Dynamin 2 regulates actin-mediated spindle migration in mouse oocytes

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Running Title: Dynamin2 in mouse oocytes

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Abstract

Background information: During meiosis, a bipolar spindle forms in the central cytoplasm of an oocyte and then moves to the cortex to extrude the first polar body. This is dependent on the regulation of actin and actin-related molecules. Dynamin 2, a large GTPase known to regulate clathrin-mediated endocytosis, is involved in actin recruitment and actin-based vesicle mobility. In this study, we investigated the role of Dynamin2 in oocyte meiosis.

Results: Dynamin 2 was localized at the cortex and around the spindles of oocytes. Disrupting Dynamin 2 activity by RNAi or an inhibitor resulted in polar body extrusion failure. Using time-lapse microscopy to monitor aberrant oocyte cytokinesis, the chromosomes first separated, but then re-joined. Actin expression in oocytes was decreased; and actin cap formation was disrupted, which was confirmed by the disappearance of cortical granule (CG) free domains. In addition, live cell imaging showed that spindle migration had failed and that spindles were arrested centrally in oocytes. This may have been due to Dynamin-binding protein Profilin and actin-related protein 2/3 (ARP2/3) complexes, which exhibited dispersed signals after disrupting Dynamin 2 activity.

Conclusions: Thus, our results indicate that Dynamin 2 regulates spindle migration and polar body extrusion during mouse oocyte meiosis through an actin-based pathway.

Keywords: Oocyte; Dynamin; actin; spindle migration; cytokinesis

Introduction

Asymmetric oocyte division is essential for fertilization and the oocyte-embryo transition. During meiosis I, the spindle migrates towards the oocyte cortex, and as the spindle comes close to the periphery, the cortex is reorganized (Brunet and Verlhac, 2011). Microvilli disassemble and microfilaments accumulate under the plasma membrane to form an actin cap. In addition, within this area cortical granules (CG) are redistributed to form a CG-free domain (Longo and Chen, 1984, Tremoleda *et al.* , 2001). Once this migration is complete, cytokinesis begins and the oocyte extrudes the first polar body. Cortical reorganization and polarization are critical for the formation and extrusion of the polar body.

The entire process of asymmetric division is mediated by microtubule and microfilament cytoskeletons. In mouse oocytes, after the germinal vesicle envelope breaks down, microtubules become reorganized around congregated chromosomes into a bipolar spindle (Schuh and Ellenberg, 2008). Subsequently, actin filaments generate cytoplasmic streaming to push the spindle towards the actomyosin cap (Yi *et al.* , 2011). Several molecules, including small GTPases and actin nucleation factors, are known to be involved in this process. Cdc42, Formin, Arp2/3 complex and Myosin are involved in spindle positioning and Ran, Rac, Fyn are involved in cortical reorganization, which further regulates asymmetric division in mammalian oocytes (Sun and Kim, 2013, Sun *et al.* , 2011b).

In mammals, there are three distinct isoforms of the large guanosine triphosphatases (GTPase) Dynamin: brain-specific Dynamin 1; ubiquitously-expressed Dynamin 2; and brain- and testis-enriched Dynamin 3 (Schmid *et al.* , 1998, Urrutia *et al.* , 1997). All Dynamins comprise three conserved domains: a highly conserved GTPase domain; a middle

domain; and a GTPase effector domain (GED). Dynamin 2 has an additional PH domain and a proline-rich domain (PRD) (Mears and Hinshaw, 2008). Dynamin 2 binds several proteins that regulate the actin cytoskeleton, including Cortactin (McNiven *et al.* , 2000), Profilin (Gareus *et al.* , 2006, Witke *et al.* , 1998), Syndapin (Kessels *et al.* , 2001), and Murine Abp1 (Qualmann *et al.* , 1999). And a Dynamin-Coractin-Arp2/3 pathway was shown to involve into the actin assembly (Krueger *et al.* , 2003, Schafer *et al.* , 2002). Recent work also indicated that a direct dynamin-actin interaction regulated the actin cytoskeleton (Gu *et al.* , 2010). The middle domain of Dynamin 2 could bind to γ-tubulin and is critical for centrosome cohesion (Thompson *et al.* , 2004). Dynamins play important roles in most cellular processes, including clathrin-mediated endocytosis, membrane remodeling, actin recruitment, and actin-based vesicle mobility (Hinshaw, 2000, Orth and McNiven, 2003, Schafer, 2004). Moreover, Dynamin 2 was shown to associate with microtubules for the regulation of cell cycle in mitosis (Ishida *et al.* , 2011). In *C. elegans* and Dictyostelium discoideum, Dynamin localized at the cleavage furrow membranes and accumulated at the midbody of dividing embryos, and was required for cytokinesis (Masud Rana *et al.* , 2013, Thompson *et al.* , 2002).

Although several work showed the important roles of Dynamin 2 in other cell systems, its role in mammalian oocyte maturation and the underlying mechanisms are uncertain. In this study, we investigated the role of Dynamin 2 in oocyte meiosis and its relationship with Profilin. Our results show that there may be a Dynamin 2-Profilin-ARP2/3-actin pathway in mouse oocytes that is involved in meiotic spindle migration and polar body extrusion.

