—Original—

Localization of γ-Tubulin in Mouse Eggs during Meiotic Maturation, Fertilization, and Early Embryonic Development

Xiao-Qian MENG[1,2], Heng-Yu FAN[1], Zhi-Sheng ZHONG[1], Gang ZHANG[1,2], Yun-Long LI[2], Da-Yuan CHEN[1] and Qing-Yuan SUN[1]

[1]*State Key Laboratory of Reproductive Biology, Institute of Zoology, The Chinese Academy of Sciences, Beijing, 100080,* [2]*Key Laboratory of Animal Resistance, School of Life Science, Shandong Normal University, Jinan, 250014, China*

Abstract. γ-Tubulin, a member of the tubulin superfamily, is a peri-centriolar component which is considered to be essential for microtubule nucleation. The dynamics of γ-tubulin during mouse oocyte meiotic maturation, fertilization, and early cleavage as well as the co-localization of γ-tubulin and α-tubulin during the formation of the meiotic I spindle were studied by confocal microscopy. We found that γ-tubulin was evenly distributed in the germinal vesicle (GV) stage oocyte. After germinal vesicle breakdown (GVBD) γ-tubulin dots were localized in both the cytoplasm and the vicinity of the condensed chromosomes, and aligned at both poles of the meiotic spindle at prometaphase I and metaphase I. At anaphase I and telophase I, γ-tubulin was detected between the separating chromosomes, while it was absent in the midbody. At the MII stage, γ-tubulin was again accumulated at the spindle poles. α-Tubulin had a similar distribution pattern as γ-tubulin in the cytoplasm and radiated from γ-tubulin foci close to the chromosomes during the meiotic spindle formation. After fertilization, γ-tubulin was translocated from spindle poles to the area between separating chromatids and distributed around the pronuclei. It aggregated into some dots during the interphase, but was distributed on the mitotic spindle poles in early embryos. Our results suggest that γ-tubulin is essential for microtubule nucleation and spindle formation during mouse oocyte meiosis, fertilization, and early embryo cleavage.
Key words: γ-Tubulin, Mouse, Egg, Meiosis, Spindle

Almost all animal cells possess a centrosome, a structure generally composed of two centrioles surrounded by pericentriolar material (PCM), from which an interphase network of microtubules and the mitotic bipolar spindle are nucleated. Thus, the centrosome is considered as the microtubule-organizing center (MTOC) [1]. However, several examples of meiotic spindles and early embryonic mitotic spindles have been formed without centrosomes, displaying a barrel shape and a non-astral spindle. Even in the absence of centrioles,

aggregates of components recognized by anti-PCM antibodies are present and electron dense material can be observed [2, 3]. These are thus commonly considered as MTOCs. It has been found that γ-tubulin, a highly conserved member of the tubulin superfamily, is localized at MTOCs and has been shown to be involved in microtubule nucleation [4, 5]. Genetic results from fungi and *Drosophila* indicate that γ-tubulin is essential and required for spindle function [6–9]. Furthermore, *in vitro* experiments demonstrated unequivocally that γ-tubulin plays an important part in microtubule nucleation from centrosomes [10]. Most soluble γ-

tubulin is in a large complex, the γ-tubulin ring complex (γ-TuRC), which has been purified from *Xenopus* eggs [11]. γ-TuRCs nucleate microtubule assembly at the minus end of microtubules [11, 12] and act as a minus end capping protein [13].

Recent evidence suggests that γ-tubulin may have additional functions beyond microtubule nucleation. Studies in *S. pombe* have shown that γ-tubulin has a function in altering interphase microtubule arrays and is also necessary for anaphase A (chromosome to pole movement) in mitosis [14]. In *Drosophila melanmogaster*, observations of a mutant γ-tubulin revealed that γ-tubulin was not absolutely required for microtubule nucleation in meiotic cells, but was important for the organization of microtubules into functional meiotic spindles [15]. Furthermore, there are some data indicating that γ-tubulin has roles in spindle elongation [16, 17], check-point regulation [14, 18, 19] and microtubule dynamics [14, 16].

Although numerous studies have revealed the roles of γ-tubulin in mitotic division, its functions during meiosis are not fully understood, especially in mammalian oocytes. Mammalian oocytes are ideal models for the study of the meiotic cell cycle, during which considerable chromosomal and cytoplasmic changes occur, including chromosome condensation, GVBD, spindle organization, polar body emission etc. In mouse metaphase II-arrested oocytes, γ-tubulin foci have been found at the meiotic spindle poles and the cytoplasmic MTOCs [20, 21]. In pig oocytes, γ-tubulin is localized along spindle microtubules at metaphase I and until early anaphase I, when it becomes aggregated around the spindle poles [22]. These spindle associated localizations of γ-tubulin reflect its function in microtubule nucleation.

In the present study, we aimed to reveal the dynamics of γ-tubulin during mouse oocyte meiotic maturation, fertilization and early embryo development and to investigate the interaction between γ-tubulin and α-tubulin during meiosis I spindle formation. For this purpose, we used immunofluorescent confocal analysis with antibodies against α-tubulin and γ-tubulin.

Materials and Methods

Chemicals

All the chemicals used in this experiment were purchased from Sigma Chemical Company (St. Louis, MO) except for those specifically noted.

