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γ-Tubulin reorganization during mouse fertilization and early development

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SUMMARY

γ-Tubulin, a component of spindle pole bodies in fungal cells and pericentriolar material in vertebrate cells , is thought to play a role in the nucleation of microtubule growth and to define their polarity. In contrast to the adult somatic cells, microtubules are nucleated in the absence of centrioles in mammalian oocytes and early embryos. By studying acentriolar mouse oocytes and their early development following fertilization, we show that γ-tubulin antibody crossreacts with a 50,000 M_r protein in unfertilized mouse oocytes and demonstrate that γ-tubulin distribution is rearranged dramatically during fertilization. In unfertilized mouse oocytes, γ-tubulin is concentrated in the broad spindle poles of meiotic spindle (MII) and as the distinct foci which form the centers of the cytoplasmic microtubule asters (cytasters). The integrity of these γ-tubulin foci and their cytoplasmic location is maintained during the drug- or cold-induced depolymerization of microtubules. γ-Tubulin is also found in the basal body of the mouse sperm. During fertilization, the γ-tubulin is found at the cytastral centers as well as in the incorporated sperm basal body complex, and the γ-tubulin foci coalesce at the perinuclear microtubule organizing regions of the two pronuclei at the first mitotic prophase. During mitosis, γ-tubulin is found associated with broad bands that form the poles of the first mitotic spindle. By the late preimplantation stage, when newly generated centrioles have been reported to arise, γ-tubulin remains localized at the centrosome of mitotic cells. The presence of γ-tubulin in all microtubule organizing structures provides support for its proposed role as a component of microtubule organizing centers during the complex choreography of all microtubule assemblies.

Key words: γ-tubulin, fertilization, oocytes

INTRODUCTION

Microtubule arrays are organized by the centrosome (reviewed by Mazia, 1984, 1987; Kalnins, 1992), a perinuclear microtubule organizing center (MTOC) from which microtubules emanate. During cell division, the duplicated centrosomes move apart and serve as poles of the microtubule spindle, ensuring the correct segregation of the duplicated chromosomes. The meiotic spindles of mammalian oocytes, which lack centrioles, contain at their poles a band of electron-dense material from which the spindle microtubules emanate (Szollosi et al., 1972; Calarco-Gillam et al., 1983). In addition, several discrete centriole-less cytoplasmic microtubule assemblies, called cytasters, play an important role in the orchestration of further development (Maro et al., 1985; Schatten et al., 1986). The fertilization of mouse oocytes is followed by a complex array of microtubule reorganization leading to the migration of the two parental pronuclei to the center of the newly fertilized embryo, where the first mitotic spindle assembles (Schatten et al., 1985). More than seven subsequent cell divisions occur in the absence of the centrioles, reported to appear in blastocysts (Magnuson and Epstein, 1984). Therefore, early mouse embryos offer an opportunity to elucidate the mechanisms of microtubule nucleation and spindle morphogenesis in the acentriolar centrosome.

Studies of microtubule assembly *in vitro* reveal that although the assembly of purified tubulin α-β dimers can occur *de novo*, it is preferred to occur from the nucleating structures (for example, see review by Kirschner and Mitchison, 1986). In cultured fibroblasts, microtubule assembly is preferred to occur from the centrosomal sites and the free assembly of microtubules is suppressed in the presence of centrosomes (for review see Brinkley, 1985). Nucleation from these structures also results in microtubule arrays of uniform polarity orientation (Heidemann et al. 1980; Euteneuer and McIntosh, 1981). Further, the microtubules assembled *in vitro* have a variable protofilament lattice in sharp contrast to the predominantly 13-protofilament lattice structure of each microtubule within the centrosomal microtubule arrays (Evans et al., 1985; Scheele et al., 1982; Chretien et al., 1992). These observations point to a unified mechanism of *in vivo* microtubule assembly in both the centriolar centrosomes and the acentriolar centrosomes.

γ-Tubulin, a recently described protein (Oakley and Oakley, 1989), is shown to be associated with microtubule nucleating structures such as the spindle pole bodies in fungal cells (Oakley et al., 1990; Stearns et al., 1991; Horio et al., 1991) and to be in the centriolar centrosomes of vertebrate cells (Zheng et al., 1991; Stearns et al., 1991). We have recently reported that microinjection of a γ-tubulin antibody into cultured mammalian cells blocks the assembly of the mitotic spindle from the centrosomal spindle poles, implicating γ-tubulin function in the nucleation of microtubules from these structures (Joshi et al., 1992). In light of all the available data, it appears that γ-tubulin may serve as the key component of all the microtubule nucleating structures independently of their association with the centrioles.

