Inhibition of ectopic microtubule assembly by the kinesin-13 KLP-7MCAK prevents chromosome segregation and cytokinesis defects in oocytes

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SUMMARY STATEMENT
Kinesin-13 microtubule depolymerases prevent ectopic microtubule assembly and spindle disorganization when centrosome activity is low or absent such as in oocytes.

ABSTRACT
In most species, oocytes lack centrosomes. Accurate meiotic spindle assembly and chromosome segregation -essential to prevent miscarriage or developmental defects- thus occur through atypical mechanisms that are not well characterized. Using quantitative in vitro and in vivo functional assays in the C. elegans oocyte, we provide here novel evidence that the kinesin-13 KLP-7 promotes the destabilization of the whole cellular microtubule network. By counteracting ectopic microtubule assembly and disorganization of the microtubule network, this function is strictly required for spindle organization, chromosome segregation, and cytokinesis in meiotic cells. Strikingly, when centrosome activity was experimentally reduced, the absence of the kinesin-13 proteins KLP-7 or MCAK also resulted in ectopic microtubule asters during mitosis in C. elegans zygotes and HeLa cells, respectively. Our results highlight the general function of kinesin-13 microtubule depolymerases in preventing ectopic, spontaneous microtubule assembly, when centrosome activity is defective or absent, which otherwise leads to spindle microtubule disorganization and aneuploidy.
INTRODUCTION

Sexual reproduction relies on meiosis, a specialized type of cell division, which generates haploid germ cells or gametes. The genome size reduction that occurs during gametogenesis involves two successive cell divisions termed meiosis I and II preceded by a single round of genome replication (Dumont and Brunet, 2010). Chromosome gain or loss during meiosis generates aneuploid embryos after fertilization. Aneuploidy is hence a major obstacle in achieving reproductive success, as the vast majority of embryos formed from aneuploid oocytes are non-viable leading to miscarriage (Nagaoka et al., 2012).

Accurate chromosome segregation is driven by the microtubule-based spindle. In somatic cells and spermatocytes, spindle microtubules are primarily assembled from the centrosomes, which duplicate once per cell cycle to form the two spindle poles (Walczak and Heald, 2008; Heald and Khodjakov, 2015). Chromosome alignment on the spindle and segregation in anaphase then occurs through the interaction between spindle microtubules and kinetochores (Cheeseman, 2014). Chromosome spatial segregation is followed by their definitive physical separation during cytokinesis of the newly formed daughter cells (Green et al., 2012).

The generation of oocytes involves three major adaptations to the classical mechanism of cell division (Ohkura, 2015). First oocytes of most species lack conventional centriole-containing centrosomes (Szollosi et al., 1972). Spindle assembly in oocytes involves specific mechanisms such as chromatin-dependent microtubule assembly (Heald et al., 1996; Dumont and Desai, 2012). The second important adaptation of the meiotic cell division process as yet only observed in C. elegans oocytes is the kinetochore-independent chromosome segregation (Dumont et al., 2010). The precise mechanism of this atypical segregation is still unclear but involves microtubule-dependent forces exerted on chromosomes (Dumont et al., 2010; Muscat et al., 2015; McNally et al., 2016). The third specific adaptation of oocyte meiosis is polar body extrusion (PBE), which corresponds to an extremely asymmetric partitioning of the oocyte creating a tiny polar body with most of the cytoplasm maintained in the oocyte (Zhang et al., 2008; Dorn et al., 2010; Fabritius et al., 2011; Maddox et al., 2012).
All three major specific adaptations to the process of cell division essential for successful oocyte meiosis involve microtubules. Consequently, spindle microtubule dynamics in oocytes must be tightly regulated both temporally and spatially to successfully execute the meiotic cell division program. This is achieved primarily through the combined activities of MAPs and microtubule motors (Alfaro-Aco and Petry, 2015). Critical among those are the microtubule depolymerizing kinesin-13 family members (Walczak et al., 2013). Kinesin-13s use the energy from ATP hydrolysis to depolymerize microtubules and play essential roles in spindle assembly and chromosome segregation during mitosis (Wordeman and Mitchison, 1995; Walczak et al., 1996; Desai et al., 1999). Human mitotic cells depleted of the kinesin-13 MCAK assemble spindles with abnormally long and stable microtubules that correlate with a high frequency of chromosome mis-attachments (Maney et al., 1998; Kline-Smith et al., 2004; Rogers et al., 2004; Domnitz et al., 2012). In mitotic C. elegans embryos, the unique kinesin-13 family member KLP-7 (hereafter KLP-7\textsuperscript{MCAK}) prevents assembly of an abnormally high number of astral microtubules and thus protects against an excessive increase in astral cortical pulling forces (Srayko et al., 2005). Accordingly, in KLP-7\textsuperscript{MCAK}-depleted embryos, mitotic spindles break apart during anaphase and sister chromatids separate prematurely (Grill et al., 2001).

During oocyte meiosis, kinesin-13s have also been involved in multiple aspects of cell division including the control of meiotic spindle length and proper chromosome alignment (Zou et al., 2008; Illingworth et al., 2010; Radford et al., 2012; Do et al., 2014). In the C. elegans oocyte, KLP-7\textsuperscript{MCAK} has been proposed to limit metaphase spindle pole numbers by correcting improper kinetochore-microtubule attachments, but its precise function throughout the two meiotic divisions remains elusive (Connolly et al., 2015; Han et al., 2015). Here we show the critical meiotic function of KLP-7\textsuperscript{MCAK} in preventing ectopic microtubule assembly that otherwise leads to spindle disorganization and chromosome segregation defects. For this we provide the first high-resolution time-resolved comprehensive view of meiotic divisions. Specifically, we show that KLP-7\textsuperscript{MCAK} acts by globally destabilizing microtubules within the meiotic spindle in metaphase and the central spindle in anaphase, as well as throughout the oocyte
cell cortex. We also demonstrate that KLP-7\textsuperscript{MCAK} or MCAK activities prevent ectopic cytoplasmic aster formation during mitosis in the \textit{C. elegans} zygote or in HeLa cells respectively, when centrosome function is impaired. Our data therefore suggest that the function of kinesin-13 is essential to delimit the proper local assembly of microtubules in dividing cells when centrosome activity is reduced or absent, and thus for accurate spindle assembly.

**RESULTS**

\textit{In utero imaging: KLP-7\textsuperscript{MCAK} is required for the initial step of meiotic spindle assembly and bipolarization}

We sought to test the role of KLP-7\textsuperscript{MCAK} during meiotic spindle assembly. We first verified that our RNAi-mediated depletion strategy led to a strong embryonic lethality phenotype comparable to the $\textit{klp-7}\textsuperscript{MCAK}$ deletion mutant (hereafter $\textit{klp-7}\Delta$), and completely removed KLP-7\textsuperscript{MCAK} from oocytes (Fig. S1A, B). We also verified that expressing a functional GFP-tagged RNAi-resistant KLP-7\textsuperscript{MCAK} protein rescued this embryonic lethality (Fig. S1C, D). We then analyzed NEBD and meiotic spindle assembly in control and KLP-7\textsuperscript{MCAK}-depleted oocytes during both meiotic divisions \textit{in utero} in immobilized worms expressing GFP-tagged $\beta$-tubulin or the microtubule minus-end and spindle pole marker protein ASPM-1\textsuperscript{Asp} and mCherry-tagged histone 2B (H2B) (van der Voet et al., 2009). Spindle assembly can be separated in 4 distinct phases (Wolff et al., 2016). In both control and KLP-7\textsuperscript{MCAK}-depleted oocytes before NEBD, microtubules were excluded from the nucleus (Fig. 1A, B and S2A, B). In controls, after NEBD (evidenced by the diffusion of the soluble pool of fluorescent H2B away from the nucleoplasm), microtubules progressively invaded the nucleus to form a diffuse nuclear cloud around chromosomes (Fig. S2A, B and Movie S1). In KLP-7\textsuperscript{MCAK}-depleted oocytes and consistent with the cytoplasmic localization of GFP::KLP-7\textsuperscript{MCAK} in control oocytes at this stage, more microtubules were visible around the nucleus before NEBD relative to controls (Fig. 1A, S2A, B and Movie S1 and S2). Strikingly, the first phase of spindle assembly never took place in KLP-7\textsuperscript{MCAK}-depleted oocytes and the diffuse microtubule cloud that formed at NEBD around chromosomes in controls was completely absent (Fig. 1C, D). Instead, ectopic
microtubules persisted after NEBD around the breaking nuclear envelope. Thus KLP-7\textsuperscript{MCAK} is essential for the formation of a microtubule cloud around chromosomes after NEBD.

