Cytoskeletal alteration of mammalian oocytes during meiotic maturation, fertilization and parthenogenesis

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Microtubules and microfilaments are major cytoskeletal components in mammalian ova that provide the framework for chromosomal movement and cellular division. Extensive changes of cytoskeletal organization occurs during maturation and fertilization. The changes in cytoskeletons are essential for the normal meiotic maturation and for the formation of the biparental diploid genome of the embryo, and thus are repeated at each cell cycle during embryonic development. Disturbance of the cytoskeletal organization could result in abnormal gamete development and early embryonic death.

Maturation.

During meiotic maturation in mammalian oocytes, considerable chromosomal and cytoplasmic changes occur including germinal vesicle breakdown (GVBD), chromosomal condensation, polar body extrusion, and the formation of the meiotic spindle. These structural changes are associated with changes in the organization of microtubules and microfilaments during specific phases of the cell cycle. Microtubules, homologous polymers of α-, β-, and γ-tubulin, are dynamic and intrinsically polar filaments. The organization of microtubules is controlled by centrosomes located at the spindle poles and at kinetochores on chromosomes(Le Guen and Crozet, 1989) In the mouse, cell cycle transition after GVBD is accompanied by extensive reorganization of the microtubule network (Kubiak et al., 1992, Verlhac et al., 1994). However, the change in microtubule assembling during meiosis of mouse oocytes may not be typical of that of other mammalian species. Unfertilized mouse oocytes contain numerous centrosomal foci in addition to spindle associated centrosomes. After fertilization the centrosomal foci are attracted to the surfaces of both the male and female pronuclei and are involved in pronuclear movement and in the formation of the mitotic spindle (Schatten et al., 1985; Maro et al., 1985). In contrast, in most mammals functional microtubules appear to be developed from the centrosome introduced by the sperm (Yllera-Frandez et al., 1992, Le Guen and Crozet, 1989; Long et al., 1993, Breed et al., 1994). Although cytoplasmic microtubules are not observed in most mammalian oocytes, treatment with taxol, a drug that nucleate microtubules in rabbit (Yllera-Ferandez et al., 1992) and sheep oocytes (Le Guen and Crozet, 1989). They suggested that some mammalian oocytes possess centrosomal (or microtubule organization center, MTOC) material scattered in the cytoplasm. The distribution of microfilaments has also been studied in mammalian oocytes. In matured mouse (Maro et al., 1984) and rat (Zernickka-Goetz et al., 1993) oocytes, microfilaments are located mainly in the cell cortex overlying the meiotic spindle. This domain rich in

microfilaments seems to be responsible for the maintenance of the meiotic spindle and chromosomes in a peripheral position (Webb et al., 1986).

Very recently, Kim et al. (1995a) studied microtubule and microfilament dynamics in pig oocytes during meiotic maturation. The study has focused on the intergrated organization between cytoskeletal components and chromatin during maturation. Figure 1. summarized microtubule and microfilament reorganization during meiotic maturation. The study has demonstrated that both microtubule and microfilament dynamics are intergrated and interact with chromosomal changes during oocyte maturation. The condensed chromatin may recruit cytoplasmic material dispersed in the cytoplasm after GVBD and evoke microtubule assembly, which is necessary for meiosis and maintenance of the metaphase plate. Microfilaments are involved in chromosomal movement to a peripheral position after GVBD which may be important for continued embryonic development after fertilization.

Fertilization

Observations made during fertilization in bovine oocytes showed that an aster of microtubules is seen adjacent to the incorporated sperm head (Navara et al., 1994). This sperm aster enlarges during sperm decondensation and extends into the total cytoplasm at the time of pronuclear apposition. Movement of both pronuclei by pulling them toward the center of the cell. This observation is consistent with the findings in most animals (Schatten, 1994). In the mouse, however, the centrosome pronuclei are organized from the centrosomal foci which preexisted in the cytoplasm of the unfertilized oocyte (Maro et al., 1985). Szollosi and Hunter (1973) have studied ultrastructural aspects of fertilization in the pig by electron microscopy. They observed the appearance of clusters of electron-dense, filamentous materials in the cytoplasm and in the absence of a centriole associated with the incorporated sperm, suggesting a maternal origin of centrosomes in porcine zygotes. However, I have recently observed, unequivocally, a sperm aster immediately after sperm penetration (Kim et al., 1994). Further, multiple sperm asters form in polyspermic oocytes after in vitro fertilization providing additional evidence that the sperm centrosome organizes microtubules in the pig.