To begin to test this hypothesis, we investigated the presence and distribution of γ-tubulin during early mouse embryogenesis, which occurs in the absence of the centrioles. We demonstrate the presence of a M_r 50,000 polypeptide in mouse oocytes recognized by γ-tubulin antibody and show the reorganization of γ-tubulin antigen during oocyte maturation and early embryogenesis. During oocyte maturation, γ-tubulin is found assembled at the meiotic spindle poles as well as in distinct foci at the centers of the cytoplasmic microtubule asters. Following fertilization, the γ-tubulin foci coalesce at the perinuclear microtubule organizing regions of the two pronuclei prior to the first mitotic division. And finally, γ-tubulin associates with the broad bands at the poles of the first mitotic spindle. During further cell divisions, γ-tubulin appears progressively focussed at the poles of subsequent mitotic spindles and becomes restricted to the newly generated centrioles within the cells of the trophoblast outgrowth. Our demonstration that γ-tubulin is a component of the centriolar sperm basal bodies and the acentriolar MTOCs of the embryos supports its proposed role in microtubule nucleation in all eukaryotic cells.

MATERIALS AND METHODS

Oocytes, zygotes and embryos were obtained from ICR mice (Sprague-Dawley, Indianapolis) following the procedures described by Simerly et al. (1990). γ-Tubulin was localized using a rabbit antibody prepared against a synthetic 17 amino acid oligopeptide sequence known to be conserved among all γ-tubulins examined (Joshi et al., 1992). Microtubule patterns were observed using a monoclonal antibody to β-tubulin (E-7; Chu and Klymkowsky, 1989) and DNA was detected with 2.5 μg/ml DAPI. The γ-tubulin antibody was microinjected into the oocytes and zygotes, as described by Simerly et al. (1990), and these cells as well as the embryos were processed for immunofluorescence localization of microtubules following the methods of Schatten et al. (1985). The pre-immune rabbit serum did not detect centrosomes in mouse oocytes or sperm. Uninjected or sham injected oocytes used as controls verified that the β-tubulin signal did not bleed through the γ-tubulin channel to artifactually affect the observations.

Microtubules were depolymerized in unfertilized oocytes using either 10 μM nocodazole or cold treatment (0˚C for 90 minutes). To trace recovery, the oocytes were transferred to 37˚C culture medium and fixed 2.5 minutes post-warming (Schatten et al., 1988).

For immunoblotting, 1000 oocytes were harvested from ICR mice as described previously (Schatten et al., 1985). These oocytes were boiled for 10 minutes in 15 μl of SDS-Laemmli buffer (Laemmli, 1970). The entire protein sample was analyzed by immunoblotting using γ-tubulin IgG (40 μg/ml) and antibody binding was detected by the Western-Light chemiluminescence procedure (Tropix; Bedford, MA) as previously described (Joshi et al., 1992). After obtaining the autoradiograph, bound antibodies were subsequently removed from the nitrocellulose membrane by incubating in 2% SDS and 100 mM β-mercaptoethanol in TBS (3 mM potassium chloride, 140 mM sodium chloride, 25 mM Tris-HCl, pH 7.4) at 70˚C for 30 minutes. The protein blot was then reprobed with preimmune IgG (40 μg/ml) and processed for detection with the Western-Light protocol as previously described (Joshi et al., 1992).

RESULTS

Detection and distribution of γ-tubulin in unfertilized oocytes

The γ-tubulin antibody recognizes a single polypeptide of M_r 50,000 in mouse oocytes (Fig. 1, lane 1) as well as γ-tubulin in HeLa cytoskeletal extracts (Fig. 1, lane 2; Joshi et al., 1992). The immunoblot of the mouse oocytes did not recognize the preimmune antibody (Fig. 1, lane 3) when destained and reprobed with 40 μg/mlof rabbit preimmune IgG antisera.

Our next goal was to test if γ-tubulin was present in the microtubule organizing centers that are not associated with morphological centrioles. To do this, we determined the γ-

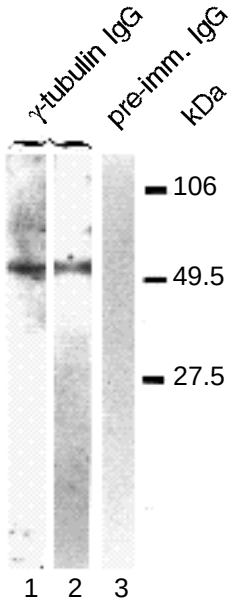

Fig. 1. γ-Tubulin crossreacts with a 50,000 M_r protein in mouse oocytes. Lane 1, immunoblot transfer of 1000 mouse metaphase-II-arrested oocytes run on a 12.5% SDS-PAGE gel and probed with purified IgG against γ-tubulin. Lane 2, HeLa cell extract from 10^5 to 2×10^5 cells run simultaneously with the mouse oocytes. Lane 3, mouse immunoblot stripped with 2% SDS, 100 mM mercaptoethanol and reprobed with the rabbit preimmune IgG antisera.