During the second phase, GFP::KLP-7\textsuperscript{MCAK} progressively accumulated on chromosomes and the assembled diffuse network of microtubules became bundled and coalesced around chromosomes in control oocytes (Fig. 1A, B, E and Movie S2). In parallel, the chromosomes became clustered together. In controls, this second phase correlated with the appearance of ASPM-1\textsuperscript{Asp} foci around chromosomes (Fig. 1F, S1C and Movie S3). Progressive microtubule bundling and cross-linking led to the formation of a multipolar spindle with several ASPM-1\textsuperscript{Asp} foci around chromosomes (Connolly et al., 2015). GFP::KLP-7\textsuperscript{MCAK} also concentrated on these multiple poles (Fig. 1E). In controls, this phase was accompanied by the dispersal of chromosomes on the forming spindle and their subsequent congression and alignment. In KLP-7\textsuperscript{MCAK}-depleted oocytes, the ectopic perinuclear microtubules seen in phase 1 coalesced around chromosomes during phase 2 to form a multipolar spindle that displayed multiple ASPM-1\textsuperscript{Asp} foci as in controls (Fig. 1F, S2C and Movie S3). Thus although the oocytes started phase 2 in absence of a diffuse microtubule cloud around chromosomes and with ectopic perinuclear microtubules, KLP-7\textsuperscript{MCAK}-depleted oocytes formed a seemingly normal multipolar spindle early in meiosis I.

During phase 3 in controls, the multipolar microtubule structure was slowly shaped into a bipolar spindle and ASPM-1\textsuperscript{Asp} and GFP::KLP-7\textsuperscript{MCAK} concentrated at the spindle poles. In KLP-7\textsuperscript{MCAK}-depleted oocytes, the multiple microtubule foci persisted and a bipolar spindle was almost never observed until after anaphase onset (see below). Similar imaging experiments in a strain expressing the nuclear envelope component Lamin 1 (LMN-1) tagged with GFP and mCherry-tagged β-tubulin revealed that the entire process of initial meiotic spindle assembly took place within the limits of the rupturing nuclear envelope (Fig. 1G, S1D and Movie S4). In both controls and KLP-7\textsuperscript{MCAK}-depleted oocytes nuclear envelope remnants were visible around the spindle up until the end of the bipolarization phase. Thus KLP-7\textsuperscript{MCAK} plays a critical role in phase 3, and is required for bipolar spindle formation at this stage.
In controls, phase 4, as previously described, corresponded to an extensive spindle pole disassembly (Yang et al., 2003). KLP-7\textsuperscript{MCAK}-depleted oocytes did not usually reach bipolar spindle assembly, but \textit{klp-7(RNAi)} did not affect the overall timing of nuclear envelope disassembly or anaphase onset (Fig. 1B, G, S1D and Movie S4). After anaphase onset, spindle bipolarity in KLP-7\textsuperscript{MCAK}-depleted oocytes was almost always rescued, due largely to “polar clustering”. Thus although disruption of KLP-7\textsuperscript{MCAK} activity leads to multipolar spindle assembly prior to anaphase, spindle bipolarity appears to be established prior to or during meiotic anaphase and cytokinesis (see also below).

\textit{High-resolution ex utero imaging: KLP-7\textsuperscript{MCAK} is required for normal meiotic spindle microtubule organization and full chromosome segregation}

To further investigate the contribution of KLP-7\textsuperscript{MCAK} in acentrosomal spindle assembly and function, we filmed \textit{ex utero} fertilized oocytes expressing GFP-tagged $\beta$-tubulin and mCherry-tagged H2B, which allows for higher spatial resolution (Fig. 2A and Movie S5). Control oocytes had all completed meiosis I spindle bipolarization at the time of dissection. In KLP-7\textsuperscript{MCAK}-depleted or deleted oocytes, spindle microtubule density (measured by average GFP::$\beta$-tubulin intensity) was increased as compared to controls at every steps of meiosis I and II (Fig. 2B and Movie S5). In controls, the barrel-shaped meiosis I spindle displayed a few extremely short microtubules extending outward (Fig. 2C). In contrast, KLP-7\textsuperscript{MCAK}-depleted oocytes displayed disorganized spindles with numerous long microtubules or microtubule bundles extending out toward the cytoplasm and the oocyte cortex (Fig. 2C). In agreement with a previous study and with our \textit{in utero} analysis, we found that spindles assembled in KLP-7\textsuperscript{MCAK}-depleted oocytes were multipolar (Connolly et al., 2015)(Fig. 2D). However, we found that the supernumerary poles were always resolved and incorporated into one of the two dominant spindle poles at or just after anaphase onset.

We next monitored the progression and accuracy of meiotic chromosome segregation in the presence and absence of KLP-7\textsuperscript{MCAK} in embryos (Fig. 2E). The overall timing of divisions was not significantly different between control and KLP-7\textsuperscript{MCAK}-depleted or deleted oocytes (Fig. 2H). In control oocytes, chromosomes aligned on tight metaphase plates during metaphase I and II.
Segregating chromosomes remained tightly clustered during both anaphase I and II, which usually ended with successful polar body extrusion. In contrast and consistent with the spindle disorganization we described above, KLP-7\textsuperscript{MCAK} depletion or deletion led to visible chromosome alignment and segregation defects during both meiotic divisions (Fig. 2E). Chromosome masses always separated after anaphase onset and lagging chromosomes were evident in most oocytes. Chromosome counting in fixed meiosis II oocytes that succeeded in first polar body extrusion revealed significant aneuploidy (control, 6 chromosomes in 14/14 oocytes; \textit{klp-7(RNAi)}, 4 chromosomes in 2/15 oocytes, 5 in 5/15, 6 in 7/15 and 7 in 1/15; \textit{klp-7Δ}, 4 chromosomes in 2/16 oocytes, 5 in 6/16, 6 in 6/16 and 7 in 2/16). As expected, KLP-7\textsuperscript{MCAK} depletion or deletion did not affect chromosome number during meiosis I (control, 6 chromosomes in 24/24 oocytes; \textit{klp-7(RNAi)}, 6 chromosomes in 20/20 oocytes; \textit{klp-7Δ}, 6 chromosomes in 20/20 oocytes).

We also found that KLP-7\textsuperscript{MCAK} is required for full chromosome segregation in meiosis I. Kymographs of anaphase I revealed that chromosome masses in KLP-7\textsuperscript{MCAK}-depleted or deleted oocytes separated at a rate comparable to controls during the first 2 min following anaphase onset (Fig. 2F, G). This timing corresponds approximately to the duration of anaphase A during meiosis I in \textit{C. elegans} oocytes (McNally et al., 2016). Chromosomes in controls continued to separate during anaphase B for the following 3 min and reached a maximal distance of 5.5 μm. In striking contrast, chromosome masses abruptly slowed down in KLP-7\textsuperscript{MCAK}-depleted or deleted oocytes 1.5 min after anaphase onset and chromosome separation paused at a distance of about 2.5 μm (Fig. 2G). Abnormal chromosome segregation was frequently followed by unsuccessful polar body extrusion and formation of a multi-pronucleate polyploid embryo (Fig. 2E). Thus, KLP-7\textsuperscript{MCAK} is essential for anaphase B chromosome movements and for the overall accuracy and success of meiotic chromosome segregation.

\textbf{KLP-7\textsuperscript{MCAK} promotes meiotic central spindle assembly and polar body extrusion}

The defects we observed in chromosome segregation and polar body extrusion in KLP-7\textsuperscript{MCAK}-depleted or deleted oocytes prompted us to analyze anaphase I
central spindle organization and function, which is indeed essential for both processes in the C. elegans oocyte (Dumont et al., 2010; Fabritius et al., 2011). Deconvolution microscopy on fixed oocytes in anaphase I showed obvious central spindle microtubule organization defects in KLP-7\textsuperscript{MCAK}-depleted oocytes (Fig. 3A).