Oocyte collection and culture

Female Kunming mice at 4–6 weeks age were injected with 10 IU pregnant mare's serum gonadotropin (PMSG). Approximately 48 h later, GV-stage oocytes were collected from the ovaries by puncturing antral follicles with a needle (27 gauge). Cumulus-free and GV-intact oocytes were cultured in M2 (Sigma, St. Louis, MO) medium at 37 C in a humidified atmosphere of 5% CO_2.

Cumulus cell-enclosed, metaphase II-arrested eggs were obtained from mice of the same strain. After intraperitoneal injections of 10 IU PMSG and hCG 48 h apart, animals were sacrificed by cervical dislocation 16 h after hCG injection. The cumulus cell masses surrounding the eggs were removed by brief exposure to 300 IU/ml hyaluronidase in M2 medium.

In vivo and in vitro fertilization

In vivo fertilized zygotes were collected 16 h post-hCG from the oviduct ampullae of superovulated females that had been mated with the same strain of males. After removing cumulus cells with 300 IU/ml hyaluronidase in M2 medium, zygotes were cultured in M16 (Sigma) medium. Two-cell embryos were flushed from the oviducts of copulated mice 44–46 h after hCG injection. Zygotes and embryos were cultured in M16 medium at 37 C in a humidified atmosphere of 5% CO_2. Embryos at different stages of mitosis were collected for confocal microscopy.

On the day of MII stage oocyte collection, male mice were also sacrificed by cervical dislocation. The cauda epididymides were dissected away and minced slightly into pre-warmed M16 medium at 37 C for sperm capacitation. Oocytes were inseminated in a 50 μl drop of M16 medium with capacitated spermatozoa (1×10^6 cells/ml). The emission of the second polar body and the formation of the pronuclei were observed with an inverted microscope.

Confocal microscopy

After removal of zona pellucida (ZP) in acidified

Tyrode's solution (pH 2.5), oocytes or embryos were fixed in 4% paraformaldehyde in PBS for 30 min and then incubated in incubation buffer (0.5% TritonX-100 in 20 mM Hepes, pH 7.4, 3 mM MgCl$_2$, 50 mM NaCl, 300 mM sucrose, 0.02% NaN$_3$) for 30 min. After placing in methanol for 5 min at –20 C, the eggs or embryos were washed in PBS containing 0.1% Tween 20 and 0.01% Triton X-100 three times and then blocked in 1 mg/ml bovine serum albumin (BSA) in PBS for 1 h. The oocytes or embryos were incubated with 1:400 rabbit anti-γ-tubulin antibody (Sigma T3559) for 1 h before three washes, 5 min each, and then incubated with 1:100 FITC-conjugated goat anti-rabbit IgG for 1 h. After another three washes, the oocytes or embryos were stained with 10 mg/ml propidium iodide (PI) for 10 min. Finally, the eggs or embryos were mounted on glass slides and examined using a TCS-4D laser scanning confocal microscope (Leica Microsystems, Bensheim, Germany).

When eggs were double labeled for α-tubulin and γ-tubulin, they were incubated with 1:100 TRITC-conjugated goat anti-rabbit IgG for 1 h after incubation in rabbit anti-γ-tubulin antibody and three washes as described above. After another three washes the eggs were incubated with 1:200 FITC-conjugated anti-α-tubulin antibody (Sigma F2168) for 1 h. Finally, after three washes, the eggs were mounted on glass slides and examined.

The spindle organization was determined by incubating the eggs in 1:50 diluted FITC-anti-α-tubulin for 1 h after fixation and permeablization as described above.

Experimental design

Experiment 1. The localization of γ-tubulin during the meiotic maturation, *in vitro* fertilization and early embryo cleavage was studied. Eggs at different stages were fixed for confocal microscopy.

Experiment 2. To investigate the interrelationship between meiosis spindle formation and γ-tubulin localization, oocytes at different stages of spindle formation were collected for double-staining confocal microscopy.

Results

Subcellular localization of γ-tubulin during oocyte maturation

During oocyte maturation, the localization of γ-tubulin was examined using anti-γ-tubulin antibody (Fig. 1 and Fig. 2). In fully-grown GV stage oocytes, γ-tubulin was diffusely localized in both the cytoplasm and the germinal vesicle (Fig. 1A). After GVBD, when chromatin assembles to form chromosome clusters, some distinct γ-tubulin foci were detected in the vicinity of the chromosomes as well as in the cytoplasm (Fig. 1B). In prometaphase I, γ-tubulin became more aggregated on or close to the chromosomes and fewer cytoplasmic γ-tubulin foci were observed (Fig. 1C). Before the condensed chromosomes organized at the equatorial plate, the γ-tubulin foci moved away and aligned at both poles of the metaphase I spindle (Fig. 1 D and E). When the chromosomes aligned on the metaphase plate, γ-tubulin was detected as two dots associated with the spindle poles (Fig. 1F). During the formation of the meiosis I spindle, γ-tubulin was also present as some asters in the cytoplasm in addition to its spindle-associated localization (Fig. 1 D, E and F). In anaphase I and telophase I, following the separation of chromosomes, γ-tubulin changed from a highly aggregated form to an amorphous form and progressively localized between the separating chromosomes, but was completely excluded from the midbody (Fig. 1 G, H and I). At the MII stage, γ-tubulin again accumulated at the spindle poles before the formation of the metaphase II plate, becaming two spots when the chromosomes aligned at the equator completely (Fig. 1 J and K). An MII oocyte without primary antibody treatment was used as a negative control for γ-tubulin staining (Fig. 1L).

