were formed in dynein-depleted cells, suggesting that perhaps other microtubule motors do contribute to centrosome separation in these cells.

The function of dynein in antagonizing Eg5 does not appear to be active before NEB. In fact, centrosomes separate even less during prophase in cells in which both dynein and Eg5 are inhibited compared with Eg5 alone. This suggests that dynein might actually cooperate with Eg5 to pull centrosomes apart before NEB, although we cannot exclude that the defect in prophase centrosome separation after DHC RNAi is an indirect effect of the defect in centrosome–nuclear attachment in DHC-depleted cells. In any case, it is striking that dynein/Eg5 doubly inhibited cells are completely unable to separate their centrosomes in prophase, but still form bipolar

spindles at very high frequencies. The decreased centrosome separation in prophase might therefore even lead to an underestimation of the restoration of spindle bipolarity in STLC-treated cells upon dynein depletion.

There are two possible explanations for a role of CLIP-170 in spindle bipolarity. First, CLIP-170 could act to load dynein onto spindle microtubules, as has been shown in yeast (Sheeman *et al*, 2003), alternatively CLIP-170 could physically link dynein to surrounding microtubules, thereby coupling dynein-dependent movement on one microtubule to neighbouring microtubules, resulting in the generation of a minus-end-directed force (Figure 5). We favour the latter explanation, because (1) dynein localization is not detectably altered in CLIP-170-depleted cells and (2) other functions of dynein are not perturbed after CLIP-170 depletion. In the latter model, a complex of dynein–dynactin and CLIP-170 would crosslink antiparallel microtubules in which one microtubule, bound to CLIP-170, would function as a dynein cargo and the other as the track over which dynein can walk. This type of interactions could occur in the middle of the spindle, where most microtubules have an antiparallel configuration. However, at steady state, dynein localizes mostly to spindle poles, which is inconsistent with this model. Perhaps dynein is loaded onto microtubules at the zone of microtubule overlap, where it interacts with CLIP-170 at a nearby microtubule plus-end and then walks towards the spindle pole, thereby producing a brief microtubule minus-end-directed force. Continuous loading at the spindle mid-zone and streaming towards the pole would produce a continuous minus-end-directed force, but at the same time would lead to a relative accumulation of dynein at the spindle pole. It is important to note that the dynein complex contains many proteins that can directly bind microtubules and it is very well possible that multiple proteins within the dynein complex cooperate to link dynein to surrounding microtubules, but our results suggest that at least one of these proteins, CLIP-170, could have an important function in this crosslinking.

Finally, we show that Lis1 also antagonizes Eg5 during bipolar spindle assembly. Lis1 is known to stimulate the ATPase activity of dynein (Mesngon *et al*, 2006), but Lis1

Figure 5 Model of dynein, Lis1 and CLIP-170 function in bipolar spindle assembly. Dynein binds along the lattice of spindle microtubules together with Lis1 near the zone of antiparallel microtubule overlap. When a growing microtubule plus-end, decorated with CLIP-170, passes the dynein complex, CLIP-170 can interact with the dynein complex, possibly through its interaction with Lis1. Minus-end-directed movement of dynein now pulls the two centrosomes together (left). In contrast, when a CLIP-115-decorated microtubule plus-end passes the dynein complex, it does not interact with Lis1 and no minus-end-directed force is produced (middle). When Eg5 is inhibited, a complex of dynein, Lis1 and CLIP-170 pulls centrosomes together, resulting in monopolar spindle formation (right).

has also been suggested to link CLIP-170 to the dynein-dynactin complex (Coquelle *et al*, 2002; Tai *et al*, 2002). It is possible that both functions of Lis1 contribute to its role in minus-end-directed force production within the spindle. Taken together, these observations lead us to propose a model in which CLIP-170 functions together with Lis1 and dynein, linking dynein to surrounding microtubules, thereby translating dynein-dependent minus-end-directed movement to minus-end-directed force production within the spindle (Figure 5).

Materials and methods

Antibodies

The CLIP antibodies have been described earlier (Hoogenraad *et al*, 2000; Coquelle *et al*, 2002) and were used at 1:2500. α -Tubulin antibody (Sigma) was used at 1:7500, γ -tubulin antibody (Sigma) and anti-DIC (Sigma) were used at 1:200, CREST antibody (Cortex Biochem) was used at 1:2000, Hec1 antibody (Upstate) antibody was used at 1:200, HSET antibody (Santa Cruz) was used at 1:100 for western blot and 1:40 for immunofluorescence and the Eg5 antibody (Abcam) was used at 1:1000 for western blot and 1:200 for immunofluorescence. Secondary antibodies for immunofluorescence were Alexa-488, Alexa-568 and Alexa-647 (Molecular Probes) and HRP-conjugated anti-rabbit/mouse (Dako) for western blot.