To specifically understand KLP-7\textsuperscript{MCAK} function in meiotic central spindle assembly and in polar body cytokinesis, we analyzed microtubule organization and density over time by live imaging during anaphase I. For this we filmed oocytes expressing GFP-tagged β-tubulin and mCherry-tagged H2B (Fig. 3B, S3A). We noticed that the anaphase microtubule structures were more dense during anaphase in KLP-7\textsuperscript{MCAK}-depleted oocytes. Specifically, the segregating chromosome masses were devoid of microtubules in controls but remained embedded in a microtubule mesh throughout anaphase in KLP-7\textsuperscript{MCAK}-depleted oocytes. Consistent with this we found that the microtubule minus end marker ASPM-1\textsuperscript{Asp} was abnormally concentrated around the segregating chromosomes throughout anaphase (Fig. 3C, and Sup. Table 1). Thus ectopic microtubules assembled in the vicinity of chromosomes persisted throughout anaphase in KLP-7\textsuperscript{MCAK}-depleted oocytes leading to central spindle defects.

To test if these defects would directly affect central spindle component localization, we analyzed the dynamic recruitment of the central spindle microtubule bundling protein SPD-1\textsuperscript{PRC1} and of the Centralspindlin complex subunit CYK-4\textsuperscript{MgcRacGAP}. Both proteins are normally specifically recruited on central spindle microtubules during anaphase where they are essential for microtubule organization and cytokinesis (Mishima et al., 2002; Verbrugghe, 2004; Glotzer, 2005; Maton et al., 2015). The dense and disorganized microtubules of the central spindle in KLP-7\textsuperscript{MCAK}-depleted oocytes correlated with a reduction in the recruitment of these two proteins to the meiotic central spindle and with ectopic CYK-4\textsuperscript{MgcRacGAP} on chromosomes (Fig. 3D, E, Movies S6 and Sup. Table 1). Thus the lack of KLP-7\textsuperscript{MCAK} leads to the mislocalization of critical central spindle components, which likely contributes to the observed central spindle defects.

In control oocytes, recruitment of central spindle components ultimately leads to the formation of a contractile actomyosin ring that promotes plasma
membrane furrowing and cytokinesis (Maddox et al., 2012). To test if the improper central spindle component localization in KLP-7McAKe-depleted oocytes correlated with defects in actomyosin organization, we analyzed oocytes expressing GFP-tagged myosin II (NMY-2) and mCherry-tagged H2B during anaphase I. Consistent with previous findings, in control oocytes NMY-2myosin II formed a disc above the segregating chromosomes that progressively evolved into a cylinder, which ultimately formed the meiotic midbody between the segregated chromosomes (Dorn et al., 2010) (Fig. 3F, Movie S6 and Sup. Table 1). In KLP-7McAKe-depleted oocytes, a normal disc of NMY-2myosin II was initially visible above chromosomes but it strikingly almost never evolved into a cylinder. Instead the set of chromosomes that would normally end up in the first polar body reentered the oocyte cytoplasm and was surrounded by a thick layer of cortical NMY-2myosin II. Altogether, these results show that KLP-7McAKe is essential for meiotic cytokinesis and polar body extrusion through its function in central spindle organization.

**KLP-7McAKe prevents formation of ectopic cortical microtubule asters**

A recent study analyzing feeding-RNAi-mediated depletion of KLP-7McAKe or a KLP-7McAKe loss of function temperature sensitive mutant showed that the multipolar spindle phenotype observed when KLP-7McAKe activity is decreased could be rescued upon co-depletion of the NDC-80 kinetochore component (Connolly et al., 2015). This study, concluded that KLP-7McAKe is involved in destabilizing improper kinetochore-microtubule attachments established during early prometaphase similar to the function of its vertebrate ortholog MCAK during mitosis. This in turn would release tension within meiotic spindles that would otherwise lead to extra spindle pole formation. Although we found a similar rescuing effect of the *ndc-80(RNAi)* on early prometaphase spindles assembled in *klp-7McAKe*-deleted oocytes, we noticed that these spindles were still disorganized during most of prometaphase/metaphase (Fig. S4A, B). Furthermore, depleting the core kinetochore scaffold protein KNL-1 did not rescue spindle bipolarity when KLP-7McAKe is absent (Fig. S4A, B). Altogether, these results suggest that destabilizing kinetochore-microtubule attachments in oocytes is not sufficient to stably rescue bipolar spindle formation when KLP-
$7^\text{MCAK}$ is absent.

Thus to understand the origin of the extra spindle poles that assemble in KLP-$7^\text{MCAK}$-depleted or deleted oocytes, we performed *ex utero* live imaging of the spindle assembly process in oocytes. In line with a previous study, we noticed the presence of numerous ectopic microtubule asters near the cell cortex of KLP-$7^\text{MCAK}$-depleted or deleted oocytes (Fig. 4A, B) (Han et al., 2015). Ectopic cortical asters persisted throughout meiosis but disappeared at anaphase II onset (Fig. 4C). These asters displayed rapid movements at the cortex and tended to cluster together. Importantly, we observed a significant number of asters that aggregated at the metaphase spindle (Fig. 4D). Cortical asters were positive for ASPM-1 Asp and when they were localized near the meiotic spindle they contributed to generating supernumerary spindle poles (Fig. 4E, F). Thus we found that KLP-$7^\text{MCAK}$ is required to prevent ectopic microtubule assembly at the cell cortex, which otherwise leads to the formation of extra spindle poles and participates to the observed multipolar spindle phenotype.

*KLP-$7^\text{MCAK}$ is globally required for normal microtubule dynamics during meiosis*

The longer and denser meiotic spindles and ectopic cortical microtubule asters suggested that microtubules are overall more stable after KLP-$7^\text{MCAK}$ depletion. To test this hypothesis, we performed FRAP (Fluorescence Recovery After Photobleaching) experiments of the entire metaphase I spindle in control and KLP-$7^\text{MCAK}$-depleted oocytes that were genetically arrested in metaphase I (see materials and methods, Fig. 5A and Movie S7). Control metaphase I spindles were highly dynamic and recovered 86% of their initial fluorescence with a halftime of recovery (or $t_{1/2}$) of 22.2 sec. Meiotic spindles assembled in KLP-$7^\text{MCAK}$-depleted oocytes also recovered almost completely (87% of initial fluorescence), however recovery was delayed as compared to controls with a $t_{1/2}$ of 33.3 sec (Fig. 5B). Thus although the proportion of fully stable spindle microtubules that did not recover fluorescence over the course of the quantification period was not significantly ($p=0.9765$) different in control or KLP-$7^\text{MCAK}$-depleted oocytes, microtubules were on average more stable in the later ($p=0.0338$).
To determine the origin and dynamics of the ectopic cortical microtubule asters, we performed spinning disc cortical live imaging in control and \textit{klp-7MCAK} deleted oocytes expressing GFP-tagged $\beta$-tubulin (Fig. 5E, F). At the cell cortex, a dynamic cortical microtubule meshwork that slid rapidly in parallel with the cortex was visible in both control and \textit{klp-7MCAK}-deleted oocytes (Fig. 5C, D, E). However, the microtubule meshwork was more dense in the absence of KLP-7MCAK suggesting global microtubule stabilization leads to the observed ectopic cortical microtubule asters (Fig. 5C). Consistent with this, cortical microtubules were overall less dynamic in \textit{klp-7MCAK}-deleted oocytes as evident by the increased time spent in pause (not growing or shrinking, $t_{\text{cont}} = 28.1 \pm 29.4$ s, $t_{\text{klp-7\Delta}} = 68.9 \pm 35.11$ s, Fig. 5F) and the overall reduction of all microtubule dynamics parameters (Fig. 5G, H, I) (Lacroix et al., 2014). By applying a simple model of microtubule dynamics to our data, we calculated that the average theoretical length of microtubules in absence of KLP-7MCAK was higher than in control oocytes at steady state ($L_{\text{control}} = 6 \mu\text{m}$, $L_{\text{klp-7\Delta}} = 8.2 \mu\text{m}$, see Material and Method section), which is consistent with our live observations (Verde et al., 1992). Thus KLP-7MCAK increases the cortical microtubule dynamics in the \textit{C. elegans} oocyte preventing ectopic microtubule aster formation.