Subcellular localization of γ-tubulin during fertilization and early embryo cleavage

In our experiment, the oocytes extruded their second polar body 2 h after insemination, and completed pronuclear formation 6–8 h after insemination. As shown in Fig. 2, the chromatids separated and moved to the spindle poles after sperm penetration. During this process, the localization of γ-tubulin was similar to that in meiosis I, changing from aggregated dots to an amorphous form and progressively localizing to the area between the separated chromatids (Fig. 2 A, B and C). With the completion of meiosis II and the formation of the male and female pronuclei, slight γ-tubulin dots were observed around the female pronuclei (Fig. 2D). When the pronuclei
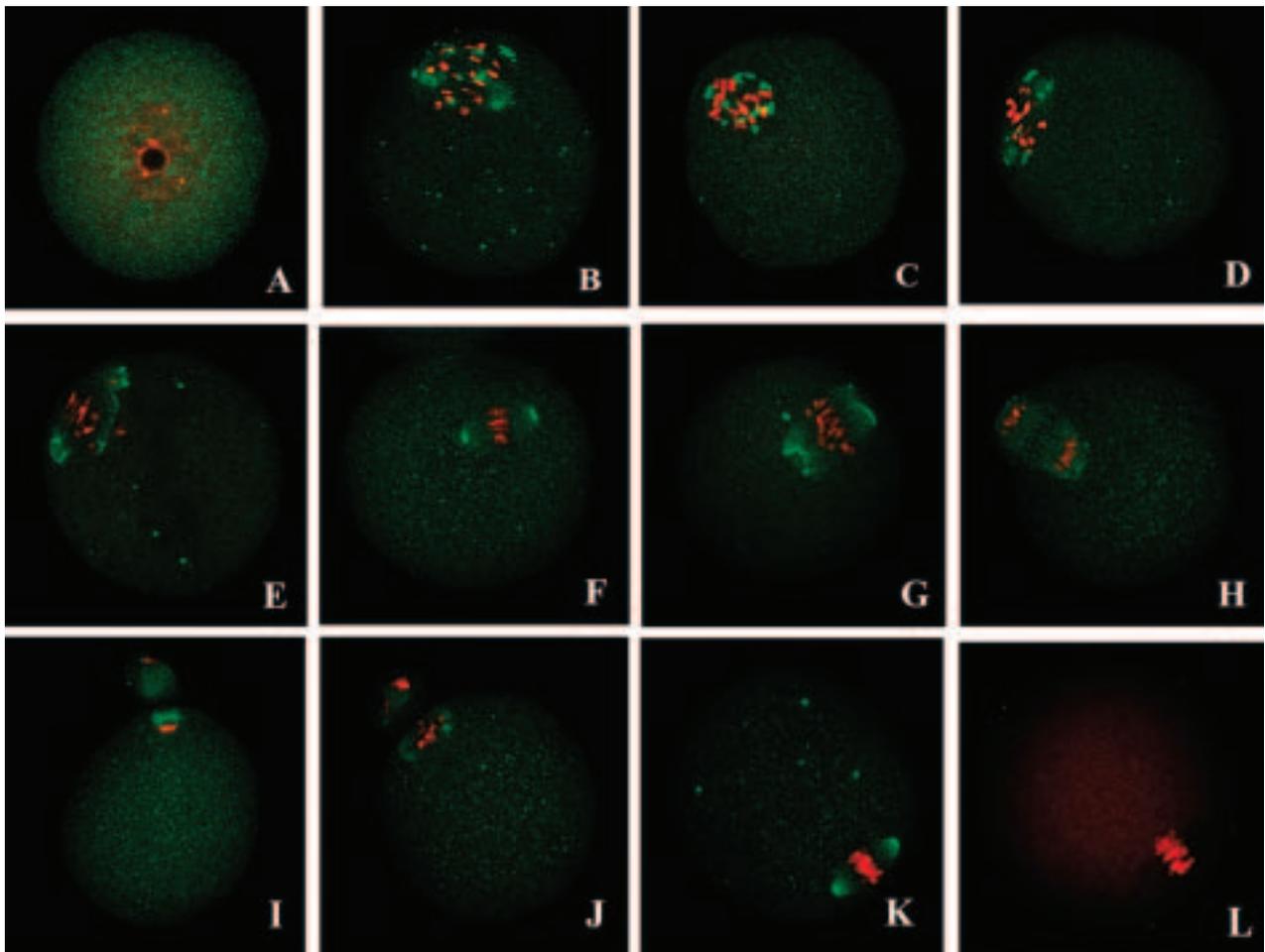
Fig. 1. Immunofluorescent localization of γ-tubulin during meiotic maturation of mouse oocytes. Green, γ-tubulin; red, chromatin. γ-Tubulin was distributed evenly in the GV oocyte (A). When GVBD occurred, γ-tubulin concentrated into some dots, and localized both in the cytoplasm and in the vicinity of the chromosomes (B). γ-Tubulin dots become more aggregated around the condensed chromosomes (C). γ-Tubulin began to migrate to the spindle poles (D). Then, γ-tubulin concentrated on the two poles of the spindle before the chromosomes arranged in the middle of the spindle (E). At metaphase I, γ-tubulin accumulated as two dots at the spindle poles (F). At the early anaphase I, when the chromosomes begin to separate, γ-tubulin became less accumulated at the spindle poles (G). Following the separation of the chromosomes, γ-tubulin was localized along the whole spindle (H). At telophase I, γ-tubulin was localized between the separated chromosomes (I). At early metaphase II, γ-tubulin re-aggregated and began to migrate to the meiosis II spindle poles (J). γ-Tubulin was localized at the poles of the spindle as well as in the cytoplasm in the MII oocyte (K). An MII oocyte was used as a negative control for the γ-tubulin staining, in which no first antibody was used but the fluorescent second antibody was used as in the experimental group (L). Original magnification × 630 (the same as in Figs. 2 and 3).