Results

Dynamin 2 localizes at the cortex and around the spindle during oocyte maturation

To investigate the roles of Dynamin 2 in oocyte development, Dynamin 2 subcellular expression and localization at different stages of mouse oocyte meiosis were determined by immunofluorescent staining. As shown in Figure 1A, Dynamin 2 was clearly enriched at the periphery of the oocyte during the entirety of the cell development process. In addition, Dynamin 2 appeared nearby to germinal vesicles at the GV stage, and at the MI and MII stages its signals were concentrated around the spindle. After double staining for Dynamin 2 and actin, Dynamin 2 showed a localization pattern similar to that of actin in the oocyte cortex (Fig. 1B). As shown in Fig. 1C for co-staining with Dynamin and tubulin, Dynamin 2 accumulated around the spindle and was enriched at the spindle periphery. In addition, the specific expression of Dynamin 2 in the vicinity of the spindle disappeared after nocodazole treatment (Fig. 1D).

Disrupting Dynamin 2 expression results in polar body extrusion failure

We injected siRNA into oocytes to knock down Dynamin 2 mRNA expression. We also cultured oocytes with dynasore, an inhibitor of Dynamins, at different concentrations for 12 h. After Dynamin 2 RNAi, its mRNA level was significantly decreased (37.6 ± 12.5% vs. 100%; Fig. 2A), and Dynamin 2 protein expression was also decreased (Fig. 2B); while after the inhibitor treatment Dynamin 2 no longer exhibited its specific localization in oocytes, as it was homogeneously distributed (Fig. 2C). Disrupting Dynamin 2 activity also resulted in oocyte polar body extrusion failure (Fig. 2D). After Dynamin RNAi, only 40.7 ± 16.1% (n =

99) of oocytes extruded their first polar body as compared with 78.3 ± 7.2% (n = 108) of control oocytes ($p<0.05$). With dynasore treatment at 100 μM and 200 μM, only 41.34 ± 15.86% (n = 100) and 24.02 ± 6.38% (n=72) of oocytes extruded their first body, respectively, as compared to 72.93 ± 9.58% (n = 144) of control oocytes ($p<0.05$) (Fig. 2D).

We also monitored the dynamic changes in oocytes using time-lapse microscopy. As shown in Fig. 2E, in a control oocyte, the spindle migrated to the cortex and the oocyte extruded a polar body. In contrast, polar bodies were not extruded in dynasore-treated oocytes. Two aberrant phenotypes were observed: 1) chromosomes did not segregate; and 2) chromosomes segregated, but reunited after separation.

Disrupting Dynamin 2 activity results in actin disorganization

The failure of oocyte maturation prompted us to further explore Dynamin 2 involvement during this process. We used time-lapse microscopy to observe actin dynamics both in control and dynasore-treated oocytes. As shown in Fig. 3A, by live cell imaging a control oocyte exhibited the strong signal of actin at the membrane; in contrast, in a treated oocyte, actin signals were weak and gradually decreased.

Next, we used immunofluorescent staining to examine actin expression after treatment. As shown in Fig. 3B, on the same slide, actin signals of most control oocytes were much stronger than those of treated oocytes at the plasma membrane. The same result was found in the cytoplasm after enhancing image contrast. General fluorescence intensity analysis confirmed this: Control oocytes: 41.87 ± 6.67 (n = 62) vs. Treated oocytes: 28.05 ± 5.02 (n = 57); ($p<0.05$) (Fig. 3C).

We also examined actin cap formation and cortical granule free domains (CGFDs). As shown in Fig. 3D, in control oocytes, actin caps formed at the cortex at a location close to spindles, whereas no specific actin cap formation was observed in treated oocytes. Similar results were obtained for CGFDs in oocytes. In control oocytes, CGFDs formed in the cortex close to spindles, whereas no specific CGFDs formed in treated oocytes (Fig. 3E). This indicated that cortical reorganization failed after suppressing Dynamin 2 activity.

Spindle migration fails after disrupting Dynamin 2 activity

Because actin is closely associated with spindle mobility in mouse oocytes, aberrant actin expression after Dynamin 2 inhibition prompted us to investigate spindle movement. The time-lapse microscopy results in Fig. 4A show that in a control oocyte, the spindle moved to the cortex after 9 h in culture. However, in a treated oocyte, the spindle did not move and was arrested in the place where it had formed. Fluorescence microscopy also provided a complete view for the position of a spindle during oocyte maturation; a large proportion of oocyte chromosomes localized within the central cytoplasm (Fig. 4B). The rate of aberrant spindle migration in treated oocytes was significantly higher than that in control oocytes. For statistical comparisons, 19.21 ± 14.94% (n = 142) of control oocytes showed central spindle positions, whereas in dynasore-treated oocytes, 36.27 ± 15.25% (n=101; with 100 μM) and 40.79 ± 8.08% (n = 95; with 200 μM) showed centrally arrested spindles ($p<0.05$) (Fig. 4C).

Profilin and ARP2 localization is disrupted after disrupting Dynamin 2 activity

To explore a possible regulatory mechanism for Dynamin 2 during mouse oocyte maturation, we examined the localizations of Profilin and ARP2, which may take part in this process. As shown in Fig. 5A, Profilin accumulated at the periphery of an oocyte and in the vicinity of a spindle during the MI stage, whereas after suppressing Dynamin 2 activity, Profilin aggregation disappeared. An immunofluorescence curve confirmed this result. Similar results were found for ARP2; there was no specific localization for ARP2 in a treated oocyte, whereas ARP2 signals were observed at the periphery and in the vicinity of a spindle in a control oocyte (Fig. 5B).