\textbf{Kinesin-13 depolymerases KLP-7 and MCAK prevent ectopic microtubule assembly when centrosome activity is reduced or absent}

Strikingly, the ectopic asters that formed in oocytes in absence of KLP-7MCAK were never observed in mitotic embryos. Instead during mitosis, the absence of KLP-7MCAK leads to increased astral microtubule density and a corresponding increase in astral microtubule pulling forces at centrosomes (Fig. S3B) (Srayko et al., 2005). To test if this difference between oocytes and zygotes could be linked to the presence of functional centrosomes in the zygote, we analyzed the effect of reducing centrosomal activity following depletion of the scaffold component SPD-5 in \textit{klp-7MCAK}-deleted zygotes. In absence of functional centrosome in \textit{spd-5(RNAi)} zygotes when KLP-7MCAK is present, cytoplasmic asters were never observed and the few microtubules that assembled following NEBD always radiated from the condensed chromosomes (Fig. 6A) (Hamill et al., 2002). Strikingly however, when SPD-5 was depleted in \textit{klp-7MCAK}-deleted zygotes,
numerous cytoplasmic ectopic asters assembled at NEBD at distance from chromosomes (Fig. 6A, B). These asters subsequently coalesced around the condensed chromosomes to form a single larger microtubule structure (not shown). Thus in absence of functional centrosomes in C. elegans zygotes, KLP-7MCAK activity is essential to prevent ectopic microtubule assembly.

To test if this function of KLP-7MCAK is a general feature of kinesin-13, we tested the effect of MCAK depletion in HeLa cells when centrosome activity is reduced during microtubule regrowth after nocodazole washout (Cavazza et al., 2016). 45 mins after removing nocodazole, microtubules reassembled from 2 microtubule-organizing centers (MTOCs) on average in control cells (2.16±0.06 MTOCs)(Fig. 6C, D). In contrast, 3 MTOCs (3.13±0.12 MTOCs) could be detected in most MCAK-depleted cells. Importantly, the effect of depleting MCAK was specific to cells in which centrosome activity was reduced by the nocodazole treatment. Thus ectopic microtubule nucleation centers are activated in human cells with reduced centrosome activity when MCAK levels are decreased. Altogether, these results suggest that preventing ectopic microtubule assembly in cells with reduced or absent centrosome activity is a previously uncharacterized general and conserved function of kinesin-13 depolymerases (Fig. 6E).

DISCUSSION

**KLP-7MCAK is essential for the formation of a functional spindle in the C. elegans oocyte**

Previous studies of meiotic spindle assembly in the C. elegans oocyte have been performed at relatively low spatial and temporal resolutions, did not provide temporal information and/or missed the very early steps of spindle assembly (Yang et al., 2003; Connolly et al., 2015; Wolff et al., 2016). Here we provide a precise quantitative picture covering the full time window of interest and the first time-resolved analysis of the entire process of meiotic spindle formation in this system. Oocytes of most species lack centriole-containing centrosomes and microtubules assemble through the chromatin-dependent pathway or from acentriolar MTOCs (Dumont and Desai, 2012). In these acentrosomal oocytes,
microtubules can be seen originating locally from the chromatin itself or from discrete organizing centers (Huchon et al., 1981; Gard, 1992; Dumont et al., 2007; Schuh and Ellenberg, 2007; Colombie et al., 2008). In contrast, we found that in the *C. elegans* oocyte, microtubules, which are excluded from the nucleus before NEBD, assemble in the nuclear space after NEBD to form a diffuse cloud. This result is consistent with qualitative observations made in previous studies and with the lack of discrete MTOCs in this system (Yang et al., 2003). We found that spindle assembly is constrained within the space of the rupturing nuclear envelope. We thus propose that in *C. elegans* oocytes the space delimited by the nuclear envelope remnants acts as a diffuse microtubule-organizing center. In KLP-7<sup>MCAK</sup>-depleted oocytes, NEBD occurred normally but the microtubule nuclear cloud did not form and an excess of microtubules persisted around the breaking nuclear envelope throughout phase 1. This suggests that the function of KLP-7<sup>MCAK</sup>, which is cytoplasmic before and at NEBD, is to destabilize these perinuclear microtubules in order to release free tubulin necessary for the formation of the nuclear cloud.

Following formation of the microtubule nuclear cloud, bundling and cross-linking activities led to microtubule coalescence around meiotic chromosomes. This second step is likely to be under the control of microtubule motors previously implicated in successful meiotic divisions such as dynein, the two redundant kinesin-14 family members KLP-15/16<sup>NCD</sup> and the kinesin-12 family member KLP-18<sup>XKLP2</sup> (Dernburg et al., 2000; Piano et al., 2000; Colaiacovo et al., 2002; Segbert et al., 2003; Wolff et al., 2016). We found that in absence of a nuclear cloud of microtubules in KLP-7<sup>MCAK</sup>-depleted oocytes, the ectopic perinuclear microtubules are instead bundled and coalesce around chromosomes to form a seemingly normal multipolar spindle. However the subsequent organization of microtubules into a bipolar spindle was impaired. Instead, abnormally dense multipolar spindles with long, disorganized, and stable microtubules were formed.

Following bipolar spindle formation and chromosome alignment on a tight metaphase plate, drastic microtubule reorganization occurs that ultimately leads to chromosome segregation and polar body extrusion. We previously showed that chromosome segregation in the *C. elegans* oocyte is driven by an
atypical kinetochore-independent mechanism (Dumont et al., 2010). Instead in this system, central spindle organization is critical for chromosome segregation (Muscat et al., 2015; McNally et al., 2016). In agreement, we show here that KLP-7MCaK depletion leads to disorganized central spindles that correlate with impaired chromosome segregation. Specifically, anaphase B, which normally accounts for most of the segregation process, does not occur. In line with this result, central spindle elongation was proposed to be specifically important for anaphase B chromosome movements (McNally et al., 2016). Live imaging of the minus-end marker GFP::ASPM-1Asp at this stage, showed that microtubule minus-ends are distributed all over the disorganized central spindle instead of being concentrated toward chromosomes and generate an antiparallel microtubule overlap. We suspect that KLP-7MCaK is required to generate this overlap by preventing excessive and/or ectopic microtubule elongation from chromosomes where it is concentrated during meiotic anaphase (Fig. 1E)(Han et al., 2015).

SPD-1PRC1 and the CentralSpindlin complex (including CYK-4MgRacGAP) have been shown to preferentially interact with overlapping microtubule plus-ends, which might explain their delocalization in the denser and disorganized central spindle assembled following klp-7(RNAi) (Bieling et al., 2010; Davies et al., 2015).

In the absence of KLP-7MCaK, another striking defect in the organization of the microtubule network is the formation of multiple microtubule asters at the oocyte cortex. Although a cortical meshwork of microtubules is present in control oocytes, asters are normally not present at the cortex. A cytoplasmic pool of KLP-7MCaK may be responsible for reducing the stability of this microtubule meshwork and prevent ectopic aster formation. We observed that cortical asters located near the meiotic spindle, often joined the spindles and contributed to the formation of the multipolar spindle. The minus-end directed motor dynein, present throughout the cortex of the oocyte, is probably responsible for the aster aggregation we observed (Crowder et al., 2015). Asters incorporated in the spindle could saturate the activity of microtubule motors and thus prevent normal spindle bipolarization.
Kinesin-13 depolymerases prevent ectopic microtubule assembly when centrosome function is low or absent