enlarged and became opposed to each other, many γ-tubulin dots were distributed on the pronuclei or in the vicinity of the pronuclei (Fig. 2E). At the metaphase of the first mitosis, γ-tubulin was again detected on the spindle poles (Fig. 2F). By the completion of the first cell cycle, two blastomeres had formed and both of them entered the interphase. At this time, γ-tubulin dots were distributed around the nucleus (Fig. 2G). At the three and four cell stages, faint γ-tubulin foci were

observed in the cytoplasm or in the vicinity of the interphase nucleus (Fig. 2H).

Co-localization of α- and γ-tubulin during the formation of meiosis I spindle

γ-Tubulin was distributed evenly in GV intact mouse oocytes (Fig. 1A). α-Tubulin condensation was detected at the periphery of the GV (Fig. 3A). Shortly after GVBD, diffusely distributed γ-tubulin assembled in an area that might have been the
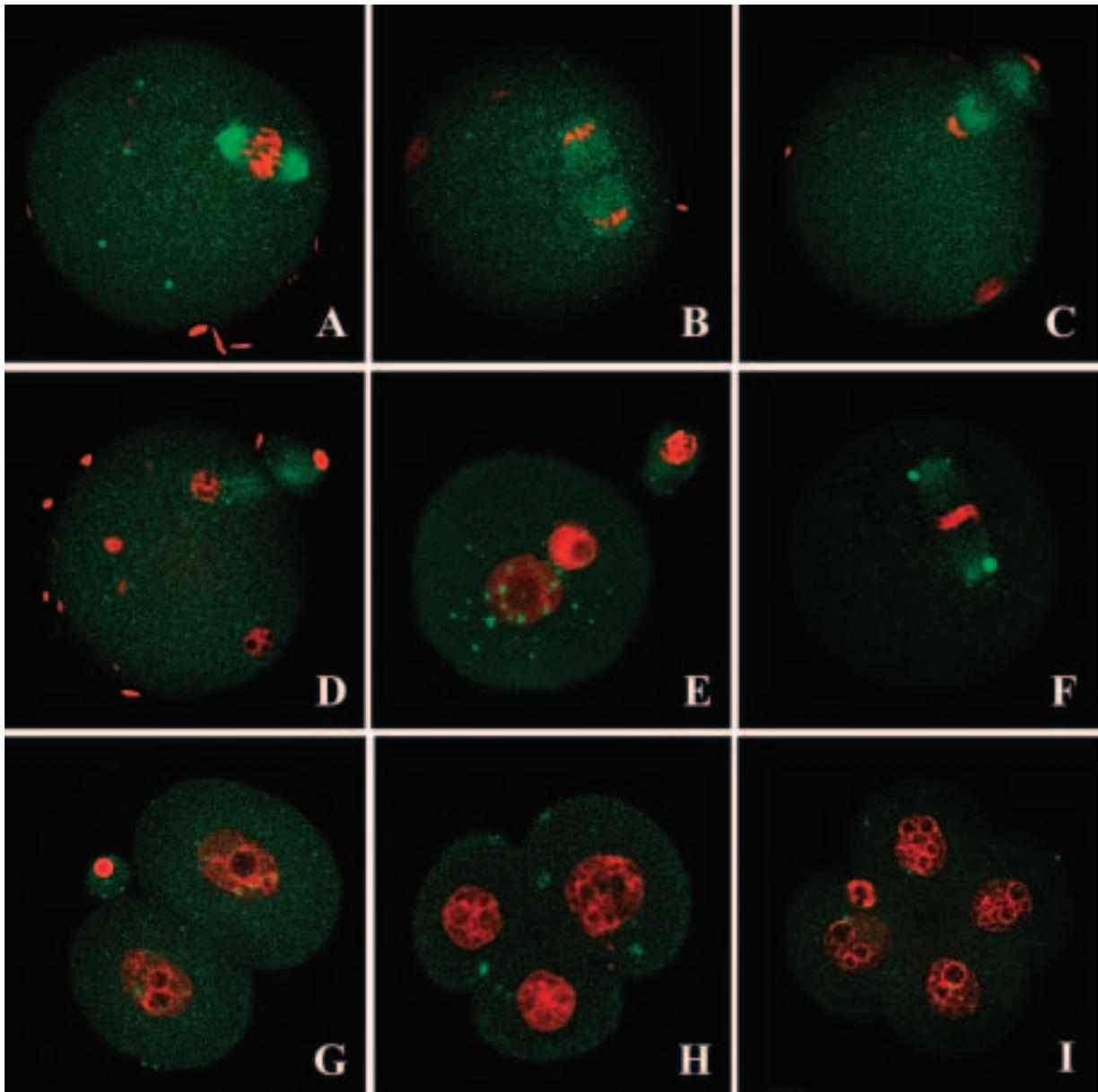
Fig. 2. Localization of γ-tubulin after fertilization and in early embryos. γ-Tubulin became less aggregated shortly after insemination (A). When the chromosomes separated, γ-tubulin was detected along the whole meiosis II spindle (B). During the extrusion of the second polar body and the formation of the pronuclei γ-tubulin was localized to the area between the separated chromatid, absent in midbody (C and D). Many γ-tubulin dots were detected around the adjacent pronuclei (E). γ-Tubulin aggregated near the spindle poles during the first mitosis (F). γ-Tubulin accumulated around the nucleus at the interphase of two, three and four cell stage embryos (G, H, I).