Discussion

In this study, we have provided evidence that the large GTPase Dynamin 2 regulates spindle migration and cytokinesis in mouse oocytes. Our results demonstrated that the roles of Dynamin 2 in oocytes were associated with actin and might have occurred by regulating Profilin and ARP2/3 complexes.

We first showed that during mouse oocyte meiotic maturation, Dynamin 2 was located at the periphery of oocytes and that after GVBD it was localized around the spindle. This localization pattern was similar to that of actin, as actin localized at the cortex of an oocyte and around the meiotic spindle. Fmn2, a key actin nucleation factor for oocyte asymmetric division, was recruited to the endoplasmic reticulum structures surrounding the meiotic spindle (Yi *et al.* , 2013). Thus, we assumed that Dynamin 2 may also be involved in actin-related processes during the maturation of mouse oocytes.

To confirm our hypothesis, we knocked down Dynamin 2 mRNA expression using RNAi and cultured oocytes with a Dynamin inhibitor for 12 h to disrupt Dynamin functions. These results showed that suppressing Dynamin 2 activity resulted in polar body extrusion failure. Time-lapse microscopy showed that cytokinesis had also failed, as chromosomes first segregated and then realigned.

Next we explored a possible cause for polar body extrusion failure. Actin provides the driving force for cells to undergo cytokinesis (Hall, 1998) and chromosome congregation (Lenart *et al.* , 2005). In the mouse oocyte, an "actin cloud" around meiotic spindles drives spindle migration (Bezanilla and Wadsworth, 2009). In addition, Dynamins, particularly the Dynamin 2 isoform, which localizes with actin filaments, are often found at locations where cell membranes undergo remodeling (Schafer, 2004). Thus, we hypothesized that the dynamic changes of actin might be related to Dynamin 2 in mouse oocytes.

Actin expression in dynasore-treated oocytes was significantly decreased in the cytoplasm and at the membrane compared to control oocytes Fluorescence intensity analysis confirmed this. The failure to form actin caps and cortical granule free domains, which are polarized characteristics of oocytes, indicated the disruption of oocyte polarization, a process that depends on actin dynamics. Similar results were found for the small G protein Cdc42. In MII oocytes, Cdc42 inhibition causes a loss of a polarized F-actin cap and a failure to protrude the second polar body (Dehapiot *et al.* , 2013). Taken together, our results suggested that aberrant actin expression after inhibiting Dynamin 2 might have been the cause for defective polar body extrusion.

Spindle migration and anchoring are dependent on actin microfilaments in mammalian oocytes (Li *et al.* , 2008). Since the aberrant actin expression was observed, we investigated whether actin-based spindle migration was the cause of defective polar body extrusion. Live cell imaging showed that after disrupting Dynamin 2 activity, the spindle did not move to the cortex and was arrested in the central cytoplasm. Spindle migration is important for first polar body extrusion and our several previous studies have confirmed that disrupting spindle migration results in oocyte polarization failure (Sun *et al.* , 2011a, Sun *et al.*, 2011b, Sun *et al.* , 2011c). Therefore, we concluded that polar body extrusion failure after Dynamin 2 inhibition was caused by blocked spindle movement and actin degradation.

To explore a possible regulatory mechanism for Dynamin 2 in mouse oocyte maturation, we examined the activity of a Dynamin-binding protein, Profilin, and an actin-related protein (ARP2/3) complex. A previous study showed that Profilin bound to a large number of proline-rich proteins, including Dynamins, and played a role in regulating actin dynamics during mitosis. The ARP2/3 complex is also involved in numerous actin-based cellular processes. Inhibiting ARP2/3 complex activity results in disrupting oocyte polarization during mouse oocyte meiotic maturation (Sun *et al.*, 2011b). Our results showed that after suppressing Dynamin 2 activity, the specific localizations of both Profilin and ARP2 in oocytes were disrupted. Taken together, our results suggest that Dynamin 2 involvement during the meiotic maturation of mouse oocytes might be mediated through a Dynamin-Profilin-ARP2/3-actin pathway.

In conclusion, our results indicate that Dynamin 2 regulates actin-based spindle migration and cytokinesis in mouse oocytes, and this regulation may be mediated via a Dynamin-Profilin-ARP2/3-actin pathway (Fig. 6).

Materials and Methods

Antibodies and chemicals

Goat polyclonal anti-Dynamin2 antibody was purchased from Santa Cruz (Santa Cruz, CA), whilst mouse monoclonal anti-Profilin 1 antibody was purchased from Abcam (Abcam, UK). ARP2 antibody was a gift from Prof. Qing-Yuan Sun of the Chinese Academy of Sciences, China. Dynasore was purchased from Calbiochem (Merck KGaA, Darmstadt, Germany). Mouse polyclonal anti-α-tubulin-FITC antibody and Phalloidin-TRITC was purchased from Sigma (St Louis, MO). Alexa Fluor rabbit anti-goat 488 and donkey anti-goat 568 antibodies were from Invitrogen (Carlsbad, CA). Goat anti-rabbit IgG/FITC, goat anti-rabbit IgG/TRITC, goat anti-mouse IgG/FITC and IgG/TRITC were from Zhongshan Golden Bridge Biotechnology Co., Ltd (Beijing, China).

