Supplementary Information

Design and implementation of a targeted RNAi screen for genes involved in acentrosomal spindle assembly

We sought to identify proteins required for acentrosomal spindle assembly by performing a targeted RNAi-based screen for defects in oocyte spindle formation in *C. elegans*. Our screening strain contained transgenes expressing GFP::tubulin and GFP::histone to allow visualization of microtubules and chromosomes in live worms. The screening strain also contained a temperature-sensitive mutation affecting the anaphase promoting complex (APC), which was used to enrich for spindles arrested at metaphase of meiosis I[1] [strain AV335 (*emb-27(g48)II; unc-119(ed3) ruIs32[unc-119(+) pie-1::GFP::H2B]III; ruIs57[unc-119(+) pie-1::GFP::tubulin]*)]. Approximately 800 genes that were previously annotated as embryonic lethal in a genome-wide screen[2] were chosen to be tested in our targeted screen, as this class contained two genes, *mei-1* and *mei-2*, previously shown to be required for normal organization of the oocyte spindle[3-5].

A schematic of the RNAi screen is shown in Figure S1A. Individual clones selected from an RNAi feeding library[2, 6] were used to inoculate 3 ml overnight LB/100 μg/ml Amp cultures. These saturated cultures were then used to seed NGM plates containing 100 μg/ml Amp and 1 mM IPTG; plates were left at room temperature overnight to induce dsRNA formation. The next day, L1 worms (synchronized by bleaching adults[7] and then letting the embryos hatch overnight on a plate without food) were put on the RNAi plates, and the worms were grown to adulthood by placing the plates at 15°C for 5 days.

Prior to screening worms for spindle defects, the RNAi plates were shifted to 25°C for 5-7 hours to arrest embryos in metaphase of meiosis I. Live worms were then mounted on a slide and their fertilized embryos were screened (using the 63X objective of a Zeiss Axioplan II microscope) for defects in oocyte meiotic spindle organization. Although the APC arrest induced a row of embryos arrested at metaphase I, only the most recently arrested embryos were analyzed to avoid potential problems associated with prolonged arrest. This primary screen successfully identified *mei-1* and *mei-2* as genes required for proper spindle morphology, validating the rationale for the screen. Candidates from the primary screen are being rescreened to confirm perturbation of spindle organization and to better define the nature of the spindle defects elicited by RNAi. Rescreening is being conducted using high-resolution Deltavision deconvolution imaging in the absence of the APC arrest.

In the primary screen stage, we identified two candidates (*klp-18* and C28C12.2) for which RNAi caused the formation of abnormal aster-like structures (Fig. S1B). For both of these genes, high-resolution imaging at the rescreening stage and subsequent immunofluorescence analysis demonstrated that the aster-like structures elicited by RNAi were monopolar spindles (Fig. 1). KLP-18 was known to be required for normal oocyte spindle formation[8], but previous analysis had not revealed the organization of the aberrant structures that formed following KLP-18 depletion. Our high-resolution imaging allowed us to ascribe a specific role for this protein in promoting spindle bipolarity. Since C28C12.2 is a novel gene, we named it *mesp-1* (meiotic spindle 1). MESP-1 is a member of a rapidly evolving protein family whose members exhibit high sequence divergence within the *Caenorhabditis* genus and that includes multiple paralogues in other *Caenorhabditis* species. MESP-1 does not contain any recognized predicted functional domains.

Supplementary References

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Supplemental Movie legends

Movie S1. Full Z-stack of the image projection displayed in Figure 2A, showing lateral microtubule bundles ensheathing the chromosomes in a monopolar spindle, imaged using a strain expressing GFP::histone and GFP::tubulin. Image stacks were obtained at 0.3 μm Z steps. Images are displayed at 2 frames per second.

Movie S2. Full Z-stack of the image projection displayed in Figure 2B, showing lateral microtubule bundles ensheathing the chromosomes in a bipolar spindle, imaged using a strain expressing mCherry::histone (red) and GFP::tubulin (green). Image stacks were obtained at 0.3 μm Z steps. Images are displayed at 2 frames per second.

Supplemental Figure legends

Figure S1. Design and implementation of the RNAi screen. (**a**) Gravid adult worms expressing GFP::tubulin and GFP::histone and containing a temperature-sensitive mutation affecting the Anaphase Promoting Complex (*emb-27ts*) were bleached to release embryos. These embryos were allowed to hatch overnight to synchronize L1-stage worms, which were then transferred to RNAi plates and incubated for 5 days at 15°C. Gravid adult worms from these plates were then shifted to 25°C for 5-7 hours to induce the APC arrest, resulting in embryos arrested at metaphase I. Live worms were then mounted for microscopy and visually screened for defects in the organization of the oocyte spindle. (**b**) Examples of normal and abnormal spindles observed

in the primary screen. Metaphase I-arrested embryos expressing GFP::tubulin and GFP::histone in control (left) and *klp-18(RNAi)* (right) worms. The most recently arrested embryo (+1) was screened for defects in spindle morphology. In the control, a bipolar spindle with chromosomes aligned at the metaphase plate is clearly visible in the +1 embryo. In the *klp-18(RNAi)* worm, microtubules are found in abnormal aster-like structures (rather than bipolar spindles). Spindles in +2 embryos often appeared to have collapsed due to the prolonged arrest and were therefore excluded from analysis. Following the primary screen, candidates were rescreened without the APC arrest using high-resolution imaging (Fig. 1).