The ectopic asters observed in oocytes in absence of KLP-7MCAK disappeared abruptly at anaphase II and were never observed in mitotic embryos. During mitosis, KLP-7MCAK-depletion leads to increased astral microtubule density but does not lead to ectopic cortical aster formation (Srayko et al., 2005). We hypothesize that this difference is linked to the large size of the embryonic mitotic spindle as compared to the tiny oocyte spindles, and to the absence of functional centrosomes in oocytes, which are the dominant microtubule organizing centers during mitotic divisions (Hannak et al., 2002). Oocytes and the single celled fertilized zygote share a common cytoplasmic composition including the same concentration of tubulin heterodimers. The large astral spindle in zygotes contains a higher microtubule mass than tiny meiotic spindles in oocytes. This leads to a lower cytoplasmic free tubulin heterodimer concentration in zygotes as compared to oocytes. The cytoplasmic tubulin concentration in oocytes is thus likely closer to the in vivo critical concentration at which microtubules can spontaneously nucleate and form microtubule asters. KLP-7MCAK depolymerase activity must restrain this spontaneous microtubule assembly in oocytes. During mitosis, the centrosomes would thus act as a microtubule polymerization-buffering system and prevent overall spindle disorganization. In contrast, in oocytes depleted of KLP-7MCAK, free tubulin heterodimers are incorporated in all existing microtubule networks including the perinuclear microtubules in unfertilized oocytes, and the cortical meshwork and the spindle after fertilization ultimately leading to its disorganization. Consistent with this interpretation we showed that, in absence of KLP-7MCAK, microtubule asters spontaneously assembled during mitosis in the one-celled zygote only when free tubulin heterodimer concentration was experimentally increased through reduction of centrosome activity. Similarly, we observed a higher number of MTOCs in human tissue cultured cells when centrosome activity was reduced (during microtubule regrowth after nocodazole washout) after kinesin-13 MCAK depletion than in control cells. Similar ectopic asters have been observed in Drosophila oocytes depleted of the kinesin-13 KLP10A.
(Radford et al., 2012; Do et al., 2014). We thus propose that, when centrosome activity is reduced or absent, global microtubule destabilization by a kinesin-13 family member(s) is essential to prevent formation of ectopic microtubule asters, which otherwise lead to spindle disorganization and chromosome mis-segregation (Fig. 6E). This, previously uncharacterized function of kinesin-13 proteins, defines a new level in the regulation of microtubule assembly \textit{in vivo}, which is particularly important for the generation of euploid oocytes that lack centrosomes. As kinesin-13 motors are highly conserved across evolution, this new paradigm likely applies to other species and could further our understanding of human reproduction and the etiology of sterility.
MATERIALS AND METHODS

C. elegans strains and RNAi

C. elegans strains are listed in Table S2 and were maintained at 16°C or 23°C (Oegema et al., 2001). Primers for dsRNA production are listed in Table S3 (Oegema et al., 2001). L4 hermaphrodites were microinjected with dsRNA and incubated at 20°C for 48 hours before processing.

HeLa cell culture and treatment

HeLa cells, tested monthly for mycoplasma contamination using a luminometer detection method (Lonza), were maintained in DMEM (Lonza) supplemented with 10% FBS, penicillin/streptomycin (Gibco) at 37°C in a humidified atmosphere with 5% CO₂. Cells were plated on glass coverslips coated with poly-L-lysine (Sigma-Aldrich). RNAi experiments were conducted using RNAi MAX transfection reagent (Invitrogen) according to the manufacturer’s guidelines. Previously published siRNA oligos were used to deplete MCAK (Domnitz et al., 2012). After 48 hours of siRNA treatment, the cells were incubated for 2-3 hours with 300 ng/ml nocodazole. The nocodazole was then washed out 5 times with fresh DMEM and cells were left for 45 minutes in fresh DMEM. Cells were then briefly washed in PBS and fixed in PHEM (60 mM Pipes, 25 mM Hepes, 10 mM EGTA, and 2 mM MgCl₂, pH 6.9) containing 4% formaldehyde for 10 minutes. Immunofluorescence was conducted using antibodies against mouse anti-β-tubulin (Sigma) and human anti-Ndc80 antibody (kind gift from Iain Cheeseman). DNA was then counterstained with 1 µg/mL of Hoechst.

Images were acquired on a DeltaVision Core deconvolution microscope (Applied Precision) equipped with a CoolSnap HQ2 CCD camera. Twenty Z-sections were acquired at 0.3 µm steps using a 100x 1.4 NA Olympus U-PlanApo objective without binning. Maximal projections of stacks of interest after image deconvolution (SoftWorks) are presented. Equivalent exposure conditions were used between controls and drug-treated cells. Experiments were repeated 3 times. The number of spindle poles or the presence of ectopic microtubule foci in the cytoplasm was visually assessed and quantified.
Live imaging and metaphase I arrest

For in utero live imaging experiments, adult worms were anaesthetized using 100 mg Tricaine (Sigma-Aldrich, E10521) and 10 mg Tetramisol hydrochloride (Sigma-Aldrich, T1512) diluted in 1 mL of M9 buffer. Immobilized worms were then mounted on a 2% agarose pad in M9 buffer between a slide and a coverslip. Live imaging was performed using a Nikon CFI APO LBDA S 40x/NA1.25 water objective on a spinning disk confocal microscope (Roper Scientific) equipped with a CoolSNAP HQ2 camera (Photometrics) and acquisition parameters were controlled by MetaMorph 7 software (Molecular Devices). 4 z sections every 2 µm were acquired at 20 seconds intervals. Imaging on ex utero oocytes was performed as described in (Dumont et al., 2010).

FRAP experiments were performed on ex utero oocytes using a Nikon CFI APO LBDA S 60x/NA1.4 oil objective with 2x2 binning on a spinning disk confocal microscope equipped with the iLas Pulse FRAP/Photoactivation module (Roper Scientific). The extensive disassembly of microtubules observed during the spindle shrinkage phase could preclude measuring fluorescence recovery. To avoid this caveat and to measure fluorescence recovery in a steady state, we performed the FRAP experiments in the mat-2(ax76ts) temperature-sensitive (ts) strain that arrests in metaphase I when shifted at the restrictive temperature (26°C)(Golden et al., 2000). Stacks of 4 z-sections with a spacing of 2 µm were acquired every 3 sec in the GFP channel before a single FRAP event of the entire surface of the metaphase spindle. After the FRAP event, images were acquired every 3 sec for the first 120 sec, then every 10 sec for the following 100 seconds and every 20 sec for the last 500 seconds. A maximum projection of the 4 z-sections is presented for each time point. The average fluorescence was measured in a box around the metaphase spindle (Fspin) and in a box away from the spindle in the cytoplasm (Fcyt). Normalization, correction and fitting of the measured fluorescence intensities were performed using the Prism6 software (GraphPAD Software). Although we verified that the imaging conditions we used did not lead to any significant photobleaching on embryos that did not undergo a FRAP event, the data were corrected for the any potential photobleaching occurring during acquisition by multiplying each time point by Fcyt(0)/Fcyt(t). In order to be able to compare different experiments, the last prebleach and first
postbleach time points were normalized to 1 and 0 respectively
\(F_{\text{corNormalized}}(t)=F_{\text{cor}}(t)-F_{\text{corPost}}/F_{\text{corPre}}-F_{\text{corPost}}\). The mean value of
\(F_{\text{corNormalized}}\) was then calculated for individual embryos at each time point. The corresponding plot was fitted to a mono-exponential function and the half-time for recovery was extracted.

**Image analysis and microtubule length calculation**

Image analyses and quantifications were performed using the Fiji (Schindelin et al., 2012) and Icy software (de Chaumont et al., 2012). Kymographs were generated using the Multi Kymograph tool in Fiji.

For estimating the average length of microtubules at steady state, we used a simple mathematical model that links microtubule length distribution to dynamics parameters (Verde et al., 1992). In this model, the average length \(<L>\) is equal to \((R_{\text{shrink}} \times R_{\text{growth}}) / ((R_{\text{shrink}} \times R_{\text{cat}})-(R_{\text{growth}} \times R_{\text{res}}))\).

**Antibodies and immunofluorescence microscopy**

Immunofluorescent staining was performed as described in (Dumont et al., 2010). The rabbit anti-KLP-7\(^{MCAK}\) antibody was custom produced, validated in this study and used at 1 \(\mu\text{g/\mu l}\).