Oocyte collection and culture

All animal manipulations were conducted according to the guidelines of the Animal Research Committee of Nanjing Agricultural University, China. Mice were housed in a temperature-controlled room with proper darkness-light cycles, fed with a regular diet, and maintained under the care of the Laboratory Animal Unit, Nanjing Agricultural University, China. The mice were killed by cervical dislocation. This study was specifically approved by the

Committee of Animal Research Institute, Nanjing Agricultural University, China. Germinal vesicle-intact oocytes were collected from ovaries of 4- to 6-week-old mice and cultured in M2 medium (Sigma Chemical Co., St. Louis, MO) under paraffin oil at 37^{o}C in a 5% CO_2 atmosphere. After different times in culture, oocytes were used for immunostaining.

Oocyte nocodazole treatment

For nocodazole treatment, 10 mg/ml of nocodazole in DMSO stock was diluted with M2 medium to a final concentration of 20 μg/ml. After incubation for 10 min, oocytes were used for immunofluorescence microscopy.

Real-time quantitative PCR analysis

Analysis of Dynamin2 gene expression was measured by real-time quantitative PCR and the $\Delta\Delta C_T$ method. Total RNA was extracted from 50 oocytes using a Dynabead mRNA DIRECT kit (Invitrogen Dynal AS, Norway). First strand cDNA was generated with a cDNA synthesis kit (Takara) using Oligo(dT)12–18 primers (Invitrogen). A cDNA fragment of Dynamin 2 was amplified using the following primers: Forward, CCC TAG TGG ACA TGA CAA TGA A; Reverse, AGG ATG CAG TTC CTG TTC TG. We used a DyNAmo HS SYBR Green qPCR kit (FINNZYMES) with an Applied Biosystems 7500 Real-Time PCR System under the following conditions: 95^{o}C for 10 sec, and 38 cycles of 95^{o}C for 5 sec and 59^{o}C for 32 sec.

Dynamin 2 siRNA injection

Approximately 5–10 pl of Dynamin 2 siRNA (Santa Cruz, sc-35237) was microinjected into the cytoplasm of a fully grown GV oocyte using an Eppendorf FemtoJet (Eppendorf AG, Hamburg, Germany) with a Nikon Diaphot ECLIPSE TE300 inverted microscope (Nikon UK Ltd., Kingston upon Thames, Surrey, UK) equipped with a Narishige MM0-202N hydraulic three-dimensional micromanipulator (Narishige Inc., Sea Cliff, NY). After injection, oocytes were cultured in M16 medium containing 5 μM milrinone for 24 h, and then washed five times (2 min each wash) in fresh M16 medium. Oocytes were then transferred to fresh M16 medium and cultured under paraffin oil at 37^{o}C in a 5% CO_2 atmosphere. Control oocytes were microinjected with 5–10 pl of negative control siRNA. Spindles, actin cap phenotypes, and chromosome localization were examined using a confocal microscope (Carl Zeiss LSM 700 META, Germany).

Oocyte dynasore treatment

After collection, oocytes were cultured in M2 medium containing 100 or 200 μM Dynasore for 12 h. Spindle phenotypes and chromosome localization were examined using a confocal microscope (Zeiss LSM 700 META, Germany).

Western blot analysis

A total of 150 mouse oocytes were placed in Laemmli sample buffer (SDS sample buffer and 2-Mercaptoethanol) and heated at 100°C for 5 min. Proteins were separated by SDS-PAGE and then electrophoretically transferred to polyvinylidene fluoride membranes. After transfer, membranes were blocked in PBST (PBS containing 0.1% Tween 20) containing 5%

non-fat milk for 1 h, followed by incubation at 4°C overnight with goat polyclonal anti-Dynamin2 antibody (1:1000) and tubulin antibody (1:2000). After washing 3 times in PBST (10 min each), membranes were incubated at 37°C for 1 h with HRP conjugated Pierce anti-goat IgG (1:10,000). Finally, membranes were processed using an enhanced chemiluminesence detection system (Amersham, Piscataway, NJ).

Immunostaining and confocal microscopy

For immunostaining, oocytes were fixed in 4% paraformaldehyde in PBS for 30 min at room temperature and then transferred to a membrane permeabilization solution (0.5% Triton X-100) for 20 min. After 1 h in blocking buffer (1% BSA in PBS), oocytes were incubated overnight at 4^{o}C with 1:25 goat anti-Dynamin 2 (1:100 mouse anti-Profilin, 1:50 rabbit anti-ARP) antibody. After three washes in wash buffer (0.1% Tween 20 and 0.01% Triton X-100 in PBS), oocytes were labeled with FITC-anti-goat IgG or TRITC-anti-goat IgG (1:100) for 1 h at room temperature. For α-tubulin-FITC and Phalloidin-TRITC staining, after incubation for 1 h, oocytes were washed three times in PBS containing 0.1% Tween 20 and 0.01% Triton X-100 for 2 min. The samples were then co-stained with Hoechst 33342 (10 μg/ml in PBS) for 10 min, followed by three washes in wash buffer. Oocytes were mounted on glass slides and examined with a confocal laser-scanning microscope (Zeiss LSM 700 META, Germany). At least 20 oocytes were examined for each experimental condition.