Cell culture and transfection

U2OS and HeLa cells were cultured in DMEM (Gibco) with 6% FCS, 100 U/ml penicillin and 100 μ g/ml streptomycin. siRNA was transfected using reverse transfection with Hiperfect (Qiagen) according to the manufacturer's guidelines. siRNA sequences and transfection concentrations are listed in Supplementary Table S1.

References

Akhmanova A, Hoogenraad CC (2005) Microtubule plus-end-tracking proteins: mechanisms and functions. *Curr Opin Cell Biol* **17**: 47–54

Blangy A, Arnaud L, Nigg EA (1997) Phosphorylation by p34cdc2 protein kinase regulates binding of the kinesin-related motor HsEg5 to the dynactin subunit p150. *J Biol Chem* **272**: 19418–19424

Blangy A, Lane HA, d'Herin P, Harper M, Kress M, Nigg EA (1995) Phosphorylation by p34cdc2 regulates spindle association of human Eg5, a kinesin-related motor essential for bipolar spindle formation *in vivo*. *Cell* **83**: 1159–1169

Coquelle FM, Caspi M, Cordelieres FP, Dompierre JP, Dujardin DL, Koifman C, Martin P, Hoogenraad CC, Akhmanova A, Galjart N, De Mey JR, Reiner O (2002) LIS1, CLIP-170's key to the dynein/dynactin pathway. *Mol Cell Biol* **22**: 3089–3102

Corthesy-Theulaz I, Pauloin A, Pfeffer SR (1992) Cytoplasmic dynein participates in the centrosomal localization of the Golgi complex. *J Cell Biol* **118**: 1333–1345

DeBonis S, Skoufias DA, Lebeau L, Lopez R, Robin G, Margolis RL, Wade RH, Kozielski F (2004) *In vitro* screening for inhibitors of the human mitotic kinesin Eg5 with antimitotic and antitumor activities. *Mol Cancer Ther* **3**: 1079–1090

Draviam VM, Shapiro I, Aldridge B, Sorger PK (2006) Misorientation and reduced stretching of aligned sister kinetochores promote chromosome missegregation in EB1- or APC-depleted cells. *EMBO J* **25**: 2814–2827

Dzhindzhev NS, Rogers SL, Vale RD, Ohkura H (2005) Distinct mechanisms govern the localisation of *Drosophila* CLIP-190 to unattached kinetochores and microtubule plus-ends. *J Cell Sci* **118**: 3781–3790

Galjart N (2005) CLIPs and CLASPs and cellular dynamics. *Nat Rev Mol Cell Biol* **6**: 487–498

Ganem NJ, Compton DA (2004) The KinI kinesin Kif2a is required for bipolar spindle assembly through a functional relationship with MCAK. *J Cell Biol* **166**: 473–478

Confocal microscopy

Cells were grown on 10 mm glass coverslips and fixed in 3.7% formaldehyde/0.5% triton in PBS for 5 min, washed once with PBS and subsequently incubated in ice-cold methanol for 5 min. Coverslips were blocked in PBS with 3% BSA for 30 min. All primary antibodies were incubated at room temperature overnight and secondary antibodies were incubated for 1 h at room temperature. DAPI was added to all samples before mounting using Vectashield mounting fluid (Vectorlabs). Confocal images were acquired on a Zeiss LSM510 META (Carl Zeiss) with a Plan Apochromat $\times 63$ NA 1.4 objective. Z-planes were acquired with 1 μ m intervals. Brightness and contrast were adjusted with Photoshop 6.0 (Adobe). Images are maximum intensity projections of all Z-planes.

Time-lapse microscopy

Cells were plated on four-well glass-bottom slides (Labtek). Slides were imaged on a Zeiss Axiovert 200M microscope equipped with a Plan-Neofluar $\times 63/1.25$ oil in a permanently heated chamber with 5% CO₂. Images were acquired every 3–5 min using a Photometrics Coolsnap HQ charged-coupled device camera (Scientific, Tucson, AZ) and a YFP filter cube (Chroma Technology Corp.). Z-stacks were acquired with 2 μ m interval between Z-slices. Images were processed using Metamorph software (Universal Imaging, Downingtown, PA).

Supplementary data

Supplementary data are available at *The EMBO Journal* Online (<http://www.embojournal.org>).

Acknowledgements

We thank Helder Maiato and Arne Lindqvist for critically reading the paper and the Medema lab members for helpful discussions. We also thank Livio Kleij for maintaining the time-lapse and confocal microscopes. MET and RHM are supported by a VICI grant from ZonMw (918.46.616).

