The conserved kinase SRPK regulates karyosome formation and spindle microtubule assembly in Drosophila oocytes

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Summary

In Drosophila oocytes, after the completion of recombination, meiotic chromosomes form a compact cluster called the karyosome within the nucleus, and later assemble spindle microtubules without centrosomes. Although these oocyte-specific phenomena are also observed in humans, their molecular basis is not well understood. Here, we report essential roles for the conserved kinase SRPK in both karyosome formation and spindle microtubule assembly in oocytes. We have identified a female-sterile srpk mutant through a cytological screen for karyosome defects. Unlike most karyosome mutants, the karyosome defect is independent of the meiotic recombination checkpoint. Heterochromatin clustering found within the wild-type karyosome is disrupted in the mutant. Strikingly, a loss of SRPK severely prevents microtubule assembly for acentrosomal spindles in mature oocytes. Subsequently, bi-orientation and segregation of meiotic chromosomes are also defective. Therefore, this study demonstrates new roles of this conserved kinase in two independent meiotic steps specific to oocytes.

Key words: Drosophila, SRPK, Kinase, Meiosis, Mitosis, Oocyte, Spindle

Introduction

Meiosis consists of recombination followed by two rounds of chromosome segregation. Oocytes have a very long intervening period between recombination and chromosome segregation: 10–40 years in humans and 2–3 days in Drosophila. During this period, Drosophila meiotic chromosomes cluster together to form a spherical body, called the karyosome, within the enlarged oocyte nucleus (King, 1970). Similar clustering of chromosomes is also observed in the growing phase of mouse and human oocytes (Parfenov et al., 1989). This conservation suggests an important role for chromosome clustering, but the precise biological significance is not yet established. In mice, the clustering of meiotic chromosomes is known to correlate with the developmental competency of the oocytes (Zuccotti et al., 1998; Zuccoti et al., 2002). In Drosophila, we proposed that clustering of meiotic chromosomes facilitates the formation of one unified spindle from multiple chromosomes (Cullen et al., 2005).

Despite being widespread across animals, only limited information is available on the molecular mechanism of chromosome clustering in the oocyte nucleus. In Drosophila, we previously found that the conserved kinase NHK-1 directly regulates karyosome formation through phosphorylation of BAF, a linker between the nuclear envelope and chromatin (Lancaster et al., 2007). Furthermore, when DNA double strand breaks (DSBs) are not repaired, the meiotic recombination checkpoint (hereafter referred to as the meiotic checkpoint) prevents karyosome formation through suppression of NHK-1 (Lancaster et al., 2010).

Once oocytes are mature, the nuclear envelope breaks down and the meiotic spindle is assembled. In many animals including humans and Drosophila, oocytes do not contain centrosomes, the major microtubule organising centres in mitotic cells (McKim and Hawley, 1995). Instead, chromosomes play a central role in spindle formation in oocytes. In Xenopus egg extracts, the Ran-GTP gradient is essential for spindle assembly around chromosomes (Carazo-Salas et al., 1999; Kalab et al., 2002). In addition, several other proteins were also shown to be essential for spindle microtubule assembly in Xenopus extracts (Gruss et al., 2001; Tournebize et al., 2000; Sampath et al., 2004; Wilde and Zheng, 1999). On the other hand, spindle microtubule assembly in living oocytes is far from being fully understood. For example, in mouse and Drosophila oocytes, the spindle can be formed around chromosomes even if the Ran-GTP gradient is disrupted (Dumont et al., 2007; Cesario and McKim, 2011). In Drosophila, no mutants have been identified which severely prevent spindle microtubule assembly.

Here we report a mutation in the conserved kinase SRPK which disrupts both karyosome formation and spindle microtubule assembly in Drosophila oocytes. The karyosome defect in the mutant is independent of the meiotic checkpoint. Remarkably, spindle microtubule assembly is compromised much more...
Results and Discussion
A screen for mutants with defective karyosomes identified the conserved kinase SRPK
To gain insight into the molecular mechanism of karyosome formation, we screened a collection of mutants for abnormal karyosome morphology. The genomes were first chemically mutagenised and germline clones of mutations on the right arm of the second chromosome were generated in females using the FRT/ovo system (Fig. 1A) (Vogt et al., 2006). About 100 mutants produced eggs that failed to develop at all. These mutants were cytologically screened for abnormal karyosome morphology in germline clones. This report focuses on one mutant (2R-129-09) identified in this screen. The mutant is sterile in females and males, but is fully viable without obvious morphological defects, including ones typically associated with mitotic defects such as rough eyes or missing bristles.