Time-lapse microscopy

After microinjecting tubulin-GFP mRNA (derived from in vitro transcription of a pRN3-b5-tubulin-GFP plasmid), oocytes were incubated in M2 medium containing Hoechst 33342 (5 ng/ml; Sigma) and dynasore (200 μM) to image spindle and chromosome dynamics during oocyte maturation. Microtubule dynamics were imaged using a Perkin Elmer precisely Ultra VIEW VOX confocal Imaging System. The exposure time was set to between 200 and 800 ms, depending on the tubulin-GFP fluorescence level. Digital time-lapse images were acquired under the control of IP Lab (Scanalytics) or AQM6 (Andor/Kinetic-imaging) software. Confocal images of spindles in live oocytes were acquired with a 10x objective on a spinning disk confocal microscope (Perkin Elmer). The same treatment was used for Alexa 488-Phalloidin (1 μM).

Statistical analysis

At least three replicates were done for each experiment with results expressed as means ± SEM's. Statistical comparisons between groups were made by analysis of variance (ANOVA) followed by Duncan's multiple comparisons test. A p-value of < 0.05 was considered significant. Fluorescence intensity statistics were evaluated using Image J (NIH) software with at least 10 oocytes analyzed for each experiment.

Author contribution

QCW and SCS conceived and designed the experiments; QCW, JL and ZBW performed the experiments; QCW, ZBW and SCS analyzed the data; YZ, XD, XSC and NHK contributed reagents/materials/analysis tools; QCW and SCS wrote the paper; all authors approved the manuscript content and its final version.

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Figure 1. **Dynamin 2 expression and localization in mouse oocytes. (A) Dynamin 2 expression is enriched at the periphery of oocytes during the entire cell development process, and is concentrated around spindles after GVBD. (B) Dynamin 2 exhibits a localization pattern similar to that of actin. Bar = 5 μm. (C) Dynamin 2 concentrated at the periphery of the spindle. Bar = 5 μm. (D) After nocodazole treatment, Dynamin 2 loses its specific localization around the spindle. Bar = 20 μm.**

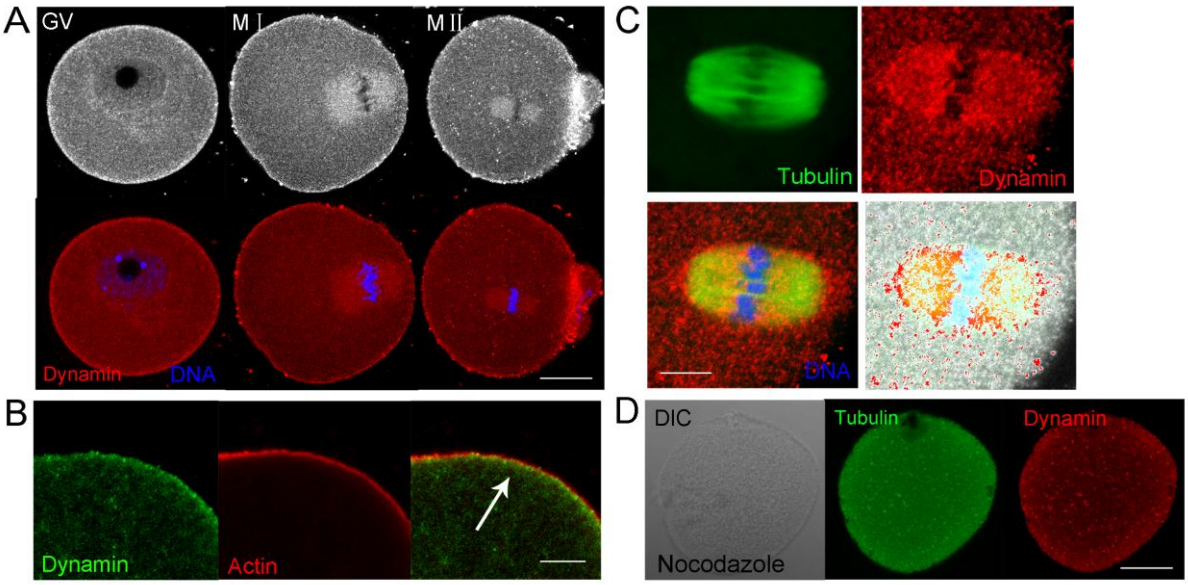

Figure 2. Dynamin 2 effects on polar body extrusion. (A) Dynamin 2 mRNA levels after siRNA microinjection. (B) Dynamin 2 protein levels after siRNA microinjection. (C) After dynasore treatment, Dynamin 2 loses its specific localization. Bar = 20 μm. (D) After Dynamin RNAi or inhibitor treatment, oocytes fail to extrude polar bodies. The Rates of polar body extrusion after Dynamin 2 RNAi and after inhibitor treatment significantly decreased after treatment. Different letters or * indicate significantly different. Bar = 80 μm. (E) Polar body extrusion as observed with time-lapse microscopy. In treated oocytes, chromosomes separate but then reunite or cannot move to the cortex. Bar = 20 μm.