**Graphs and statistical analysis**

Experiments were repeated at least twice and a minimum number of 10 oocytes were quantified for each experimental condition. All graphs and statistical analysis were done with Excel for mac 2011 (Microsoft) and GraphPad Prism 6 (GraphPad Software). Statistical significance was evaluated using unpaired t-tests with Welch’s correction or one-way ANOVA.
ACKNOWLEDGEMENTS

We thank Jeremy Cramer from Cherry Biotech (Rennes, France) for allowing us to use pre-commercial development versions of the CherryTemp system. We are grateful to Patricia Moussounda for providing technical support. We thank the CGC for worm strains and the NBRP (NIG, Japan) for the supply of the tm2143 mutant strain. We thank Iain Cheeseman for the Ndc80 bonsai antibody. E.G. is supported by an ARC (Association pour la Recherche sur le Cancer) post-doctoral fellowship. This work was supported by CNRS and University Paris Diderot and by grant NIH R01 GM117407 and NIH DP2 OD008773 to J.C.C., a CRUK Career Development Fellowship (C40377/A12840) to J.W., and grants from the ANR (Agence Nationale de la Recherche, ANR-09-RPDOC-005-01), the Mairie de Paris (Emergence) and the FRM (Fondation pour la Recherche Médicale, DEQ20160334869) to J.D.

AUTHOR CONTRIBUTIONS

Experiments were conceived by J.D and were primarily performed and analyzed by E.G. and M.S. All the strains used in this study were generated by M.S. and J.C.C. K.L., F.E., G.M., and B.L. performed some of the live imaging experiments and analyses. A.G. and J.W. conceived, performed and analyzed the experiments in HeLa cells. J.C.C., J.W. and J.D. made the figures and wrote the manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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REFERENCES


Figures

A

1. Microtubule invasion
2. Bundling and coalescence
3. Microtubule organization
4. Spindle shrinkage

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C

GFP: β-tub intensity

D

Average fluorescence intensity in the nuclear area (a.u.)

E

GFP:: KLP-7

F

GFP::KLPMCAK

G

GFP:: ASPM-1

GFP::LMN-1
Figure 1. KLP-7MCAK is required for meiotic spindle assembly.

(A) Still images from *in vivo* live imaging in GFP-tagged β-tubulin and mCherry-tagged H2B-expressing oocytes showing the 4 phases of meiosis I spindle assembly in the indicated conditions. Timings are relative to NEBD. (B) Average timings relative to NEBD of the 4 phases of spindle assembly in control and KLP-7MCAK-depleted oocytes. (C) Fluorescence intensity linescan of GFP-tagged β-tubulin across the nucleus between 1.7 min before NEBD and 3.3 min after NEBD (timings are color-coded according to the horizontal scale presented at the bottom). 0 μm corresponds to the center of the nucleus. The sample size (number of oocytes analyzed) is provided in the figure and was generated by aggregation over 6 independent experiments. Error bars represent the s.e.m. (D) Average fluorescence intensity of GFP-tagged β-tubulin and mCherry-tagged H2B in the nuclear area between 1.7 min before NEBD and 3.3 min after NEBD. Error bars represent the s.d. (E) Still images from *in vivo* live imaging in a GFP-tagged KLP-7MCAK and mCherry-tagged β-tubulin-expressing oocyte. (F) Still images from *in vivo* live imaging in GFP-tagged ASPM-1Asp and mCherry-tagged H2B-expressing oocytes showing the 4 phases (indicated by the color-coded line above each image) of meiosis I spindle assembly in the indicated conditions. Timings are relative to NEBD. (G) Still images from *in vivo* live imaging in GFP-tagged LMN-1Lam in 1 and mCherry-tagged H2B-expressing oocytes showing the 4 phases (indicated by the color-coded line above each image) of meiosis I spindle assembly in the indicated conditions. Scale bars, 10 μm.
Figure 2. KLP-7MCAK promotes proper meiotic spindle organization and chromosome segregation.

(A) Still images from live imaging in GFP-tagged β-tubulin and mCherry-tagged H2B-expressing oocytes between prometaphase I and anaphase II. Timings are relative to anaphase I onset. Scale bar, 5 μm. (B) Quantification of the spindle...
microtubule average intensity over time in the indicated conditions. Error bars represent the s.e.m. (C) Pseudocolored still images of metaphase I spindles in control and KLP-7MCAK-depleted oocytes (Left). Scale bar, 5 μm. Distribution of the length of microtubules or microtubule bundles emanating from meiosis I spindles in control and KLP-7MCAK-depleted oocytes (Right). (D) Still images from in vivo live imaging in GFP-tagged ASPM-1Asp and mCherry-tagged H2B-expressing oocytes highlighting the dynamics of spindle poles during anaphase I. Timings are relative to anaphase I onset (Left). Scale bar, 5 μm. Quantification of the spindle pole number in control and KLP-7MCAK-depleted oocytes before (-20 s) and after (+20 s) anaphase onset (Right). (E) Top row: schematic representation of chromosome segregation during meiosis I and II. (PB1, first polar body; PB2, second polar body; PN, pronucleus). Bottom rows: still images from live imaging of mCherry-tagged H2B expressing fertilized oocytes. Timings are relative to anaphase I onset. In the KLP-7MCAK depletion and deletion images, the white arrowheads indicate lagging chromosomes during anaphase I. Quantification of lagging chromosomes during anaphase I and II and of polar body extrusion (meiosis I, MI; meiosis II, MII) is shown on the right. Scale bar, 5 μm. (F) Kymographs, initiated at anaphase I onset, showing movements of chromosomes during anaphase I. The time interval between consecutive strips is 20 s. Scale bar, 2 μm. (G) Quantification of chromosome masses separation over time in control and KLP-7MCAK-depleted or deleted oocytes. Error bars represent the s.e.m. (H) Timing of meiotic divisions and of the first embryonic mitosis is not affected by depletion or deletion of KLP-7MCAK. Error bars represent the s.e.m. Unpaired t-tests with Welch’s correction were used to determine significance, (Anal-II: cont vs klp-7(RNAi) p=0.2426, cont vs Δklp-7 p=0.2081; Anal-Mito: cont vs klp-7(RNAi) p=0.8367, cont vs Δklp-7 p=0.3315).
Figure 3. KLP-7<sup>MCAK</sup> promotes meiotic central spindle assembly and polar body extrusion.

(A) KLP-7<sup>MCAK</sup> depletion leads to central spindle microtubule disorganization during meiotic anaphase. Scale bar, 5 μm. (B-E) Montage of still images from a live imaging experiments in GFP-tagged β-tubulin (B), GFP-tagged ASPM-1<sup>Asp</sup> (C), GFP-tagged CYK-4<sup>MgcRacGAP</sup> (D), GFP-tagged SPD-1<sup>PRC1</sup> (E), and mCherry-tagged...
H2B-expressing oocytes during anaphase I (Left). Scale bar, 2 μm. The time interval between consecutive strips is 20 s. Quantifications of microtubule average intensity on chromosomes (red) or in the central spindle region (green) during anaphase are shown on the right. The quantified regions of interest are schematized in the top row. (F) Montage of still images from a live imaging experiment in GFP-tagged NMY-2\textsuperscript{myosin II} and mCherry-tagged H2B-expressing oocytes during anaphase I (Left). Scale bar, 5 μm. Timings are relative to anaphase I onset. Quantification of the GFP-tagged NMY-2\textsuperscript{myosin II} average intensity during anaphase I in a region of interest surrounding the chromosomes and the anaphase central spindle is shown on the right. p values associated with Figure 3 are indicated in Table S1. Error bars represent the s.e.m.
Figure 4. KLP-7MCAK prevents formation of ectopic cortical microtubule asters that generate extra spindle poles.

(A) Pseudocolored images from a live imaging experiment in GFP-tagged β-tubulin-expressing oocytes showing the ectopic cortical asters after KLP-7MCAK depletion. Magnifications of a cortical region (white dashed box) are shown on the right. Arrows in the KLP-7MCAK image point to cortical asters. (B) Quantification of the cortical aster number 20 s before anaphase onset in control and KLP-7MCAK-depleted oocytes. Error bars represent the s.e.m. Unpaired t-test with Welch’s correction was used to determine significance (P<0.0001). (C) Quantification of the cortical average intensity of GFP::β-tubulin over time in the indicated conditions. Error bars represent the s.e.m. (D) Pseudocolored still images from a live imaging experiment in GFP-tagged β-tubulin expressing oocytes showing cortical asters movements and incorporation in the meiosis I spindle. Timings are relative to the first time point shown. (E) Pseudocolored images from a live imaging experiment in GFP-tagged ASPM-1Asp-expressing oocytes showing the ectopic cortical asters after KLP-7MCAK depletion. Arrows in the KLP-7MCAK image point to cortical asters. Scale bar, 5 μm. (F) Quantification of the cortical aster number 20 s before anaphase onset in control and KLP-7MCAK.
depleted oocytes. Error bars represent the s.e.m. Unpaired t-test with Welch’s correction was used to determine significance (P<0.0001).
Figure 5. **KLP-7MCAK** is globally required for normal microtubule dynamics during oocyte meiosis.