Gonczy P, Pichler S, Kirkham M, Hyman AA (1999) Cytoplasmic dynein is required for distinct aspects of MTOC positioning, including centrosome separation, in the one cell stage *Caenorhabditis elegans* embryo. *J Cell Biol* **147**: 135–150

Goshima G, Vale RD (2003) The roles of microtubule-based motor proteins in mitosis: comprehensive RNAi analysis in the *Drosophila* S2 cell line. *J Cell Biol* **162**: 1003–1016

Goshima G, Wollman R, Stuurman N, Scholey JM, Vale RD (2005) Length control of the metaphase spindle. *Curr Biol* **15**: 1979–1988

Green RA, Wollman R, Kaplan KB (2005) APC and EB1 function together in mitosis to regulate spindle dynamics and chromosome alignment. *Mol Biol Cell* **16**: 4609–4622

Grigoriev I, Splinter D, Keijzer N, Wulf PS, Demmers J, Ohtsuka T, Modesti M, Maly IV, Grosveld F, Hoogenraad CC, Akhmanova A (2007) Rab6 regulates transport and targeting of exocytotic carriers. *Dev Cell* **13**: 305–314

Heck MM, Pereira A, Pesavento P, Yannoni Y, Spradling AC, Goldstein LS (1993) The kinesin-like protein KLP61F is essential for mitosis in *Drosophila*. *J Cell Biol* **123**: 665–679

Hoogenraad CC, Akhmanova A, Grosveld F, De Zeeuw CI, Galjart N (2000) Functional analysis of CLIP-115 and its binding to microtubules. *J Cell Sci* **113** (Part 12): 2285–2297

Hoogenraad CC, Koekkoek B, Akhmanova A, Krugers H, Dortland B, Miedema M, van Alphen A, Kistler WM, Jaegle M, Koutsourakis M, Van Camp N, Verhoye M, van der Linden A, Kaverina I, Grosveld F, De Zeeuw CI, Galjart N (2002) Targeted mutation of Cyln2 in the Williams syndrome critical region links CLIP-115 haploinsufficiency to neurodevelopmental abnormalities in mice. *Nat Genet* **32**: 116–127

Howell BJ, McEwen BF, Canman JC, Hoffman DB, Farrar EM, Rieder CL, Salmon ED (2001) Cytoplasmic dynein/dynactin drives kinetochore protein transport to the spindle poles and has a role in mitotic spindle checkpoint inactivation. *J Cell Biol* **155**: 1159–1172