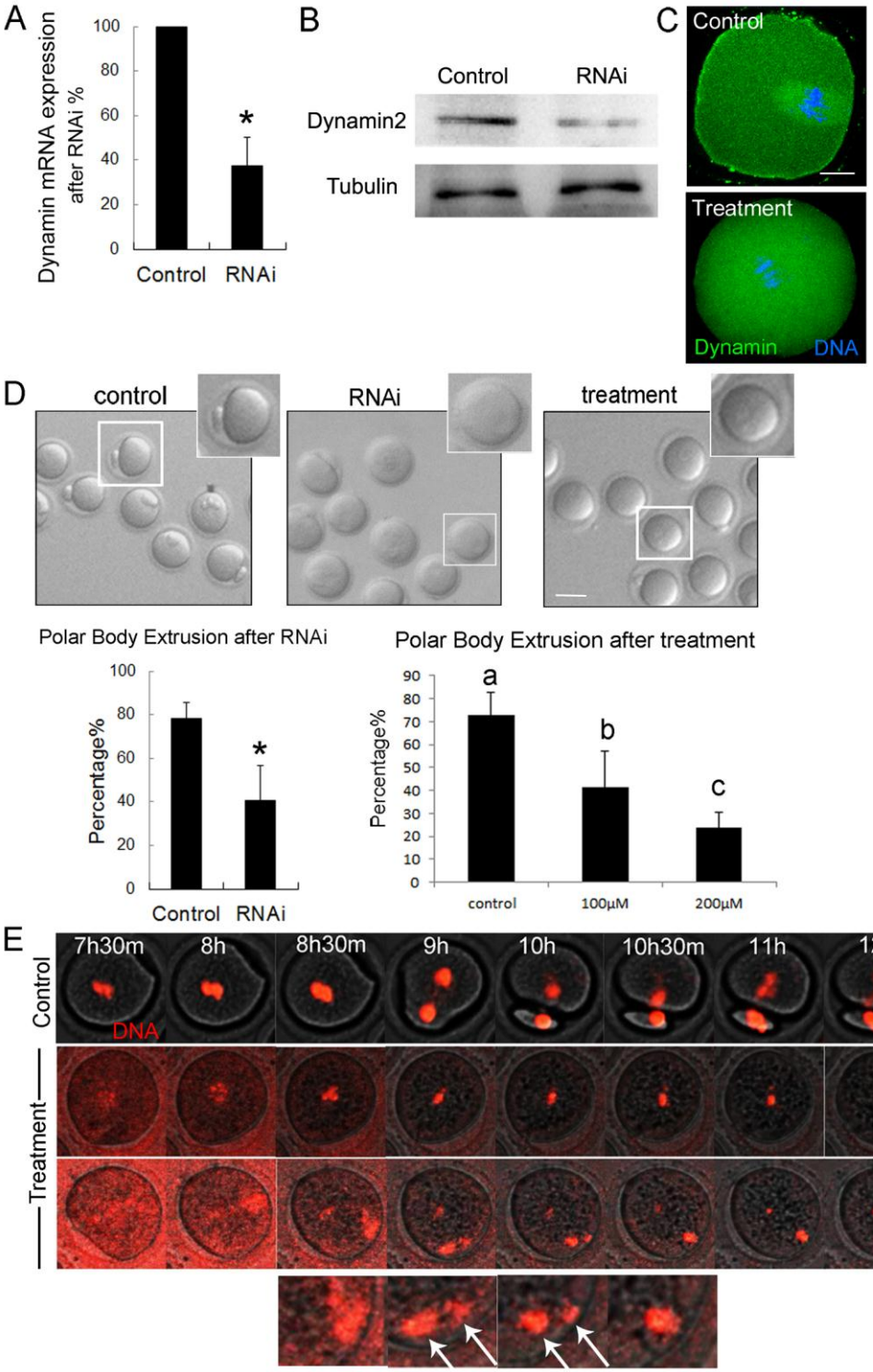

Figure 3. Changes in actin expression after suppressing Dynamin 2 activity. (A) Time-lapse microscopy results for actin expression during mouse oocyte maturation. A control oocyte exhibits distinct actin aggregation at the membrane, whereas actin signal is weak and gradually decreases of a dynasore-treated oocyte. Bar = 20 μm. (B) Actin expression at the membrane and in the cytoplasm after disrupting Dynamin 2 activity. Bar (left) = 80 μm; Bar (right) = 20 μm. (C) Actin fluorescence intensity analysis in oocytes. Actin fluorescence intensity in control oocytes is significantly higher than that in treated oocytes ($p < 0.05$). (D) Actin cap formation after disrupting Dynamin 2 activity. Actin caps formed in control oocytes, whereas no actin caps formed in treated oocytes. (E) CGs free domain formation after disrupting Dynamin 2 activity. CGFDs formed in control oocytes, whereas no CGFDs formed in treated oocytes. Bar = 20 μm.