location of the condensed chromosomes (according to the localization of γ-tubulin and the chromatin as shown in Fig. 1) and many short microtubules (α-tubulin) grew from the γ-tubulin foci. Despite the chromosome-associated localization, α-tubulin and γ-tubulin dots were also detected in the cytoplasm and these two tubulins were highly identical in the

localization (Fig. 3B). Then γ-tubulin became more aggregated and the microtubules grew longer, radiating from the γ-tubulin centers. At this stage, less tubulin foci were observed in the cytoplasm (Fig. 3C). When the γ-tubulin moved away and aligned at the meiosis spindle poles, the randomly arrayed microtubules were in the process of

becoming two polarized arrays (Fig. 3 D, E, F, and G). At the MI stage, the microtubules were organized into a fusi-form metaphase spindle and γ-tubulin was localized at the poles (Fig. 3 H and I).

Discussion

In all eukaryotic cells, there is strong evidence showing that microtubule nucleation is mediated by the γ-tubulin complex. In the present study, in addition to providing evidence of its function in microtubule nucleation, we also found that γ-tubulin may play a role in meiotic spindle formation in mouse oocytes.

The subcellular localization of γ-tubulin has been investigated in oocytes of insects, amphibian and mammals. However, the dynamics of γ-tubulin during the spindle formation have exhibited some differences among different animals. In *Xenopus* oocytes, γ-tubulin was bound to spindle microtubules during the formation of the spindle, and it was only after full elongation of the meiotic spindle that γ-tubulin became heavily concentrated at the spindle poles [23]. But in *Drosophila* oocytes, a surprising feature was that γ-tubulin was not detected at the poles of the meiotic I spindle [24]. Our results show that in mouse oocytes γ-tubulin dots are localized in the vicinity of the condensed chromosomes after GVBD and align at both poles of the meiotic spindle. In contrast, pig oocytes did not show γ-tubulin dots but amorphous γ-tubulin staining surrounding the chromatin clusters in late diakinesis after GVBD was observed. It was detected along spindle microtubules at metaphase I, and it became aggregated around the spindle poles until early anaphase I [22]. Recently, γ-tubulin was detected in the MII spindle and in the cortex of bovine oocytes [25]. These differences suggest that γ-tubulin may play roles other than microtubule nucleation and may also reflect different mechanisms of spindle formation among different animal oocytes.

Our results show that γ-tubulin is evenly distributed in GV intact mouse oocytes. This localization is different from previous reports [26, 27]. These disagreements may be due to the different treatments of the oocytes adopted by different authors. Mouse oocytes begin to mature immediately after being released from the follicular environment. Calarco [26] used isobutylmethylxanthine (IBMX) to block the initiation of maturation and examined the true time-zero sample. She found two γ-tubulin foci near the periphery of the oocyte, which moved rapidly away from the cortex along with fragmentation into smaller units when oocytes were placed in IBMX-free medium [26]. This suggests that γ-tubulin responds to the signal of the initiation of the meiotic maturation and moves quickly from the cortex of the oocytes to the germinal vesicle to allow the formation of the meiotic spindle.

The presence of cytoplasmic MTOCs, independent of the meiotic spindle, is a peculiarity of the mouse oocyte. In our experiments, γ-tubulin spots were observed in the cytoplasm during the process of meiotic spindle formation. Just before or shortly after GVBD, γ-tubulin changed rapidly from an evenly distributed form to some aggregated dots. Then with the accumulation of γ-tubulin around the chromatin clusters, fewer cytoplasm γ-tubulin dots were detected. Along with the aggregation of γ-tubulin, α-tubulin grew and then formed microtubule asters around γ-tubulin dots. These results suggest that the origin of meiotic spindle poles in the mouse oocyte is associated with MTOCs near the condensed chromosomes and that spindle γ-tubulin is recruited from the cytoplasm during the process of spindle assembly. It was interesting that α-tubulin foci were highly co-localized with γ-tubulin foci in the cytoplasm shortly after GVBD. The consistent co-localization of α-and γ-tubulin indicates that microtubules are nucleated from the γ-tubulin foci. When the γ-tubulin dots got close to the chromosomes, the microtubules had radiated from the γ-tubulin centers. We can infer from this phenomenon that microtubule nucleation could happen without chromosome regulation, while the spindle organization is probably dependent on the regulation of the chromosomes. This is consistent with previous conclusions regarding mitotic cells and pig oocytes [28, 29].