Although paternal inheritance of a functional centrosome has been suggested in most animals, it is still controversial whether the sperm itself contributes the centrosome. Since cell divisions are successful after parthenogenesis, the oocytes must contain sufficient maternal materials to organize a bipolar mitotic apparatus. This has led to the hypothesis that the sperm introduces a strong attractant for recruiting centrosomal materials stored in the oocyte (Steans and Kirschner, 1994). In a my study, treatment of unfertilized pig oocytes with taxol, a drug that promotes microtubule assembly, induced numerous microtubule foci (400-500), but taxol did not induce microtubules in fertilized eggs when treated with taxol(Kim et al., 1995e). These results suggest that the sperm aster may be produced by collecting centrosomal materials (γ-tubulin) which preexisted in the cytoplasm. Microtubule organization during fertilization in porcine oocytes is diagrammed in Figure 2A (Kim et al., 1994). After sperm penetration, centrosomal material is attracted to the sperm neck area. During pronuclear formation, the sperm aster enlarges as the decondensing male and female chromatins

move toward the center of the oocyte. After gamete union at fertilization, microtubules are less detectable in the cytoplasm. During mitotic metaphase, centrosomal material may disperse to the entire cytoplasm again where prometaphase chromatin attracts centrosomal material and forms a mitotic spindle.

The distribution of microfilaments has also been studied in mammalian ova. In mature mouse (Maro et al., 1984) and rat (Zernicka-Goetz et al., 1993) oocytes, microfilaments are located mainly in the cell cortex overlying the meiotic spindle. This domain, rich in microfilaments, seems to be responsible for the maintenance of the meiotic spindle and chromosomes in a peripheral position (Webb et al., 1986). Maro et al. (1984) found that following fertilization or parthenogenetic activation of mouse oocytes, this domain disappears and microfilaments are concentrated around the pronuclei. Recently, my study demonstrated that, in mature pig oocytes, two domains (a thick and a thin microfilament area) exist in the oocyte cortex (Kim et al.,1995b). Chromosomes were located in the thick microfilament domain of the cortex, which may be important for polar body extrusion and normal embryonic development after fertilization. The abnormalities of microfilament organization seem to be closely related with culture system during in vitro maturation (Funahashi et al., 1995) and cause abberant pattern of fertilization processes and incomplete cortical reaction after sperm penetration (Kim et al 1995c).

Parthenogenesis

In mammals, parthenogenesis is the extraordinary process in which the oocyte initiates cell division without paternal contribution. Study of parthenogenesis has contributed considerably to the understanding of many aspects of early embryonic development. Parthenogenetic activation can be induced by a variety of stimuli such as electrical shock, Ca^{++}, inositol triphosphate, heat, alcohol, cycloheximide, puromycin, ets. In the mouse, parthenogenetic embryos are capable of development through the preimplantation period and progress to the somite stages of development following implantation (Kubiac, 1989). In general, parthenotes in mouse and rabbit have well develped embryonic tissue but poorly develped extraembryonic membranes. Activation of porcine oocytes (Funahashi et al., 1994; Kim et al., 1995d) has recently been studied. A relatively high number of pig oocytes (70 to 80%) formed pronuclei after activation. However, considerably fewer activated eggs developed to morulae or blastocysts as compared to in vitro fertilized eggs (Funahashi et al., 1994). Since paternal inheritance of a functional centrosome has been suggested for most animals, it is possible that impaired development of parthenotes may be the result of the absence of fertilizing sperm centriole. The cell cycle in the parthenote occurs on schedule; the maternal chromosomes condense at mitosis and decondense during the next interphase. In the absence of a reproducing centrosome, Matia (1984) described as "a polar" or " nonpolar" mitosis in which the single cell undergoes repeated rounds of division attempts, but can only form monoasters each cycle. Curiously, parthenogenetic rabbit blastocysts display centrioles, structures not normally observed in early development in this species (Szollosi and Ozil, 1991). Recent studies in the bovine parthenogenetic oocytes (Navara et al., 1994) showed disarrayed microtubules in the cytoplasm and some microtubules extended from the remnants of

the second meiotic spindle. These parthenotes then formed bipolar spindles and divided normally. These results suggest that mammalian oocytes are able to form a functional centrosome in lieu of any contribution by the sperm. Figure 2B summarized microtubule organization during parthenogenesis in porcine oocytes(Kim et al., 1994; 1995e). After electrical activation, centrosomal material is activated and forms a network of microtubules. During pronuclear formation, the sperm aster enlarges as the decondensing male and female chromatins move toward the center of the oocyte. After pronuclear formation, microtubules are less detectable in the cytoplasm. During mitotic metaphase, centrosomal material may disperse to the entire cytoplasm again where prometaphase chromatin attracts centrosomal material and forms a mitotic spindle.