(A) Pseudocolored still images from a FRAP experiment in GFP-tagged β-tubulin-expressing oocytes artificially arrested in metaphase I. Timings are relative to the bleaching event. (B) Quantification of the fluorescence recovery over time during the FRAP experiment. Error bars represent the s.e.m. Scale bars, 5 μm. (C) Pseudocolored still images from a cortical live imaging experiment in GFP-tagged β-tubulin-expressing oocytes showing the dense microtubule meshwork present in klp-7MCAK-deleted oocytes. (D) Pseudocolored kymographs of individual cortical microtubules, showing their dynamics in control and klp-7MCAK-deleted oocytes. A schematic representation of a growth and shrinkage event in a control oocyte and of a growth and pause event in a klp-7MCAK-deleted oocyte is shown.
on the right. The time interval between consecutive strips is 250 ms. (E) Quantification of the microtubule sliding velocity in control and klp-7<sup>MCAK</sup>-deleted oocytes (n(MTs)≥210). Error bars represent the s.d. (F) Quantification of the time spent in pause by individual microtubules in control and klp-7<sup>MCAK</sup>-deleted oocytes (n(MTs)≥130). Box plots represent the 75th percentile with the median indicated as a line and error bars represent the s.d. (G) Quantification of the growth and shrinkage rates, catastrophe and rescue frequencies of individual microtubules in control and klp-7<sup>MCAK</sup>-deleted oocytes (n(MTs)≥160). Error bars represent the s.d. (H) Diamond graph representing the microtubule dynamics in control oocytes (Left). Microtubule dynamics in control and klp-7<sup>MCAK</sup>-deleted oocytes are displayed and compared using jointly normalized diamond graphs (Right). (I) Average values for the four parameters used in the diamond graphs. Unpaired t-tests with Welch's correction were used throughout the figure to determine significance (P<0.0001 for all comparisons).
Figure 6. Kinesin-13 depolymerases prevent ectopic microtubule assembly when centrosome function is low or absent.

(A) Pseudocolored images from a live imaging experiment in GFP-tagged β-tubulin-expressing klp-7Δ or control zygotes 100 s after NEBD with or without spd-5(RNAi). Multiple ectopic microtubule asters assemble in absence of KLP-7MCAK when centrosome activity is impaired after spd-5(RNAi). Scale bar, 5 µm.

(B) Quantification of the number of microtubule asters in klp-7Δ or control...
zygotes 100 s after NEBD with or without spd-5(RNAi). Error bars represent the s.d. Unpaired t-test with Welch’s correction was used to determine significance (P<0.0001). (C) Immunostaining of kinetochores (Ndc80) and microtubules in control or MCAK-depleted HeLa cells incubated or not for 3 hours in 300 nM nocodazole or 45 min after nocodazole washout. Ectopic MTOCs are observed in MCAK-depleted cells only after nocodazole washout. Scale bar, 5 μm. (D) Quantification of the MTOC number in control or MCAK-depleted cells before nocodazole treatment. (p=0.0837) or 45 min after nocodazole washout (p<0.0001). Unpaired t-test with Welch’s correction was used to determine significance. Error bars represent the s.d. (E) Model of the kinesin-13-dependent ectopic microtubule assembly when centrosome activity is low or absent. In controls (Right), the centrosomes incorporate most of the free tubulin and thus act as a microtubule polymerization-buffering system. The free tubulin concentration is low and does not allow spontaneous microtubule aster formation at distance from the centrosomes. In absence of centrosomes, the free tubulin concentration is high enough to allow spontaneous ectopic microtubule aster formation (Left), unless Kinesin-13 is active and destabilizes these ectopic asters (Middle).
A B

% embryonic lethality

control GFP::KLP-7b

klp-7Δ (RNAi)

klp-7Δ

n=1918

100% embryonic lethality

n=400

n=1482

n=1369

n=840

n=995

C D

NeckGFP Motor domain

GFP::KLP-7 RNAi-resistant re-encoded region

689 aa

KLP-7 GFP::KLP-7

α-KLP-7

KLP-7MCAKTubulin DNA

klp-7 (RNAi)

klp-7Δ

control

control

GFP::KLP-7

klp-7Δ klp-7Δ

GFP::KLP-7

Development 144: doi:10.1242/dev.147504: Supplementary information
Figure S1. Efficiency and specificity of the RNAi-mediated depletion of KLP-7\textsuperscript{MCAK}.

(A) Quantification of the embryonic lethality associated with \textit{klp-7}(RNAi) or in the \textit{klp-7} deletion (\textit{klp-7Δ}) in presence or absence of RNAi-resistant GFP::KLP-7\textsuperscript{MCAK}. Error bars represent the s.d. (B) Immunostainings of KLP-7\textsuperscript{MCAK} during metaphase I in control, \textit{klp-7}(RNAi) and \textit{klp-7Δ} oocytes (Right). A close-up of the spindle region is shown in the white dashed box. Microtubules (green) and chromosomes (red) are also stained (Left). Scale bar, 5 μm. (C) Schematic representation of the RNAi-resistant GFP::KLP-7\textsuperscript{MCAK} transgene displaying the functional domains, the re-encoded RNAi-resistant region and the region recognized by the anti-KLP-7 antibody used in this study. (D) Western blot on whole worm extracts with the indicated genotypes. The rabbit KLP-7 antibody recognized both endogenous and GFP-tagged KLP-7 proteins.
Figure S2. KLP-7MCAK function during meiotic spindle assembly.

(A) Still images from in vivo live imaging in GFP-tagged β-tubulin and mCherry-tagged H2B-expressing oocytes showing the first phase of meiosis I spindle assembly in the indicated conditions. Timings are relative to NEBD. (B) Fluorescence intensity linescan of mCherry-tagged H2B and GFP-tagged β-tubulin across the nucleus between 1.7 min before NEBD and 3.3 min after NEBD (timings are color-coded according to the horizontal scale presented at the bottom). 0 µm corresponds to the center of the nucleus. The sample size (number of oocytes analyzed) is provided in the figure and was generated by aggregation over 6 independent experiments. Error bars represent the s.e.m. (C) Still images from in vivo live imaging in GFP-tagged ASPM-1Asp and mCherry-tagged H2B-expressing oocytes showing the 4 phases (indicated by the color-coded line above each image) of meiosis I spindle assembly in the indicated conditions. Timings are relative to NEBD. (D) Still images from in vivo live imaging in GFP-tagged LMN-1Lamin1 and mCherry-tagged H2B-expressing oocytes showing the 4 phases (indicated by the color-coded line above each image) of meiosis I spindle assembly in the indicated conditions. Timings are relative to NEBD. Scale bars, 10 µm.
Figure S3. KLP-7\textsuperscript{MCAK} is essential for central spindle assembly.

(A) Fluorescence intensity linescan of mCherry-tagged H2B and the indicated GFP-tagged protein across the central spindle long axis during anaphase I. 0 µm corresponds to the position of chromosomes in metaphase (metaphase plate). The sample size (number of oocytes analyzed) is provided in the figure and was generated by aggregation over 3 independent experiments. Error bars represent the s.d.
Figure S4. Kinetochore component depletion does not stably rescue spindle bipolarity in the absence of KLP-7MCAK.