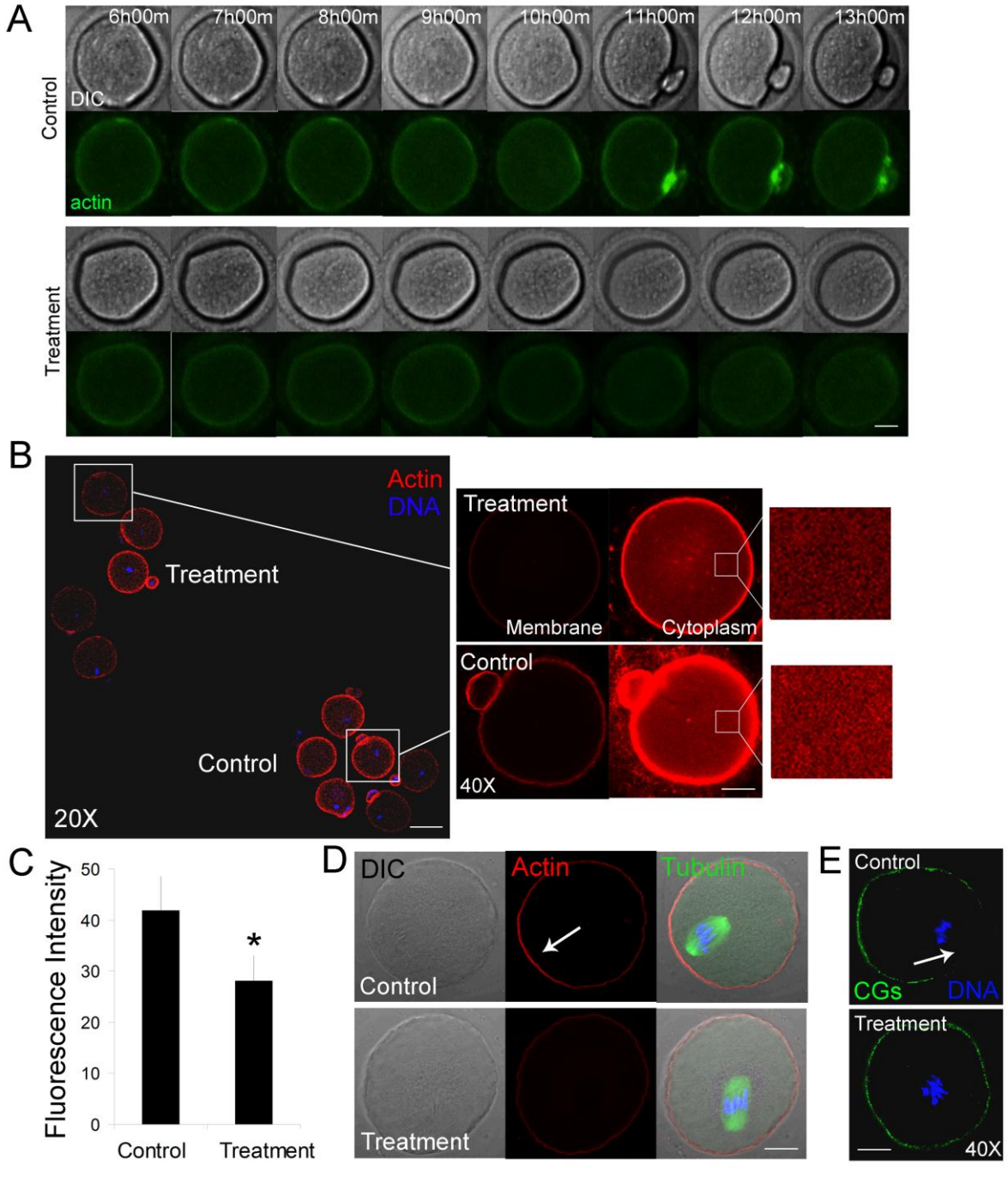

Figure 4. Dynamin 2 effects on spindle migration. (A) Time-lapse microscopy results showing that in the control oocyte the spindle moves to the cortex, whereas in the treated oocyte, the spindle fails to migrate and is arrested in the central cytoplasm. Bar = 20 μm. (B) Overall spindle locations after disrupting Dynamin 2 activity. Most control oocyte spindles localize near to the cortex, whereas in treated oocytes, spindles remain in the central oocyte. Bar (left) = 80 μm; Bar (right) = 20 μm. (C) Rate of spindle migration after disrupting Dynamin 2 activity.