The interaction of γ-tubulin with the formation of meiosis I spindle was clearly observed when the mouse oocytes were double-labeled for γ-tubulin and α-tubulin. In our culture system, γ-tubulin spots were detected migrating to the spindle poles 4 h after GVBD and aligning at both poles of the meiosis I spindle before the condensed chromosomes organized at the equatorial plate.
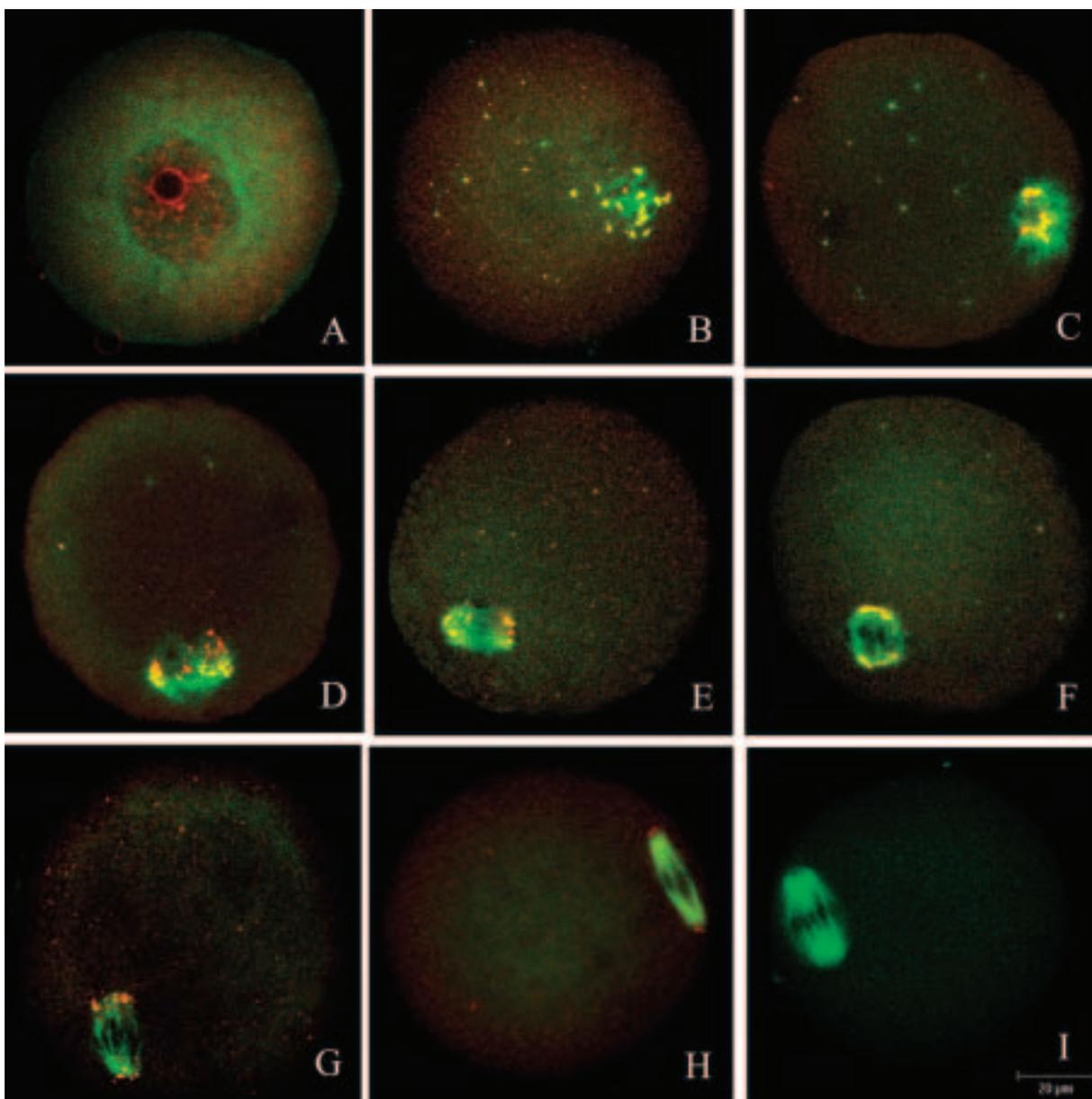
Fig. 3. Co-localization of α-tubulin and γ-tubulin during the formation of the meiosis I spindle. Green, α-tubulin; red, chromatin (A) or γ-tubulin (B-I); yellow, overlapping of green and red. α-Tubulin was localized around the germinal vesicle (A). Overlapping α-tubulin and γ-tubulin dots were detected in the cytoplasm and around the area of the nucleus . Some short microtubules (α-tubulin) radiated from the chromosome associated γ-tubulins (B). γ-Tubulin became more accumulated and many microtubules grew from the γ-Tubulin foci (C). γ-Tubulin began to move to the meiotic poles (D). γ-Tubulin concentrated on the two poles of the spindle and microtubules arranged progressively into a fusi-form spindle (E, F, and G). γ-Tubulin was localized at the meiosis I spindle poles (H). An MII oocyte was used as a negative control for the α-tubulin and γ-tubulin double staining, in which no γ-tubulin first antibody was used but the fluorescent second antibody and FITC-conjugated anti-α-tubulin IgG were used as in the experimental group (I).