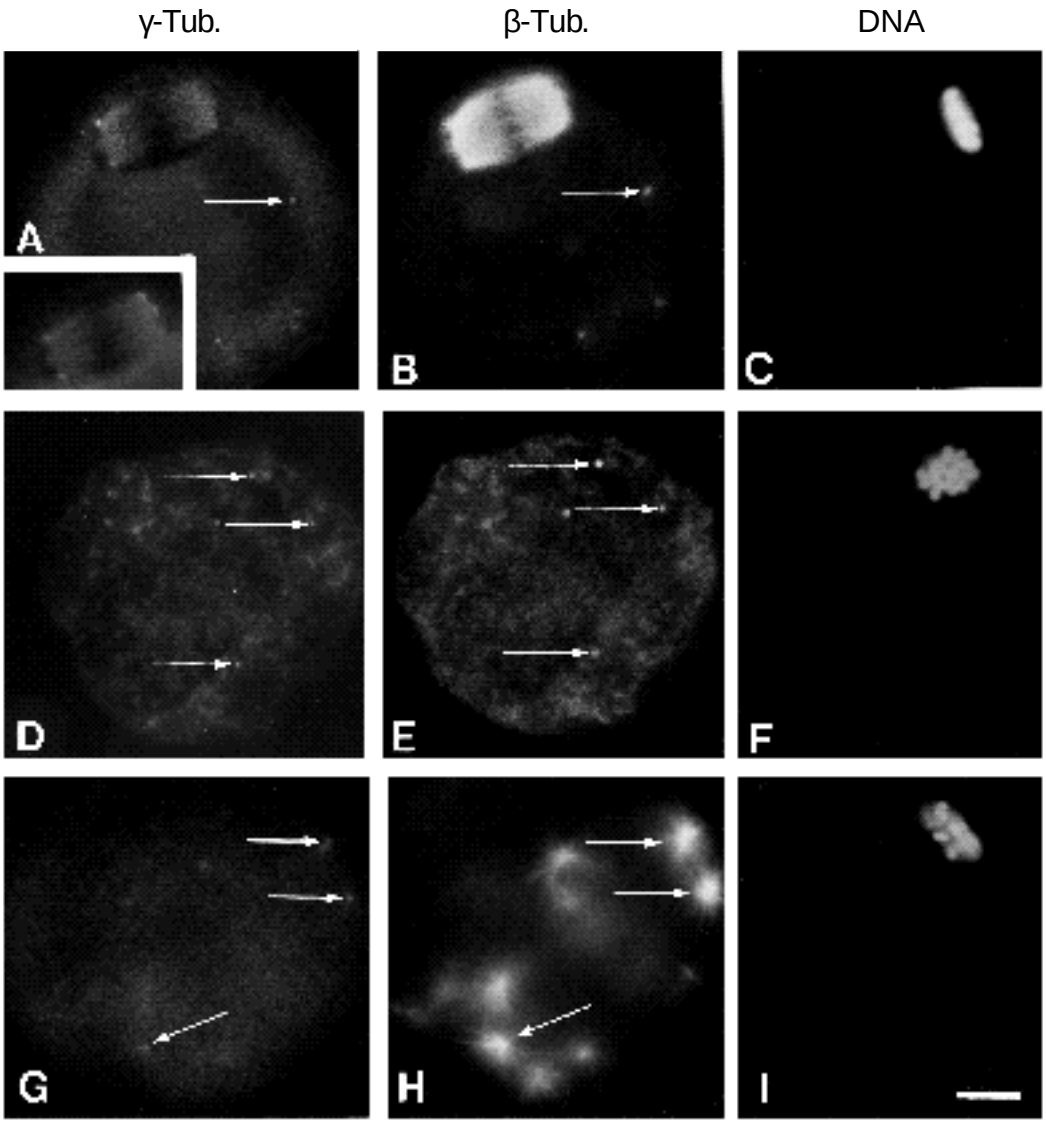

Fig. 2. γ-Tubulin in the centrosomes of unfertilized mouse oocytes. (A-C) Unfertilized oocyte. γ-Tubulin is localized exclusively at the centrosomal foci found at the poles of the metaphase-II-arrested meiotic spindle and at the center of cytoplasmic asters (arrow in A). The microtubule pattern is shown in B and the chromosomes aligned on the metaphase plate in C. (D-F) Microtubule disassembly induced by cold depolymerization (0˚C for 90 minutes). Inset: second meiotic spindle at a different focal plan showing γ-tubulin at spindle poles. γ-Tubulin remains associated with the centrosomes after the microtubules are disrupted with cold (D-F) or nocodazole treatment (data not shown). (G-I) Microtubule recovery from cold treatment. γ-Tubulin remains at the centrosomal foci in the spindle poles and cytastral centers after microtubule reassembly. A, D, G, γ-tubulin localization; B, E. H, microtubules; C, F, I, DNA detection. Bar, 10 μm.