- Hoyt MA, He L, Loo KK, Saunders WS (1992) Two *Saccharomyces cerevisiae* kinesin-related gene products required for mitotic spindle assembly. *J Cell Biol* **118**: 109–120
- Kapitein LC, Peterman EJ, Kwok BH, Kim JH, Kapoor TM, Schmidt CF (2005) The bipolar mitotic kinesin Eg5 moves on both microtubules that it crosslinks. *Nature* **435**: 114–118
- Kapoor TM, Mitchison TJ (2001) Eg5 is static in bipolar spindles relative to tubulin: evidence for a static spindle matrix. *J Cell Biol* **154**: 1125–1133
- Khodjakov A, Copenagle L, Gordon MB, Compton DA, Kapoor TM (2003) Minus-end capture of preformed kinetochore fibers contributes to spindle morphogenesis. *J Cell Biol* **160**: 671–683
- Komarova YA, Akhmanova AS, Kojima S, Galjart N, Borisy GG (2002) Cytoplasmic linker proteins promote microtubule rescue *in vivo*. *J Cell Biol* **159**: 589–599
- Lansbergen G, Komarova Y, Modesti M, Wyman C, Hoogenraad CC, Goodson HV, Lemaitre RP, Drechsel DN, van Munster E, Gadella Jr TW, Grosveld F, Galjart N, Borisy GG, Akhmanova A (2004) Conformational changes in CLIP-170 regulate its binding to microtubules and dynactin localization. *J Cell Biol* **166**: 1003–1014
- Maiato H, Rieder CL, Khodjakov A (2004) Kinetochore-driven formation of kinetochore fibers contributes to spindle assembly during animal mitosis. *J Cell Biol* **167**: 831–840
- Manning AL, Ganem NJ, Bakhoum SF, Wagenbach M, Wordeman L, Compton DA (2007) The kinesin-13 proteins Kif2a, Kif2b, and Kif2c/MCAK have distinct roles during mitosis in human cells. *Mol Biol Cell* **18**: 2970–2979
- Martin-Lluesma S, Stucke VM, Nigg EA (2002) Role of Hec1 in spindle checkpoint signaling and kinetochore recruitment of Mad1/Mad2. *Science* **297**: 2267–2270
- Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL, Mitchison TJ (1999) Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. *Science* **286**: 971–974
- Merdes A, Heald R, Samejima K, Earnshaw WC, Cleveland DW (2000) Formation of spindle poles by dynein/dynactin-dependent transport of NuMA. *J Cell Biol* **149**: 851–862
- Merdes A, Ramyar K, Vechio JD, Cleveland DW (1996) A complex of NuMA and cytoplasmic dynein is essential for mitotic spindle assembly. *Cell* **87**: 447–458
- Mesngon MT, Tarricone C, Hebbard S, Guillotte AM, Schmitt EW, Lanier L, Musacchio A, King SJ, Smith DS (2006) Regulation of cytoplasmic dynein ATPase by Lis1. *J Neurosci* **26**: 2132–2139
- Mitchison TJ, Maddox P, Gaetz J, Groen A, Shirasu M, Desai A, Salmon ED, Kapoor TM (2005) Roles of polymerization dynamics, opposed motors, and a tensile element in governing the length of *Xenopus* extract meiotic spindles. *Mol Biol Cell* **16**: 3064–3076
- Mountain V, Simerly C, Howard L, Ando A, Schatten G, Compton DA (1999) The kinesin-related protein, HSET, opposes the activity of Eg5 and cross-links microtubules in the mammalian mitotic spindle. *J Cell Biol* **147**: 351–366
- Pierre P, Pepperkok R, Kreis TE (1994) Molecular characterization of two functional domains of CLIP-170 *in vivo*. *J Cell Sci* **107** (Part 7): 1909–1920
- Robinson JT, Wojcik EJ, Sanders MA, McGrail M, Hays TS (1999) Cytoplasmic dynein is required for the nuclear attachment and migration of centrosomes during mitosis in *Drosophila*. *J Cell Biol* **146**: 597–608
- Roof DM, Meluh PB, Rose MD (1992) Kinesin-related proteins required for assembly of the mitotic spindle. *J Cell Biol* **118**: 95–108
- Sawin KE, LeGuellec K, Philippe M, Mitchison TJ (1992) Mitotic spindle organization by a plus-end-directed microtubule motor. *Nature* **359**: 540–543
- Sharp DJ, Brown HM, Kwon M, Rogers GC, Holland G, Scholey JM (2000) Functional coordination of three mitotic motors in *Drosophila* embryos. *Mol Biol Cell* **11**: 241–253
- Sharp DJ, McDonald KL, Brown HM, Matthies HJ, Walczak C, Vale RD, Mitchison TJ, Scholey JM (1999a) The bipolar kinesin, KLP61F, cross-links microtubules within interpolar microtubule bundles of *Drosophila* embryonic mitotic spindles. *J Cell Biol* **144**: 125–138
- Sharp DJ, Yu KR, Sisson JC, Sullivan W, Scholey JM (1999b) Antagonistic microtubule-sliding motors position mitotic centrosomes in *Drosophila* early embryos. *Nat Cell Biol* **1**: 51–54
- Sheeman B, Carvalho P, Sagot I, Geiser J, Kho D, Hoyt MA, Pellman D (2003) Determinants of *S.cerevisiae* dynein localization and activation: implications for the mechanism of spindle positioning. *Curr Biol* **13**: 364–372
- Tai CY, Dujardin DL, Faulkner NE, Vallee RB (2002) Role of dynein, dynactin, and CLIP-170 interactions in LIS1 kinetochore function. *J Cell Biol* **156**: 959–968
- Tanenbaum ME, Galjart N, van Vugt MA, Medema RH (2006) CLIP-170 facilitates the formation of kinetochore–microtubule attachments. *EMBO J* **25**: 45–57
- Uteng M, Hentrich C, Miura K, Bieling P, Surrey T (2008) Poleward transport of Eg5 by dynein–dynactin in *Xenopus laevis* egg extract spindles. *J Cell Biol* **182**: 715–726
- Valetti C, Wetzel DM, Schrader M, Hasbani MJ, Gill SR, Kreis TE, Schroer TA (1999) Role of dynactin in endocytic traffic: effects of dynamin overexpression and colocalization with CLIP-170. *Mol Biol Cell* **10**: 4107–4120
- Whitehead CM, Rattner JB (1998) Expanding the role of HsEg5 within the mitotic and post-mitotic phases of the cell cycle. *J Cell Sci* **111** (Part 17): 2551–2561
- Wojcik E, Basto R, Serr M, Scaerou F, Karess R, Hays T (2001) Kinetochore dynein: its dynamics and role in the transport of the Rough deal checkpoint protein. *Nat Cell Biol* **3**: 1001–1007
- Yang Z, Tulu US, Wadsworth P, Rieder CL (2007) Kinetochore dynein is required for chromosome motion and congression independent of the spindle checkpoint. *Curr Biol* **17**: 973–980