Correspondingly, the microtubules that grew from γ-tubulin changed from a randomly arrayed emanation to a fusi-like form. At metaphase I stage, the microtubules constituted the meiosis I spindle and on its two poles, two localized, highly aggregated γ-tubulin dots. Recently, evidence that γ-tubulin has a function in mitotic spindle organization has been reported [15, 30]. The kinesis

of γ-tubulin during meiosis I spindle formation suggests its roles in this process in mouse oocytes. Paluh *et al.* [14] suggested that γ-tubulin may play a role in anaphase in *S. pombe*. However, the role of γ-tubulin in anaphase in mammalian oocytes is not known. Its spindle-associated localization may reflect its function in microtubule movements.

At an early stage of fertilization, no γ-tubulin foci were observed around the sperm chromatin or early male pronucleus, which is consistent with a previous conclusion that the centrosome is maternally inherited in the mouse. γ-tubulin foci in the egg cytoplasm and spindle poles were transformed to amorphous γ-tubulin between the separating chromatids, and cytoplasmic γ-tubulin foci disappeared. When the pronuclei became enlarged and got close to each other, many γ-tubulin dots in the vicinity of the pronuclei were observed. The accumulation of γ-tubulin around the pronuclei may be responsible for extensive microtubule assembly in this region. In the pig zygote, male and female pronuclei were included in separate domains of microtubules [31]. In bovine oocytes, maternal γ-tubulin is recruited by a sperm component to reconstitute the zygotic centrosome [25]. The same conclusion was drawn from an *in vitro* experiment [32]. Although the mechanism of recruitment of γ-tubulin to the sperm component is currently unclear, it appears to be true that a model of blended zygotic centrosome composed of maternal constituents is introduced to a paternal template after insemination[32].

Gueth-Hallonet *et al.* [20] found no cytoplasmic staining of γ-tubulin in the interphase of the mouse blastomeres as a result of dispersing of γ-tubulin in the cytoplasm. In our experiment, γ-tubulin dots were obviously observed close to the pronuclei and the nucleus in the interphase of the 1–4 cell mouse embryos. This inconsistency may be due to the different antibodies used by different authors or due to the difference of the immunofluorescence analysis. The aggregation of γ-tubulin in the cytoplasm in the interphase allows mitotic spindle formation during rapid early cleavage. Taken together, the subcellular localization of γ-tubulin suggests that this protein plays an important role in microtubule nucleation and meiotic or mitotic spindle formation in mouse oocytes, fertilized eggs, and early embryos.

Ackenowledgements

References

1. **Brinkley BR**. Microtubule organizing centers. *Annu Rev Cell Biol* 1985; 1: 145–172.
2. **Calarco-Gillam PD, Siebert MC, Hubble R, Mitchison T, Kirschner M**. Centrosome development in early mouse embryos as defined by an auto-antibody against pericentriolar material. *Cell* 1983; 35: 621–629.
3. **Maro B, Houliston E, Webb M**. Non-spindle microtubule organizing centers in metaphase II-arrested mouse oocytes. *J Cell Biol* 1985; 101: 1665–1672.
4. **Oakley CE, Oakley BR**. Identification of γ-tubulin, a new member of the tubulin superfamily encoded by *mip A* gene of *Aspergillus nidulans*. *Nature* 1989; 338: 662–664.
5. **Oakley BR**. γ-Tubulin. *Curr Top Dev Biol* 2000; 49: 27–54.
6. **Oakley BR, Oakley CE, Yoon YS, Jung MK**. Gamma-tubulin is a component of the spindle pole body that is essential for microtubule function in *Aspergillus nidulans*. *Cell* 1990; 61: 1289–1301.
7. **Martin MA, Osmani SA, Oakley BR**. The role of γ-tubulin in mitotic spindle formation and cell cycle progression in *Aspergillus nidulans*. *J Cell Sci* 1997; 110: 623–633.
8. **Horio T, Uzawa S, Jung MK, Oakley BR, Tanaka K, Yanagida M**. The fission yeast gamma-tubulin is essential for mitosis and is localized at microtubule organizing centers. *J Cell Sci* 1991; 99: 693–700.
9. **Zheng YX, Jung MK, Oakley BR**. Gamma-tubulin is present in *Drosophila melanogaster* and *Homo sapiens* and is associated with the centrosome. *Cell* 1991; 65: 817–823.
10. **Oakley BR**. An abundance of tubulins. *Tren Cell Biol* 2000; 10: 537–542.
11. **Zheng Y, Wong ML, Alberts B, Mitchison T**. Nucleation of microtubule assembly by a γ-tubulin-containing ring complex. *Nature* 1995; 378: 578–583.