Microfilament organization after activation has been studied in mouse and rat oocytes (Maro et al., 1984; Zernicka-Goetz, 1993). Microfilaments are located mainly in the cell cortex overlying the meiotic spindle. In this microfilament-rich domain, the cleavage furrow forms for polar body extrusion following activation. When the oocyte enter interphase, this domain disappears and the microfilaments concentrate around pronuclei. In aged mouse and porcine oocytes, the microfilament-rich domain overlying the meiotic spindle disappears (Webb et al., 1986; Kim et al., 1995b). This is followed by migration of the spindle toward the center of the egg and spindle breakdown with the chromosomes no longer organized on a metaphase plate. These results suggest that the distribution of microfilaments in closely related to microtubule organization and is integrated and interacts with chromatin morphology.

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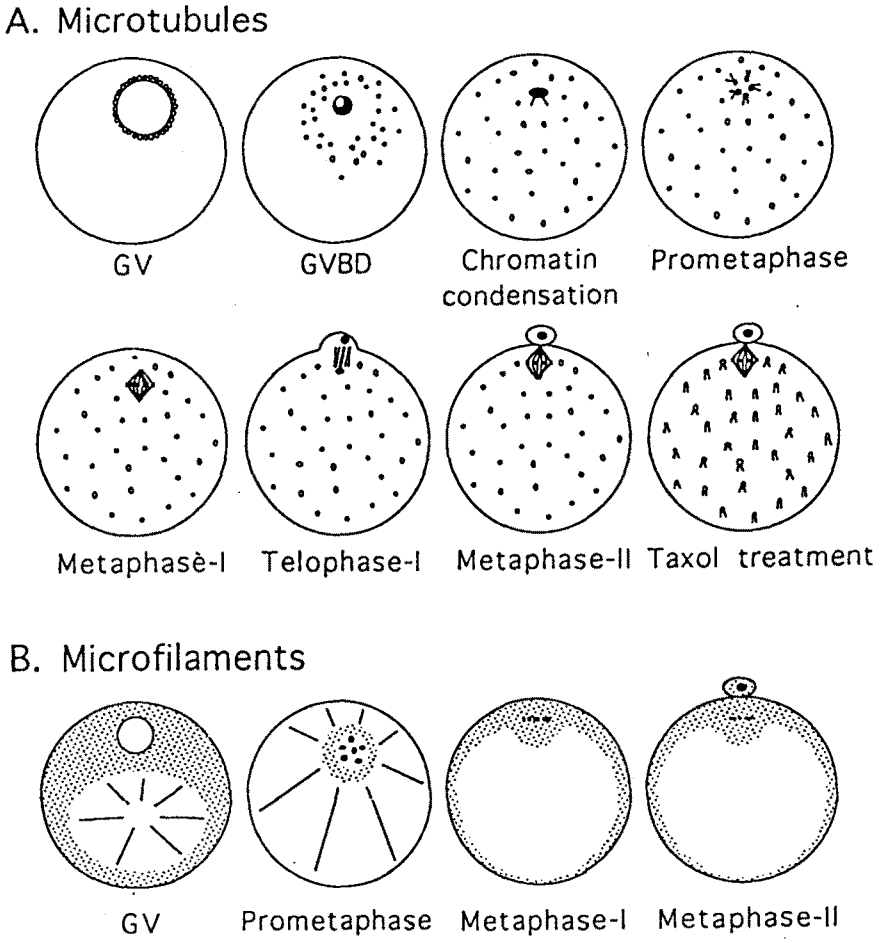