Figure S2. ASPM-1 localizes to the poles of both centrosomal and acentrosomal spindles. (**a-c**) Worms stained with Hoechst (blue), α-tubulin antibodies (green) and an antibody raised against ASPM-1 (red). The ASPM-1 antibody stains spindle poles as well as kinetochores and mid-bivalents in male spermatocyte and oocyte meiosis (**a**, **b**), and spindle poles as well as kinetochores in mitotic one-cell stage embryos (**c**). We find that *aspm-1(RNAi)* disrupts oocyte meiotic spindle organization (**b**, right) and that the ASPM-1 antibody does not stain the disorganized poles. However, residual staining at kinetochores and mid-bivalents was not eliminated, indicating that it may not correspond to ASPM-1. The fact that defined poles lacking ASPM-1 staining are present in mitotic spindles following *aspm-1(RNAi)* (**c**) indicates that pole staining is ASPM-1–dependent. The *aspm-1(RNAi)* image shown in **c** is an earlier stage embryo than the control, chosen to highlight the residual staining at individual kinetochores. Scale bar, 2 μm (**a**); 5 μm (**b,c**).

Figure S3. Microtubule density at chromosome ends is substantially lower than in the lateral microtubule bundles. (**a-c**) Assessment of relative microtubule density in the vicinity of three different chromosomes on monopolar spindles. Pixel intensities were measured within a 4 pixel by 33-34 pixel area (white boxes shown on the images at left); graphical representations of these data are shown on the right. Images depicted and analyzed are single sections selected from high-resolution image stacks (in **a-b,** the analyzed chromosomes are from the monopolar spindle shown in Fig. 2A; **c** is taken from one of the images shown in Fig. 2D). The ratios between the peak intensities and the region of minimum intensity between the bundles (adjacent to the chromosome end) are shown above each graph. Peak 1 corresponds to the peak on the left of each graph; peak 2 is on the right. Ratios were calculated following subtraction of the average pixel intensity values for domains outside the ensheathing microtubule bundles. In part **c**, only the microtubule channel (displayed in green) was analyzed.

Figure S4. Bivalent orientation on monopolar (**a**) and bipolar (**b**) oocyte spindles. Images show GFP::tubulin and GFP::histone. (**a**) Bivalent orientation was assessed by drawing a line from the center of the aster through the midpoint of the bivalent (green line) and measuring the angle between that line and the long axis of the bivalent (red line). These angles were then binned with values rounded to the nearest 10 and graphed. Most bivalents were found oriented parallel to the microtubule bundles ensheathing them. (**b**) Microtubule bundles still ensheath chromosomes (inset) and bivalent orientation is not disrupted following KLP-19 depletion. For quantification, chromosomes were classified as oriented if they were found parallel to the spindle axis. The image shows an example of a spindle formed following *klp-19(RNAi)*, where five chromosomes are oriented and one (arrowhead) is not.

Figure S5. Organization of centrosome-containing male meiotic spindles. Spermatocytes stained for DNA (blue), tubulin (green) and HIM-10 (red in **a**) or KLP-19 (red in **b**). (**a**) HIM-10 marks the kinetochore. Single channel images are shown, in addition to different combinations of merged images. The kinetochore forms a cup-like structure around the ends of meiotic bivalents, and regions of high microtubule density are seen immediately adjacent to the kinetochore staining at bivalent ends (arrowhead). In the image of the microtubules and DNA, a gap is observed (arrow) where the kinetochore is located. (**b**) KLP-19 localizes to chromosomes during spermatocyte meiosis. In prometaphase spermatocytes, KLP-19 is localized broadly over the chromosomes; at metaphase, KLP-19 is enriched at the midbivalent. In all spermatocytes (**a-b**), microtubule density appears low in the center of the spindle, where the chromosomes are located, suggesting that a substantial fraction of microtubules may terminate at the chromosomes. Scale bars, 2 μm.

Figure S6. AIR-2 localizes to a ring around meiotic chromosomes. (**a, b**) Structures stained for Aurora B kinase AIR-2 (red), DNA (blue), and tubulin (green), in Meiosis I (**a**) and Meiosis II (**b**). Shown in **a** are monopolar spindles from *klp-18(RNAi)* worms, since the bivalents are spread out and some are positioned to reveal the AIR-2 ring (arrows). In **b**, the control metaphase II spindle is tilted, so the AIR-2 rings are visible. (**c**) Oocyte spindles stained for AIR-2 (red) and DNA (blue). *icp-1(RNAi)* (right) causes removal of AIR-2 from the chromosomes. Scale bars, 5 μm.