12. **Wiese C, Zheng YN**. γ-Tubulin complexes and their interaction with microtubule-organizing centers. *Curr Opin Stract Biol* 1999; 9: 250–259.

13. **Wiese C, Zheng YX**. A new function for the γ-tubulin ring complex as a microtubule minus-end cap. *Nature Cell Biol* 2000; 2: 358–364.

14. **Paluh JL, Nogales E, Oakley BR, McDonald K, Pidoux AL, Cande WZ**. A mutation in γ-tubulin alters microtubule dynamics and organization and is synthetically lethal with the kinesin-like protein Pkl1p. *Mol Biol Cell* 2000; 11: 1225–1239.

15. **Sampaio P, Rebollo E, Varmark H, Sunkel CE, Gonzalez C**. Organized microtubule arrays in γ-tubulin-depleted Drosophila spermatocytes. *Curr Biol* 2002; 11: 1788–1793.

16. **Vogel J, Drapkin B, Oomen J, Beach D, Bloom K, Snyder M**. Phosphorylation of gamma-tubulin regulates microtubule organization in budding yeast. *Dev Cell* 2001; 1: 621–631.

17. **Vogel J, Snyder M**. The carboxy terminus of Tub4p is required for γ-tubulin function in budding yeast. *J Cell Sci* 2000; 113: 3871–3882.

18. **Hendrickson T, Yao J, Bhadury S, Corbett A, Joshi H**. Conditional mutations in γ-tubulin reveal its involvement in chromosome segregation and cytokinesis. *Mol Biol Cell* 2001; 12: 2469–2481.

19. **Vardy L, Fujita A, Toda T**. The γ-tubulin complex protein Alp4 provides a link between the metaphase checkpoint and cytokinesis in fission yeast. *Genes Cell* 2002; 7: 365–373.

20. **Gueth-Hallonet C, Antony C, Aghion J, Santa-Maria A, Lajoie-Mazenc I, Wright M, Maro B**. γ-Tubulin is present in acentriolar MTOCs during early mouse development. *J Cell Sci* 1993; 105: 157–166.

21. **Palacios MJ, Joshi HC, Simerly C, Schatten G**. γ-Tubulin reorganization during mouse fertilization and early development. *J Cell Sci* 1993; 104: 383–389.

22. **Lee J, Miyano T, Moor RM**. Spindle formation and dynamics of γ-tubulin and nuclear mitotic apparatus protein distribution during meiosis in pig and mouse oocytes. *Biol Reprod* 2000; 62: 1184–1192.

23. **Gard DL, Cha BJ, Schroeder MM**. Confocal immunofluorescence microscopy of microtubules, microtubule-associated proteins, and microtubule-organizing centers during amphibian oogenesis and early development. *Curr Top Dev Biol* 1995; 31: 383–431.

24. **Matthies HJG, Mcdonald HB, Goldstein LSB, Theurkauf WE**. An-astral meiotic spindle morphogenesis: role of the non-claret disjunctional kinesin-like protein. *J Cell Biol* 1996; 134: 455–464.

25. **Shin MR, Kim NH**. Maternal gamma (γ) -tubulin is involved in microtubule reorganization during bovine fertilization and parthenogenesis. *Mol Reprod Dev* 2003; 64: 438–445.

26. **Calarco PG**. Centrosome precursors in the acentriolar mouse oocyte. *Microsc Res Tech* 2000; 49: 428–434.

27. **Combelles CM, Albertini DF**. Microtubule patterning during meiotic maturation in mouse oocytes is determined by cell cycle-specific sorting and redistribution of gamma-tubulin. *Dev Biol* 2001; 239: 281–294.

28. **Tournebize R, Heald R, Hyman A**. Role of chromosomes in assembly of meiotic and mitotic spindles. *Prog Cell Cycle Res* 1997; 3: 271–284.

29. **Sun QY, Lai L, Wu GM, Park KW, Day BN, Prather RS, Schatten H**. Microtubule assembly after treatment of pig oocytes with taxol: correlation with chromosomes, gamma-tubulin, and MAP kinase. *Mol Reprod Dev* 2001; 60: 481–490.

30. **Prigozhina NL, Walker RA, Oakley CE, Oakley BR**. γ-Tubulin and the C-terminal motor domain kinesin-like protein, KlpA, function in the establishment of spindle bipolarity in *Aspergillus nidulans*. *Mol Biol Cell* 2001; 12: 3161–3174.

31. **Suzuki H, Takashima Y, Toyokawa K**. Cytoskeletal organization of porcine oocytes aged and activated electrically or by sperm. *J Reprod Dev* 2002; 48: 293–301.

32. **Simerly C, Zoran SS, Payne C, Dominko T, Sutovsky P, Navara CS, Salisbury JL, Schatten G**. Biparental inheritance of γ-tubulin during human fertilization: molecular reconstitution of functional zygotic centrosomes in inseminated human oocytes and in cell-free extracts nucleated by human sperm. *Mol Biol Cell* 1999; 10: 2955–2969.