tubulin distribution in unfertilized mouse oocytes, which contain two types of acentriolar MTOCs: the meiotic spindle poles and the cytastral centers. As shown in Fig. 2, γ-tubulin is associated with both of these structures. γ-Tubulin foci appeared most concentrated at the poles of the metaphase-II-arrested meiotic spindles and at the cytastral centers in unfertilized mouse oocytes (Fig. 2A). To determine the relationship of the γ-tubulin foci to the microtubule arrays, we visualized microtubules in the same cells using a monoclonal antibody to β-tubulin. As shown in Fig. 2B, the stained γ-tubulin foci corresponded to the centrosomal regions, from which microtubules emanate. The meiotic chromosomes in the metaphase-arrested mature oocyte as detected at the spindle equator are shown in Fig. 2C. We conclude that γ-tubulin is associated with the acentriolar microtubule organizing centers of unfertilized mouse oocytes.

Integrity of γ-tubulin foci after microtubule disassembly

Two alternative mechanisms can account for the focal localization of γ-tubulin at the microtubule organizing centers. γ-Tubulin may be attached at the minus ends of microtubules and the stained γ-tubulin foci may represent high concentration of the minus ends of microtubules with their attached γ-tubulin. Alternatively, γ-tubulin may be an obligatory component of the microtubule organizing centers independent of their attached microtubules. To distinguish

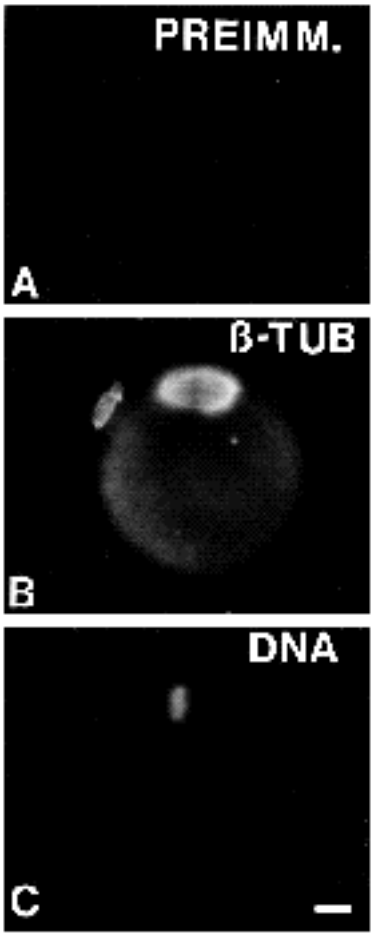

Fig. 3. Rabbit preimmune antibody staining in unfertilized mouse oocytes. Rabbit preimmune antibody (A) microinjected into the metaphase-II-arrested mouse oocyte (C) does not detect centrosomes at the meiotic poles or in the cytoplasm. (B) Total microtubule pattern detected with β-tubulin antibody. Bar, 10 μm.

between these possibilities, we investigated whether γ-tubulin remains associated with the spindle poles and as cytoplasmic foci after the microtubules have been disassembled by cold (0˚C for 90 minutes; Fig. 2D-F) or 10 μM nocodazole (data not shown). The foci detected in Fig. 2D with the γ-tubulin antibody are found on opposing sides of the chromosomes (Fig. 2F) and as punctate sources throughout the cytoplasm, representing the cytastral foci.

To ensure that the observed γ-tubulin foci represent cytoplasmic locations where microtubule growth is initiated, we followed microtubule reassembly in the meiotic spindle and the cytasters following drug removal (or return to 37˚C for 2.5 minutes) from the cold disassembly treatment. As shown in Fig. 2H, microtubule growth initiates at the γ-tubulin foci detected at the spindle poles and cytastral centers (Fig. 2G).