To identify the mutation that causes the karyosome defect in 2R-129-09, we first mapped the mutation using a series of chromosomal deletions (known as deficiencies). Two genes (srpk and dup) were disrupted by all of the three overlapping deficiencies, which did not rescue the sterility (Fig. 1B). Sequencing of the genomic region from the mutant and the parental line identified one nucleotide difference, which represents a nonsense mutation in the srpk gene. A transgene of a wild-type srpk genomic region fully rescued the sterility and the cytological defects in the mutant (Fig. 1B; supplementary material Fig. S1). This demonstrated that the mutation in srpk is the cause of the sterility and cytological defects in the mutant.

The srpk gene encodes SRPK (SR Protein Kinase), which is highly conserved and belongs to a distinct subfamily of serine/threonine kinases. The unique feature of this subfamily is a large spacer region within the kinase domain. The nonsense mutation in 2R-129-09 results in a truncated protein which lacks half of the kinase domain including subdomains VII-XI essential for kinase activity (Fig. 1C). Consistent with our results, female sterile mutations in the srpk gene (also called cuaba) have also been identified previously through a screen for abnormal oocyte polarity (Barbosa et al., 2007). However, karyosome defects have not been reported before.

SRPK is crucial for karyosome formation
To gain insight into the role of SRPK in karyosome formation, we analysed the karyosome defect in the srpk mutant in detail. In wild-type oocytes, meiotic chromosomes are clustered together to form a compact spherical karyosome. In srpk129-09/+/ mutant oocytes, the spherical karyosome morphology was frequently disrupted (79% at stage 6; Fig. 2A,B; supplementary material Fig. S2). The karyosome was often deformed with one or more lobes extended from a mass of chromosomes. In other cases, chromosomes appeared to be separated into multiple masses. Sometimes karyosomes appeared to be extensively attached to the nuclear envelope. The chromatin morphology of nurse and follicle cells was not affected (supplementary material Fig. S3).

In wild-type oocytes, the karyosome is first established at oogenesis stage 3. In mutant oocytes, karyosome defects were first observed from stage 3 and progressively worsened during oogenesis (supplementary material Fig. S2). Therefore, SRPK is crucial for both establishment and maintenance of the karyosome.

Peri-centromeric heterochromatin is clustered within wild-type karyosomes (Fig. 2C,D) (Demburg et al., 1996). Immunostaining of Heterochromatin Protein 1 (HP1) showed that heterochromatin clustering was disrupted in a large majority of mutant karyosomes, including ones with relatively normal morphology (Fig. 2C,D).

The karyosome defect in the srpk mutant is independent of the meiotic checkpoint
Karyosome defects can be induced by activation of the meiotic checkpoint pathway. The meiotic checkpoint detects DSBs caused by a failure in DNA repair or in rasiRNA processing which suppresses retrotransposition (Ghabrial et al., 1998; Klattenhoff et al., 2007). Indeed, the defects in many karyosome mutants have been shown to be caused by the meiotic checkpoint, while the karyosome defect of an nhk-1 mutant is not caused by the meiotic checkpoint (Lancaster et al., 2010).

To monitor the existence of unpaired DSBs, oocytes were immunostained for γ-H2Av (γ-H2AX in mammals), which accumulates on DSBs (Fig. 2E). In wild type, the DSBs induced during meiotic recombination were repaired by oogenesis stage 2, and γ-H2Av foci were restricted to stage 1 (Mehrotra and McKim, 2000).
H2Av foci were observed in each ovariole. st, stage; r, region.

srpk wild-type, E heterochromatin cluster at stage 3 or 4. (the oocyte nucleus. (with an anti-HP1 antibody. (reported (Staeva-Vieira et al., 2003). In the repair gene), 2006). In a mutant of srpk129-09 Fig. 2. The karyosome defect in the meiotic checkpoint. (A) Karyosomes in wild-type, srpk129-09 and mnk106 srpk129-09 oocytes. The arrowheads indicate the position of the karyosome in the oocyte nucleus. (B) The frequency of abnormal karyosomes in stage-6 oocytes (n=110). (C) Heterochromatin within the karyosome is visualised with an anti-HP1 antibody. (D) The frequency of karyosomes with a single heterochromatin cluster at stage 3 or 4. (E) γ-H2Av signals in a stage-3 or -4 wild-type, srpk and spnA oocyte nucleus. (F) The last oogenesis stage when γ-H2Av foci were observed in each ovariole. st, stage; r, region. n=33. Scale bars: 10 μm.