(A) Still images from live imaging in GFP-tagged β-tubulin and mCherry-tagged H2B-expressing oocytes in the indicated conditions between prometaphase I and anaphase I. Timings are relative to anaphase I onset. Scale bar, 5 μm. (B). Still images from live imaging in GFP-tagged β-tubulin and mCherry-tagged H2B-expressing zygotes in the indicated conditions. The same embryos were followed from metaphase of meiosis I to completion of the first mitosis. KLP-7MCAK-deleted zygotes have excessive astral pulling forces leading to central spindle rupture and abnormally fast sister chromatid segregation; NDC-80-depleted zygotes do not align chromosomes properly and display chromosomal bridges during anaphase; KNL-1-depleted embryos exhibit a “kinetochore null” phenotype characterized by clustering of chromosomes from each pronucleus, absence of a metaphase plate, and no chromosome segregation. Timings are relative to anaphase onset. Scale bar, 5 μm.
**Movie S1.** *In utero* live imaging of immobilized worms expressing GFP-tagged β-tubulin and mCherry-tagged H2B. Scale bar, 10 µm.
Movie S2. *In utero* live imaging of an immobilized worm expressing GFP-tagged KLP-7MCAK. Scale bar, 10 μm.
**Movie S3.** *In utero* live imaging of immobilized worms expressing GFP-tagged ASPM-1Asp and mCherry-tagged H2B. Scale bar, 10 μm.
**Movie S4.** *In utero* live imaging of immobilized worms expressing GFP-tagged LMN-1Lamin1 and mCherry-tagged β-tubulin. Scale bar, 10 μm.
**Movie S5.** Live imaging of fertilized oocytes expressing GFP-tagged β-tubulin and mCherry-tagged H2B. Scale bar, 10 µm.
**Movie S6.** Live imaging of oocytes expressing GFP-tagged CYK-4<sup>MgcRacGAP</sup> (Left), GFP-tagged SPD-1<sup>PRC1</sup> (Middle) or GFP-tagged NMY-2<sup>myosin II</sup> (Right), and mCherry-tagged H2B. Scale bar, 10 µm.
**Movie S7.** FRAP experiment in fertilized oocytes expressing GFP-tagged β-tubulin. Scale bar, 10 µm.
**SUPPLEMENTAL TABLE S1 – p values for Figure 3**

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</thead>
<tbody>
<tr>
<td></td>
<td>0 s</td>
<td>100 s</td>
<td>200 s</td>
<td>300 s</td>
</tr>
<tr>
<td>β-tub</td>
<td>p&lt;0.0001</td>
<td>p=0.0021</td>
<td>p=0.1251</td>
<td>p=0.0197</td>
</tr>
<tr>
<td>ASPM-1</td>
<td>p&lt;0.0001</td>
<td>p=0.0016</td>
<td>p=0.4725</td>
<td>p=0.3638</td>
</tr>
<tr>
<td>CYK-4</td>
<td>p=0.3475</td>
<td>p=0.0015</td>
<td>p=0.011</td>
<td>p=0.0426</td>
</tr>
<tr>
<td>SPD-1</td>
<td>p=0.3004</td>
<td>p=0.0024</td>
<td>p=0.7895</td>
<td>p=0.3464</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Control vs klp-7(RNAi)</th>
<th>0 s</th>
<th>100 s</th>
<th>200 s</th>
<th>300 s</th>
<th>400 s</th>
</tr>
</thead>
<tbody>
<tr>
<td>NMY-2</td>
<td>p=0.0241</td>
<td>p=0.0010</td>
<td>p=0.0005</td>
<td>p=0.0086</td>
<td>p=0.2718</td>
</tr>
</tbody>
</table>
**SUPPLEMENTAL TABLE S2 – C. elegans strains used in this study**

*unc-119(ed3)* was present in the parental strains, but these strains have not been directly sequenced to determine if the unc-119 gene contains the ed3 mutation.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>N2</td>
<td>Wild Type (Ancestral N2 Bristol)</td>
</tr>
<tr>
<td>JCC483</td>
<td>ojIs1[Ppie-1::GFP::tbb-2::3’tbb-2]; unc-119(ed3)III?; ltIs37[pAA64; Ppie-1::mCherry::his-58; unc-119 (+)]IV*</td>
</tr>
<tr>
<td>JDU320</td>
<td>klp-7(tm2143); ijmSi41[pJD457; Pmex-5::GFP::klp-7b reencoded::3’tbb-2; cb-unc-119(+)]I; ijmSi41[pJD456; Pmex-5::mCherry::tbb-2::3’tbb-2]II; unc-119(ed3) III?*</td>
</tr>
<tr>
<td>JDU106</td>
<td>ijmSi7 [pJD348; Pmex-5::GFP::tbb-2::3’tbb-2; mCherry::his-11::3’tbb-2; cb-unc-119(+)]I; mat-2(ax76)II; unc-119(ed3)III*</td>
</tr>
<tr>
<td>JDU316</td>
<td>klp-7(tm2143); ojIs1[Ppie-1::GFP::tbb-2::3’tbb-2]; unc-119(ed3) III?; ltIs37[pAA64; Ppie-1/mCherry::his-58; unc-119 (+)] IV*</td>
</tr>
<tr>
<td>JDU115</td>
<td>ijmSi11 [pJD359; Pmex-5::spd-1::sfGFP::3’spd-1; cb-unc-119(+)]I; unc-119(ed3)III?; ijmSi25 [pJD425; Pmex-5::TagRFP::zen-4::3’tbb-2; cb-unc-119(+)]III; ltIs37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)]IV*</td>
</tr>
<tr>
<td>JDU384</td>
<td>unc-119(ed3) III?; ltIs37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; zuls45[Pnmy-2::nmy-2::GFP::3’nmy-2; unc-119(+)] V*</td>
</tr>
<tr>
<td>JDU385</td>
<td>unc-119(ed3) III?; ltIs37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV; ltIs16[pASM07; Ppie-1::GFP::cyk-4::3’tbb-2; unc-119 (+)]*</td>
</tr>
<tr>
<td>JDU293</td>
<td>ijmSi65 [pJD528; Pmex-5::sfGFP::aspm-1::3’tbb-2; cb-unc-119(+) II; unc-119(ed3) III?; ltIs37 [pAA64; Ppie-1::mCherry::his-58; unc-119 (+)] IV*</td>
</tr>
<tr>
<td>JDU184</td>
<td>unc-119(ed3) III?; ijmSi49 [pJD479; Pmex-5::GFP::lmn-1::3’tbb-2]I; ijmSi41 [pJD456_Pmex-5::mCherry::tbb2::3’tbb-2]II*</td>
</tr>
<tr>
<td>JDU180</td>
<td>ijmSi40 [pJD457; Pmex-5::GFP::klp-7b reencoded::3’tbb-2; cb-unc-119(+)I; ijmSi31 [pJD446_Pmex-5::mCherry::his11::3’tbb-2]II; unc-119(ed3) III?*</td>
</tr>
</tbody>
</table>
**SUPPLEMENTAL TABLE S3**

**Primers and templates used for dsRNA production**

*Lowercase letters denote T3 and T7 sequences included for RNA synthesis*

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer 1*</th>
<th>Primer 2*</th>
<th>Template</th>
<th>Final [C]</th>
</tr>
</thead>
<tbody>
<tr>
<td>K11D9.1</td>
<td>5’ aattaacccctactaagg</td>
<td>5’ taataagactcactatagg</td>
<td>N2 cDNA</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>TGATCGAATATGGCCGTGA 3’</td>
<td>GTGCTTCTGGCAACAAACG 3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>K11D9.1</td>
<td>5’ aattaacccctactaagg</td>
<td>5’ taataagactcactatagg</td>
<td>N2 cDNA</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>CCATGCTAATGTCTCCACAG 3’</td>
<td>CGGTGAAATGGGATACG 3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>W01B6.9</td>
<td>5’ aattaacccctactaagg</td>
<td>5’ taataagactcactatagg</td>
<td>N2 cDNA</td>
<td>2.6</td>
</tr>
<tr>
<td>(ndc-80)</td>
<td>GATGACAAGTACATTGAGATATA</td>
<td>GTGGTTCAAGATTCATTTGAATATT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>CAAATGATC 3’</td>
<td>GTCCACTG 3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C02F5.1</td>
<td>5’ aattaacccctactaagg</td>
<td>5’ taataagactcactatagg</td>
<td>N2 gDNA</td>
<td>1.5</td>
</tr>
<tr>
<td>(knl-1)</td>
<td>AATCTCGAATCACCGAAATGTC 3’</td>
<td>TTCACAAACTTGGAAGCCGCTG 3’</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F56A3.4</td>
<td>5’ aattaacccctactaagg</td>
<td>5’ taataagactcactatagg</td>
<td>N2 gDNA</td>
<td>1.1</td>
</tr>
<tr>
<td>(spd-5)</td>
<td>TGTCGCAACGGTTCTGAAT 3’</td>
<td>ATGGGCAAAATTGTGCTG 3’</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>