Pre-immune rabbit serum (4 mg/ml[1]) microinjected into unfertilized oocytes arrested at metaphase II is shown in Fig. 3. To compare the fluorescence intensity of the microinjected preimmune antibody with that of γ-tubulin, uniform negative and printing exposure times were

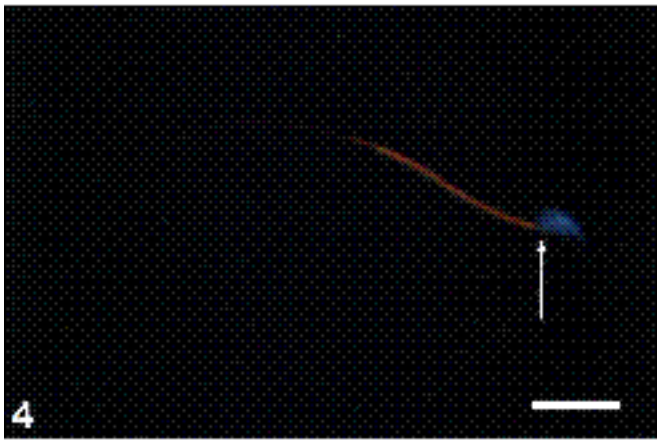

Fig. 4. γ-Tubulin in sperm. γ-Tubulin (green: arrow) is found at the basal body complex in mouse sperm. The DNA is detected in the sperm nucleus (blue) and the microtubules of the sperm tail are localized with a monoclonal antibody to β-tubulin (red). Bar, 10 μm.

employed. The preimmune serum (Fig. 3A) did not detect centrosomes or microtubules at the meiotic spindle poles or in the cytoplasmic asters when compared with the staining patterns of microtubules recognized by β-tubulin antibody (Fig. 3B).

Our next goal was to follow γ-tubulin distribution during fertilization and pre-implantation development of mouse embryos. To do this, we first determined if the sperm basal body complex also contained γ-tubulin. As shown in Fig. 4, γ-tubulin was detectable at the basal body complex (Fig. 4: green focus denoted by arrow) at the base of the sperm tail (Fig. 4: red microtubule detection) and the sperm nucleus (Fig. 4: blue DNA detection). Curiously, the sperm midpiece is stained non-specifically by the rabbit preimmune and other non-immune sera, and may not represent γ-tubulin localization.

γ-Tubulin in zygotes and embryos

γ-Tubulin is detected in zygotes at the centrosomes. In Fig. 5A, it is found at the cytastral centers (arrow) and at the perinuclear rims from which microtubules extend (Fig. 5B); this cell is at the completion of first interphase (Fig. 5C). At first mitotic prometaphase (Fig. 5F), γ-tubulin is detected at the spindle poles and also at the center of a cytaster (Fig. 5G). At metaphase (Fig. 5I), microtubules are found in the barrel-shaped anastral mitotic spindle (Fig. 5H) and as remnants of the incorporated sperm tail (Fig. 5H: inset at a different focal plane). Interestingly, γ-tubulin is detected at the spindle poles, which possess centrosomal activity, but also at the incorporated sperm basal body complex, which does not nucleate microtubules in the egg in this species.

γ-Tubulin in blastocysts

In Fig. 5K, the microtubules of the fusiform mitotic spindle in a trophectoderm cells are shown: γ-tubulin is found as punctate sources at the spindle poles in trophectoderm cells (Fig. 5J: arrows) and in cells from the inner cell mass (data not shown). Centrioles have been reported to appear

by the blastocyst stage in mouse embryos (Magnuson and Epstein, 1984), in contrast to earlier stages, and γ-tubulin can be localized by antibody labeling of fixed cells rather than requiring microinjections. This indicates that the γ-tubulin concentration found exclusively at the mitotic and interphase centrosomes is at a concentration that is either sufficient to exceed detection threshold or is more accessible at this late stage.

DISCUSSION

Centrosomes, the ubiquitous microtubule organizing centers in eukaryotic cells, are required for spindle formation during meiosis and mitosis, the orientation of cleavage axes during development, determining the direction for locomotion, as well as cell shape changes during morphogenesis and differentiation (reviewed by Mazia, 1984, 1987; and