2006). In a mutant of spnA (the homologue of the rad51 DNA repair gene), γ-H2Av foci persist well after stage 2, as previously reported (Staeva-Vieira et al., 2003). In the srpk mutant, γ-H2Av foci were restricted to stage 1 (Fig. 2F). This indicates that DSBs were repaired with normal timing in the srpk mutant.

To conclusively determine whether the karyosome defect in the srpk mutant is due to the meiotic checkpoint, the meiotic checkpoint was inactivated by a mutation in mnk (also known as chk2, which encodes a conserved kinase required for the meiotic checkpoint and the DNA damage checkpoint). The mnk mutation has been shown to rescue karyosome defects in mutants defective in DNA repair or rasiRNA processing (Abdu et al., 2002; Klattenhoff et al., 2007). Immunostaining of double mutants between srpk129-09/d and mnk106 showed that the mnk mutation failed to rescue the karyosome defect in the srpk mutant (Fig. 2A,B). This demonstrates that the karyosome defect in the srpk mutant is not caused by the meiotic checkpoint.

SRPK is essential for the assembly of spindle microtubules in mature oocytes

To identify further roles of SRPK in meiosis, we examined meiotic spindles in mature oocytes (stage-14 oocytes) by immunostaining. A mature wild-type oocyte contains a single meiotic spindle arrested in metaphase I. In oocytes, the meiotic spindle forms without centrosomes. However, the molecular requirements of microtubule assembly in oocytes remain a mystery, as no mutants have been identified that prevent microtubule assembly in oocytes.

To test the role of SRPK in spindle assembly, mature oocytes which arrest in metaphase I were immunostained for α-tubulin, the pole protein D-TACC and DNA. In a mature wild-type oocyte, a single bipolar spindle was associated with a cluster of meiotic chromosomes (Fig. 3A). In contrast, meiotic spindles were severely defective in mature oocytes of the srpk mutant (Fig. 3A,B). In all mutant oocytes, only residual microtubules were observed and a proper bipolar spindle structure did not form. Frequently (13 out of 30 oocytes), no or only a few short stubs of microtubules were associated with the chromosomes. Often (10) one or a few longer microtubule bundles were observed. In the other cases (7), a partially organised structure was formed but was very short, weak, multipolar and/or loose.

To objectively compare the amount of spindle microtubules assembled around chromosomes, the total fluorescent intensity of tubulin signals above the background was quantified in wild type and the mutant. The total spindle microtubule signal in the srpk mutant was dramatically decreased to less than 4% of the wild-type level (P<0.001, Wilcoxon test) (Fig. 3C).

Chromosomes were misaligned, but their condensation states appeared to be normal in the mutant. In some cases (5 out of 30), more than one cluster of chromosomes was observed in combination with the above defects (supplementary material Fig. S4). Distinct foci of the pole protein D-TACC were sometimes observed at the end of microtubule bundles (Fig. 3A, iv). Immunostaining of the central spindle protein Subito (the homologue of Mklp2, a kinesin-6) (Jang et al., 2005) revealed its association with spindle microtubules near chromosomes in the mutant, although the signal was weak, as expected from poorly assembled microtubules (supplementary material Fig. S5). Taken together, these observations suggest a specific defect in spindle microtubule assembly.

To test whether mutant oocytes had properly matured, we first examined disassembly of the nuclear envelope protein Lamin by immunostaining. Lamin was properly disassembled in the srpk mutant as in wild type, despite poorly assembled microtubules (Fig. 4A). To further confirm oocyte maturation, we immunostained phosphorylated Histone 3 (at serine 10), a commonly used M-phase marker. Histone 3 was phosphorylated in the srpk mutant as in wild type, despite poorly assembled spindle microtubules (Fig. 4B). Furthermore, immunoblotting showed both wild-type and the mutant stage-14 oocytes have a high level of Cyclin B, an activating subunit of Maturation Promoting Factor (Fig. 4C). These results argue strongly against the possibility that severe reduction of spindle microtubules is due to a failure to mature or maintain M-phase properly.