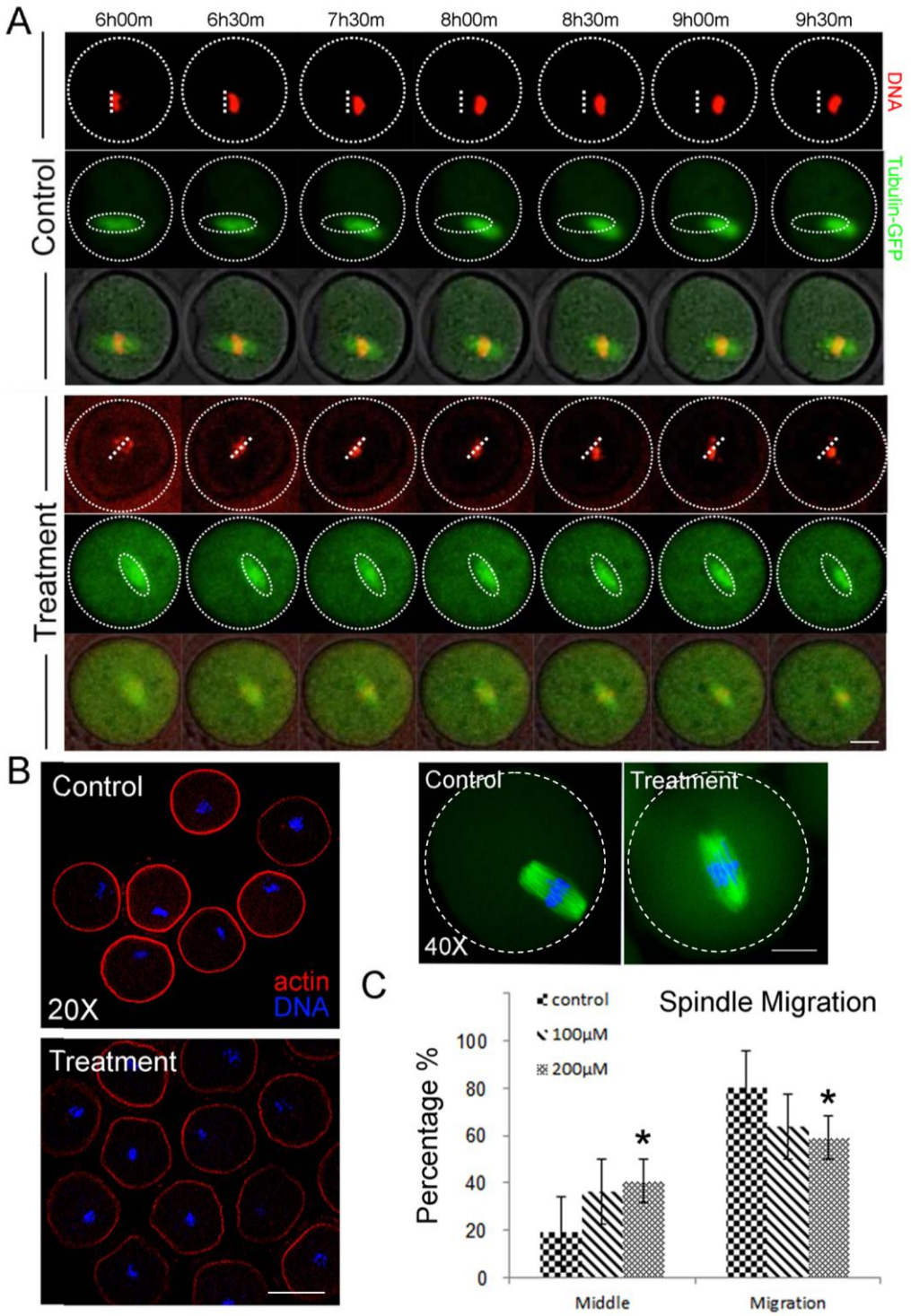

Figure 5. Localizations of Profilin and ARP2 after disrupting Dynamin 2 activity. (A) Profilin expression showed no specific localization in oocytes after inhibitor treatment, whereas in control oocytes, Profilin expression was enriched at the membrane and spindle. Immunofluorescence curve analysis was used for confirmation. (B) ARP2 expression showed no specific localization in oocytes after inhibitor treatment, whereas in control oocytes, ARP2 expression was enriched at the membrane and around chromosomes. Immunofluorescence curve analysis was used for confirmation. Bar = 20 μm.

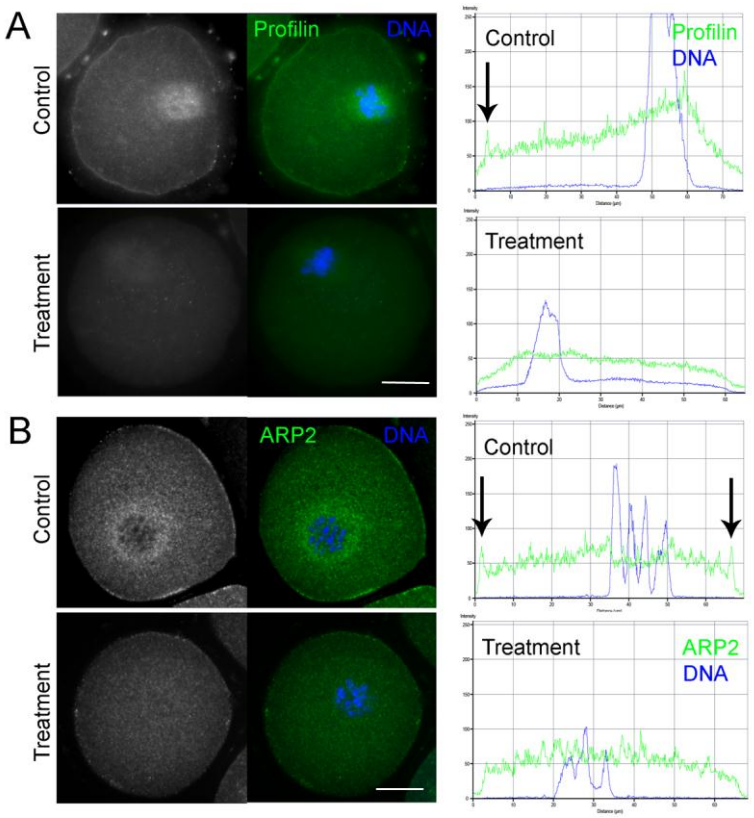

Figure 6. Diagram of Dynamin 2 function in mouse oocyte meiosis. Dynamin 2 localized at the cortex of oocyte and around the meiotic spindle, and Dynamin 2 may be involved in actin-driven spindle/chromosome migration in mouse oocytes..