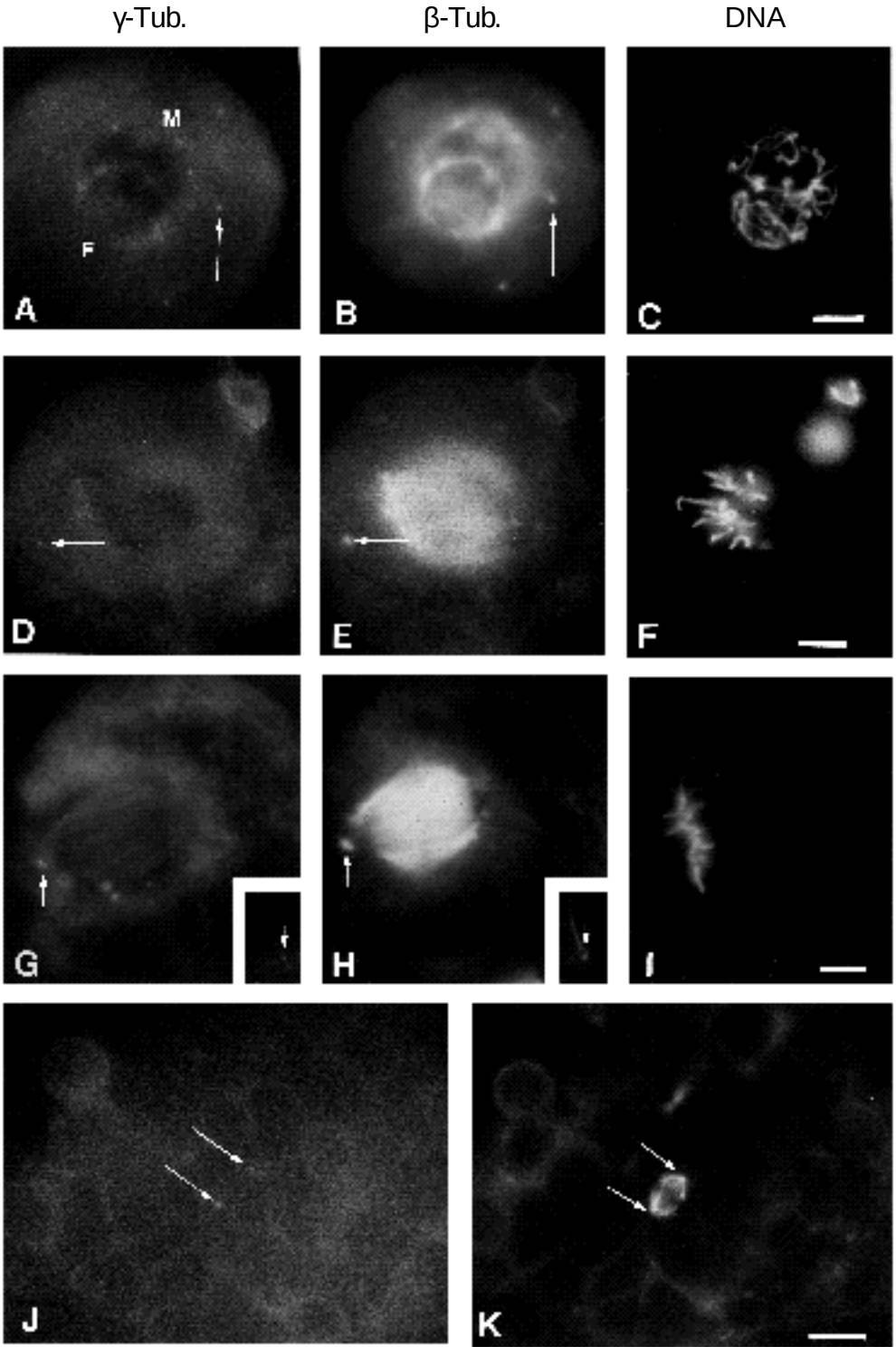

Fig. 5. γ-Tubulin in zygotes and embryos. (A-C) Fertilized oocyte towards completion of first interphase. γ-Tubulin is found predominantly at the centrosomal foci (A, arrow), from which the microtubules (B) extend. Microtubules (B) are detected in the cytasters (arrow) as well as shells surrounding the apposed male (M, in A) and female pronuclei (F, in A). The chromosomes are condensing in preparation for first mitosis (C). (D-F) First mitotic prometaphase. γ-Tubulin is detected as punctate foci at the two mitotic spindle poles (D) and at the cytastral center (arrow). The chromosomes (F) are in the process of alignment on the spindle. (G-I) First mitotic metaphase. γ-Tubulin is found at the blunt spindle poles (G, arrow), and the microtubule pattern (H) reveals the barrel-shaped anastral spindle. The incorporated sperm tail is found at a different focal plane (H, inset), and γ-tubulin is detected at the basal body complex (G, inset, arrow), which does not nucleate microtubules in the zygote. (J-K) γ-Tubulin in trophoblasts. γ-Tubulin is found exclusively at the spindle poles of trophectoderm (J) and inner cell mass cell (data not shown). The fusiform mitotic spindle is shown in K. B, E, H, K, microtubules; C, F, I, DNA detection. Bar, 10 μm.