RNA interference of srpk in Drosophila cultured cells did not reveal spindle defects in mitosis (supplementary material Fig. S6; Bettencourt-Dias et al., 2004), suggesting a specific role for SRPK in meiosis. The spindle defect seen in the srpk mutant
oocytes is very penetrant and the most severe among all mutants reported so far. These results demonstrate that SRPK is essential for spindle microtubule assembly in oocytes.

**SRPK is required for centromere bi-orientation and chromosome separation in oocytes**

We further investigated the consequence of the spindle defect in the srpk mutant. To establish whether bi-orientation of homologous centromeres is disrupted in the srpk mutant, we carried out fluorescent in situ hybridisation (FISH) using pericentromeric dodeca satellite DNA located on chromosome 3. In mature wild-type oocytes, two signals were located at the ends of the chromosome mass (Fig. 3D). These signals represent homologous centromeres pulled from two opposite poles. Each of the signals represents two tightly attached sister-centromeres. In most mature mutant oocytes, homologous centromeres were located close together and failed to achieve bi-orientation (Fig. 3D,E).

To examine meiotic progression beyond metaphase I, activated eggs were immunostained. In wild type, various stages of development were found, including a pair of tandem meiosis II spindles which shared a spindle pole (supplementary material Fig. S7A). In contrast, most srpk mutant oocytes (22 out of 42) contained figures that may correspond to products of failed chromosome segregation (supplementary material Fig. S7A, a cluster of chromosomes or a large nucleus). Few mutant oocytes contained tandem meiosis II spindles or progressed into mitotic divisions (supplementary material Fig. S7B). This confirms that meiotic chromosome segregation is defective in the mutant.
SRPK is required for karyosome formation and spindle microtubule assembly

Our study showed that a loss of SRPK results in defects in both karyosome formation and spindle microtubule assembly (Fig. 4D). We do not think that the spindle assembly defect is simply due to a general karyosome defect, as this spindle defect is unique among karyosome mutants isolated in the same screen. How does the loss of SRPK lead to these meiotic defects? Known substrates of SRPK or its homologues include the splicing factors SR proteins, the nuclear envelope-chromatin linker Lamin B Receptor (LBR), and the sperm chromatin protein protamine (Giannakouros et al., 2011). It is possible that under-phosphorylation of SR proteins may lead to the reduction of proteins required for karyosome formation or spindle assembly. However, the effect must be specific, as our preliminary results show no global changes in the protein expression pattern. As SR proteins are also known to interact with chromatin and HP1 (Loomis et al., 2009), the effect may be independent of splicing. It is also possible that phosphorylation of LBR is required for the release of chromosomes from the nuclear envelope during karyosome formation.

Interestingly, the fission yeast homologue of SRPK, Dsk1, was isolated as a high copy suppressor of a mitotic defect of a dis1 mutation (Takeuchi and Yanagida, 1993). dis1 encodes one of two homologues of Msps/XMAP215, a crucial microtubule assembly factor (Ohkura et al., 2001). Dsk1 is more active in mitosis (similar to mammalian SRPK1) and hyperphosphorylated in mitosis, suggesting a mitotic role (Takeuchi and Yanagida, 1993). The mechanism of the suppression of dis1 is still unknown, but Dsk1 may activate other microtubule assembly proteins.

In this study we have shown that the conserved kinase SRPK is involved in two critical steps in meiotic progression in oocytes. Importantly this is the first protein whose absence virtually eliminates spindle microtubule assembly in oocytes. Identification of critical regulators and substrates of this conserved kinase would lead to an understanding of the molecular regulation of these critical but poorly understood meiotic processes.

Materials and Methods

Drosophila genetics and cytology

Standard fly techniques were followed (Ashburner et al., 2005). All stocks were grown at 25°C in standard cornmeal media. Details of mutations and chromosome aberrations can be found in Lindsley and Zimm (Lindsley and Zimm, 1992) or at Flybase (http://flybase.org; McQuilton et al., 2012). Female germline clones were induced in hs-FLP122/w; FRT ovo/2;2R-129-09 (FRT G13 c px sp. *) by heat shocking (at 37°C for 45 minutes) each day for 3 days in larval stages. mGFP (Abdu et al., 2002) was a kind gift from W. Theurkauf (University of Massachusetts). The srpk129-09 phenotype was examined over a deficiency, Df(2R)ED2436. w1118 or the parental chromosome over 2R-129-09; Df(2R)ED2436 were used as wild-type controls. Immunostaining and FISH were carried out according to Lancaster et al. (2007), Cullen and Ohkura (Cullen and Ohkura, 2001) and Meireles et al. (Meireles et al., 2009). Chi-square test was used for statistical analysis unless stated otherwise.

Identification of the female sterile mutation in 2R-129-09

To map the mutation in the line 2R-129-09, a series of deficiencies on the right arm of the second chromosome were crossed with the mutant line and the lethality and female sterility of transheterozygotes were tested for each cross. Two overlapping deficiencies, Df(2R)ED2426 and Df(2R)ED2436, were found to be female sterile over 2R-129-09. After confirming the karyosome defects in the transheterozygotes, further deficiencies around the regions were tested as above. 2R-129-09 over Df(2R)ED2426, Df(2R)ED2436 or Df(2R)Exel9015 was fully viable and female sterile, and showed karyosome defects identical to female germline clones of 2R-129-09. 2R-129-09 over Df(2R)Exel6285, or Df(2R)ED2436 over the parental chromosome (FRT G13 c px sp) were fertile and did not show any karyosome defects. These results indicate that a mutation responsible for female sterility and the karyosome defect is located in the genes (dop and srpk) uncovered by Df(2R)ED2426. The entire genome region spanning dop and srpk was amplified from 2R-129-09 over Df(2R)ED2426 and the paternal chromosome over the same deficiency as a series of overlapped segments by PCR using Taq polymerase (Roche), and sequenced using Big Dye terminator (Applied Biosystems) by the Edinburgh SBS sequence facility (Gene pool). The details of PCR and sequence primers used for this study are available upon request.

To perform a rescue experiment, the BAC plasmid, CH32-130M04, in attB-P[acman]-CmR-BW (Venken et al., 2009) carrying the wild-type srpk gene was obtained from BACPAC Resource Centre (BPRC) at Oakland, USA. This plasmid was used to generate transgenic flies by Genetic Service Inc. (Sudbury, USA) using the docking vector VK33 at 65B2. The srpk/Df(2R)Exel9015 flies carrying the transgene CH32-130M04 (Pac[srpk]) were tested for male and female fertility, and a karyosome and spindle phenotype, together with siblings without the transgene.

Cytological and immunological techniques

Immunostaining of ovaries, mature non-activated oocytes and activated oocytes was carried out according to Lancaster et al. (Lancaster et al., 2007), Cullen and Ohkura (Cullen and Ohkura, 2001) and Cullen et al. (Cullen et al., 2005), respectively. Fluorescent in situ hybridisation (FISH) of ovaries was carried out according to Meireles et al. (Meireles et al., 2009) using an oligonucleotide corresponding to the dodeca satellite as a probe. The oligonucleotide (ccccctacgctgccaggttgctgccagtctccgctctccgctccgct) was 3' end-labelled by the full dideoxy method. A hundred pmol of the oligonucleotide was incubated at 37°C for 1 hour with 30 units of terminal deoxynucleotidyl transferase (Promega), 2 nmole of Alexa546-conjugated dUTP (Invitrogen) and 16 nmole of unlabelled dUTP (Promega) in 20 µl of the transferase buffer (Promega). After incubation at 70°C for 10 minutes, un-incorporated dUTP was removed by passing through a G25 Mini Quick spin column (Roche). 4 µl of this labelled oligonucleotide was hybridised with ovaries in 40 µl of the hybridisation buffer at 30°C overnight.

Unimunostained samples were examined by an Axioimager (Zeiss) attached to a confocal laser scanning head, LSM5 Exciter (Zeiss). Either one Z-plane or maximum intensity Z projections of selected planes have been shown in the figures after contrast and brightness were adjusted uniformly across the field using Photoshop (Adobe). No features were added or removed in the process.

The total tubulin intensity of the spindle was estimated using the following formula. Two areas (1,2) were drawn on the maximum intensity projection made from 2-series of images taken using the same gain (500). Area 1 includes the spindle and surrounding region, and area 2 includes mainly the spindle. The result was then taken as: [I₁-(1-I₁)N₁/N₂-L], where I₁ and N are the total pixel intensity and pixel number in the specified area, respectively, and L is the laser power.

Immunostaining of egg chambers were carried out using fluorescent secondary antibodies (Li-Cor) and detected by an Odyssey scanner (Li-Cor). Ovaries were first dissected in methanol to prevent protein degradation, and then ones at stage 1–10 and 14 were identified according to morphology.