Kalnins, 1992). The composition of the centrosome is not well understood, and in most, but not all, animals they contain centrioles. The role of centrioles in spindle morphogenesis is not clear because many cell types can divide in the absence of morphological centrioles, such as mouse oocytes and early embryos. Unfertilized mouse oocytes lack centrioles (Szollosi et al., 1972) and have pericentriole-like material at the poles of the metaphase-arrested meiotic II spindle and at the center of a dozen or so cytoplasmic asters (Calarco-Gillam et al., 1983; Maro et al., 1985; Schatten et al., 1986). The sperm introduces a basal body at fertilization (Stefanini et al. 1969; Schatten et al., 1985) but it does not serve as an MTOC within this egg. The first mitoses occur in the absence of centrioles, which appear during embryogenesis in the polar trophoblast cells and in the cells of inner cell mass later during development (Magnuson and Epstein, 1984). Mouse fertilization and early development thus offer a remarkable system in which to explore molecular components that participate in microtubule nucleation and organization. Therefore, we explored the distribution of γ-tubulin, a protein proposed to play a role in microtubule nucleation, in the mouse oocytes and in early mouse embryonic cells. Taken together, our results show that γ-tubulin is a component of all types of MTOCs observed in mouse gametes and early embryos. These MTOCs include the meiotic spindle poles, centers of cytasters in the oocytes, the sperm basal body, acentriolar poles of the early mitotic spindles, and the centriole-containing centrosome of the trophoblast cells of the embryo. Although γ-tubulin has been shown to be localized to the pericentriolar material of vertebrate cells (Stearns et al.,1991), our results demonstrate its presence in known acentriolar MTOCs and, hence, provide evidence against the possibility of adventitious association with centrioles.

γ-Tubulin was first implicated in the nucleation of microtubules by a combination of genetic and immunocytochemical observations (Oakley and Oakley, 1991; Zheng et al., 1991; Stearns et al., 1991). Null mutations in the *mipA* gene, which encodes γ-tubulin in *Aspergillus*, lead to a microtubule-less mitotic arrest, and immunofluorescence localization detected γ-tubulin in the fungal spindle pole bodies (Oakley and Oakley, 1991), and at the centrosomes of vertebrate cells. Further, an antibody generated to a conserved domain of γ-tubulin interfered with the formation of the mitotic spindles in mammalian cells (Joshi et al., 1992). All these data point to the requirement of functional γ-tubulin for in vivo microtubule nucleation. Our data demonstrating γ-tubulin as a component of all MTOCs during mouse development strongly support a key role for γ-tubulin in spindle morphogenesis.

One intriguing feature of our data is the presence of γ-tubulin immunoreactivity in the sperm basal body, which does not serve as an MTOC in the zygote. It is conceivable that γ-tubulin in sperm basal body represents a modified form of γ-tubulin that does not allow microtubule growth. Recently, Masuda et al. (1992) have demonstrated that spindle pole bodies from fission yeast contain γ-tubulin throughout the cell cycle, but these organelles nucleate microtubules only during mitosis. Further, interphase spindle pole bodies can be activated *in vitro* to nucleate microtubules by (CSF)-arrested unfertilized *Xenopus* eggs extract.

In conclusion, this investigation demonstrates the presence of γ-tubulin at the centrosomes in eggs and embryos and in the sperm's basal body. γ-Tubulin appears to be a permanent component of intracellular structures which have had or still possess microtubule organizing capabilities.

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REFERENCES

Alfa, C.A. and Hyams, J.S. (1991). ABG of microtubule assembly (news and views). *Nature* **352,** 471.

Brinkley, B.R. (1985). Microtubule organizing centers. *Annu. Rev. Cell Biol.* **1,** 145-172.

Calarco-Gillam, P.D., Siebert, M.C., Hubble, R., Mitchison, T. and Kirschner, M. (1983). Centrosome development in early mouse embryos as defined by an autoantibody against pericentriolar material. *J. Cell Biol.* **35,** 621-629.

Chretien, D., Metoz, F., Verde, F., Karsenti, E. and Wade, R.H. (1992). Lattice defects in microtubules: Protofilament numbers vary within individual microtubules. *J. Cell Biol.* **117,** 1031-1040.

Chu, D. Klymkowsky, M. (1987). Experimental analysis of cytoskeletal function in early *Xenopus laevis* embryos. *First Int. Symp. Cytoskeletal Dev*, vol. 8, pp. 331-333. ISG Press.

Euteneuer, U. and McIntosh, J.R. (1981). Polarity of some motility-related microtubules. *Proc. Nat. Acad. Sci. USA* **78,** 372-376.

Evans, L., Mitchison, T. and Kirschner, M. (1985). Influence of the centrosome on the structure of nucleated microtubules. *J. Cell Biol.* **100,** 1185-1191.

Haimo, L.T. (1985). Microtubule polarity in taxol-treated isolated spindles. *Can. J. Biochem. Cell Biol.* **63,** 519-532.

Heidemann, S.R., Zieve, G.W. and McIntosh, J.R. (1980). Evidence for microtubule subunit addition to the distal end of mitotic structures *in vitro*. *J. Cell Biol.* **87,** 152-159.

Horio, T., Uzawa, S., Jung, M.K., Oakley, B.R., Tanaka, K. and Yanagida, M. (1991). The fission yeast γ-tubulin is essential for mitosis and is localized at microtubule organizing centers. *J. Cell Sci.* **99,** 693-700.

Joshi, H.C. and Cleveland, D.W. (1989). Differential utilization of β-tubulin isotypes in differentiating neurites. *J. Cell Biol.* **109,** 663-673.

Joshi, H. C., Palacios, M. J., McNamara, L. and Cleveland, D.W. (1992). Gamma Tubulin is a centrosomal protein required for cell cycle-dependent microtubule nucleation. *Nature* **356,** 80-83.

Kalnins, V.I. (1992). *The Centrosome*. Academic Press, San Diago, California.

Kirschner, M.W. and Mitchison, T.J. (1986). Beyond self-assembly from microtubules to morphogenesis. *Cell* **45,** 329-342.

Laemmli, U.K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* **227,** 680-685.

Magnuson, T. and Epstein, C.J. (1984). Olygosyndactyly: A lethal mutation in the mouse that results in mitotic arrest very early in development. *Cell* **38,** 823-833.

Maro, B., Howle, S.K. and Webb, M. (1985). Non-spindle MTOC's in metaphase II-arrested mouse oocytes. *J. Cell Biol.* **101,** 1665-1672.

Masuda, H., Sevic, M. and Cande, W.Z. (1992). *In vitro* microtubule-nucleating activity of spindlepole bodies in fission yeast Schizosaccharomyces pombe: Cell cycle-dependent activation in Xenopus cell-free extracts. *J. Cell Biol.* **117,** 1055-1066.

Mazia, D. (1984). Centrosomes and mitotic poles. *Exp. Cell Res.* **153,** 1-15.

Mazia, D. (1987). The chromosome cycle and the centrosome cycle in the mitotic poles. *Int. Res. Cytol.* **100,** 49-92.

Oakley, B.R. (1992). γ-Tubulin: the microtubule organizer. *Trends Cell Biol.* **2,** 1-5.

Oakley, C.E. and Oakley, B.R. (1989). Identification of γ-tubulin, a new member of the tubulin superfamily encoded by mipA gene of *Aspergillus nidulans*. *Nature* **338,** 662-664.

Oakley, B.R., Oakley, C.E., Yoon, Y. and Jung, M.K. (1990). γ-tubulin is a component of the spindle pole body that is essential for microtubule function in Aspergillus nidulans. *Cell* **61,** 1289-1301.

Schatten, H., Schatten, G., Mazia, D., Balczon, R. and Simerly, C. (1986). Behavior of centrosomes during fertilization and cell division in mouse oocytes and in sea urchin eggs. *Proc.Nat. Acad. Sci. USA.* **83,** 105-109.

Schatten, G., Simerly, C., Asai, D.J., Szöke, E., Cooke, P. and Schatten, H. (1988). Acetylated α-tubulin in microtubules during mouse fertilization and early development. *Dev. Biol.* **130,** 74-86.

Schatten, G., Simerly, C. and Schatten, H. (1985). Microtubule configurations during fertilization, mitosis, and early development in the mouse and the requirement for egg microtubulemediated motility during mammalian fertilization. *Proc. Nat. Acad. Sci. USA* **82,** 4152-4156.

Scheele, R.B., Bergen, L.G. and Borisy, G.G. (1982). Control of the structural fidelity of microtubules by initiation sites. *J. Mol. Biol.* **154,** 785-500.

Simerly, C., Balczon, R., Brinkley, B.R. and Schatten, G. (1990). Microinjected kinetochore antibodies interfere with chromosome movement and mitotic mouse oocytes. *J. Cell Biol.* **111,** 1491-1504.

Stearns, T., Evans, L. and Kirschner, M. (1991). Gamma tubulin is a highly conserved component of the centrosome. *Cell* **65,** 825-836.

Stefanini, M., Ouna, C. and Zamboni, L. (1969). Ultrastructure of fertilization in the mouse. II. Penetration of the sperm into the ovum. *J. Submicrosc. Cytol.* **1,** 1-23.

Szollosi, D. (1972). Changes of Some Organelles During Oogenesis in Mammals. (ed. J.D. Biggers and A. W. Schultz) pp. 47-64. Baltimore, Maryland University Park Press.

Szollosi, D., Calarco, P. and Donahue, R.P. (1972). Absence of centrioles in the first and second meiotic spindles of mouse oocytes. *J. Cell Sci.* **11,** 521-541.

Zheng, Y., Jung, M.K. and Oakley, B.R. (1991). Gamma tubulin is present in *Drosophilamelanogaster* and *Homosapiens* and is associated with the centrosome. *Cell* **65,** 817-823.

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