The primary antibodies used in this study include antibodies against α-tubulin (mouse monoclonal DM1A; 1:500; Sigma), Lamin (mouse monoclonal ADL67.10; 1:100; Developmental Studies Hybridoma Bank, DSHB), phosphorylated H3 at serine10 (rabbit polyclonal; 1:25; Lancaster et al., 2010), phosphorylated H3 at serine10 (rabbit polyclonal; 1:500; Millipore), Cyclin B (mouse monoclonal F24; 1:100; Developmental Studies Hybridoma Bank, DSHB), D-TACC-CTD antibody (rabbit polyclonal D-TACC-CTD; 1:1000; this study). Subtilisin A (polyclonal antibody; 1:250; this study). The polyclonal rabbit D-TACC-CTD antibody was made against D-TACC-CTD expressed in E. coli from a construct kindly given by J. Raff, Oxford (Gergely et al., 2000). The polyclonal rat Subtilo antibody was made against GST-Subtilo expressed in E. coli. The secondary antibodies were purchased from Jackson Immunologicals or Molecular Probes.

RNA interference

RNA interference and immunostaining was carried out according to Dzhindzhev et al. (Dzhindzhev et al., 2005). dsRNA was produced from wild-type genomic DNA using a pair of primers (5'-CGACTCTATATAGGGAAATGGCCTGCTGCGATT-3', 5'-CGACTCTATATAGGGAAATGGCCTGCTGCGATT-3'), corresponding to E. coli β-lactamase gene was used as a control. The cells were fixed and immunostained after three and five days’ incubation with dsRNA.

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Fig. S1. A wild-type srpk transgene rescues the karyosome and spindle defects in the srpk<sup>129-09/Df</sup> mutant. (A) Karyosome morphology in the srpk mutant in the absence and presence of a transgene CH322-130M04 (Pac[SRPK]) containing wild-type srpk. (B) The frequency of abnormal karyosomes at stage 4-8. n>49. (C) Spindle morphology in the srpk mutant in the absence and presence of the transgene Pac[SRPK]. (D) The frequency of abnormal spindles in mature oocytes. n>20. All scale bars represent 10 µm.
Fig. S2. Abnormalities of karyosome morphology in the srpk mutant. Karyosome morphology was classified into four categories: deformed, dissociated, attached to the nuclear envelope (NE), and normal. (A) Examples of each category. (B) The frequency of each category in different oogenesis stages in the srpk mutant and wild type. All scale bars represent 10 µm. The difference between wild type and srpk is significant at all stages (p<0.001). The difference between stage 3 and 6 is significant in the srpk mutant (p<0.001). n>49.
**Fig. S3. Chromatin morphology in nurse and follicle cells is not affected in the srpk mutant.** DNA staining of egg chambers at stage 5 from wild type and the srpk mutant. The arrow and arrowhead indicate the nuclei of a nurse cell and a follicle cell, respectively. Bar=10 µm.

**Fig. S4. More than one meiotic chromosome cluster was observed in some srpk mutant oocytes.** Mature oocytes from the srpk mutant were stained for DNA, tubulin and D-TACC. Some contained multiple clusters of meiotic chromosomes (the arrowheads). Spindle microtubule assembly around the chromosomes was severely disrupted. Bar=10 µm.
**Fig. S5. The central spindle protein Subito properly localised in the srpk mutant.** Immunostaining of Subito, tubulin and DNA in wild-type and srpk mutant stage-14 oocytes. Subito localised to the central spindle region in the srpk mutant, although the signal was weak as expected from poor microtubule assembly. Bar=10 µm.

**Fig. S6. RNA interference of srpk in Drosophila cultured cells did not significantly affect spindle morphology or mitotic progression.** (A) Normal spindle morphologies of S2 cells after incubation with a control dsRNA or srpk dsRNA for 5 days. Bar=10 µm. (B) The frequency of abnormal spindles among mitotic cells (n≥102), and the frequency of mitotic cells (identified by phosphorylated H3 at serine 10) among all cells (mitotic index; n≥1000).
**Fig. S7. Meiotic progression is severely disrupted in the srpk mutant.** (A) Activated oocytes from wild type and srpk mutant. Activated wild-type oocytes often contain a pair of meiosis II tandem spindles with a shared pole (arrowhead) associated with D-TACC and microtubule asters. Activated srpk oocytes often contain a large chromosome cluster (middle) or a large nucleus (right) in the dorsal anterior region of the oocytes, likely due to failed chromosome segregation. Bar=10 μm. (B) The frequency of oocytes which clearly show normal meiosis II or later figures.