Figure 1. Schematic diagram of microtubule and microfilament dynamics during meiotic maturation in pig oocytes. A) Microtubule configuration: At germinal vesicle (GV) stage microtubules were not detected. The centrosomal material seems to be associated with the nuclear envelope. With germinal vesicle breakdown, the centrosomal material becomes dispersed as many foci in the cytoplasm. The condensed chromatin recruits nearby centrosomal materials, and nucleates microtubules. During prometaphase microtubules are found in association with each chromatin mass. At metaphase I, microtubules are found in the meiotic spindle. Telophase microtubules are found between two chromatins. The mature metaphase II stage oocytes have microtubules totally in the second meiotic spindle. Treatment with taxol after GVBD activated centrosomal material and induced numerous microtubule asters in the cytoplasm. B) Microfilament configuration: At germinal vesicle stage microfiaments are observed as a relatively thick uniform area around the cell cortex and are also found in a disarrayed pattern throughout the cytoplasm. After germinal vesicle breakdown, the microfilaments concentrate to the chromatin. During prometaphase, microfilaments with chromatin move to a peripheral position. At metaphase I, two domains (thick and thin microfilament area) exist in the egg cortex. Chromosomes are located in the thick microfilament domain of the cortex during oocyte maturation. (Kim et al., 1995a)

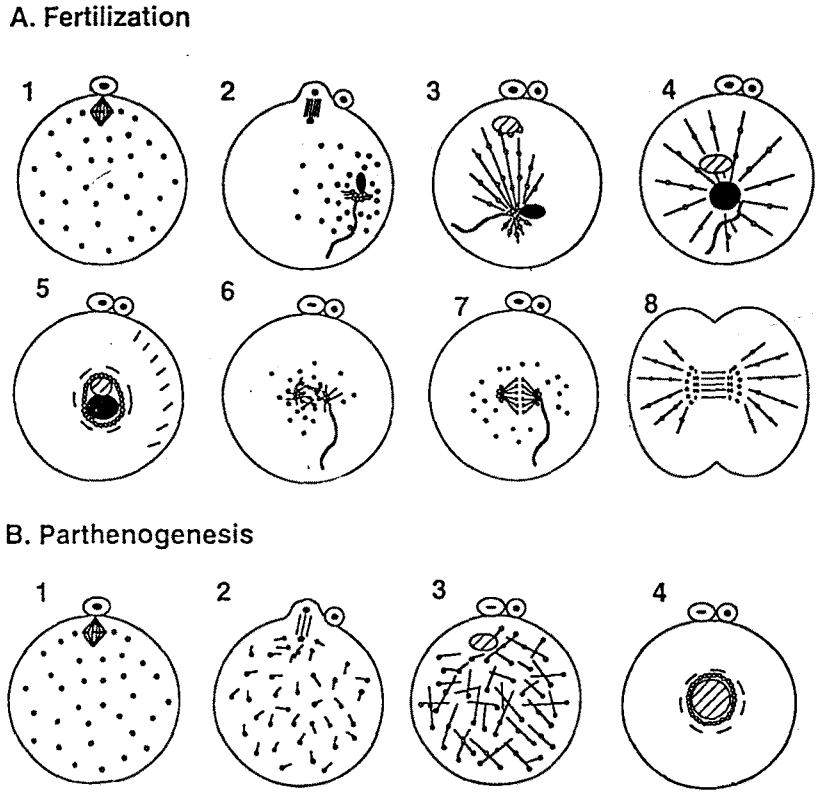

Figure 2. Schematic of microtubule configuration in the porcine oocyte after fertlization and parthenogenetic activation. A. Fertilization. The mature, unfertilized oocyte has microtubules only in the meiotic spindle. Maternal centrosomal material seems to be in the cytoplasm, perhaps in or near the cortex (A-1). After sperm incorportion, the centrosomal material is attracted to the sperm centrosome, and forms sperm aster (A-2). Sperm aster enlarges and reaches the female pronucleus (A-3).

The female pronucleus moves toward the male pronucleus, and at the same time the mae pronucleus moves to the oocyte center (A-4). During pronuclear union, microtubule matrix is less detectable in the cytoplasm. The nuclear envelope seems to retain centrosomal material (A-5). During mitotic pro-metaphase, centrosomal material is dispersed in the cytoplasm. At that time, the condensed chromatin recruits nearby centrosomal materials, and organizes microtubules (A-6). The microtubule foci form eccentric mitotic metaphase spindle, which is anastral and fusiform (A-7). At anaphase and telophase microtubules extend into the cytoplasm from the each spindle pole (A-8). B. Parthenogenesis. Parthenogenetical stimulation activated maternal centrosomal material which forms numerous microtubule foci in the cytoplasm (B-1 & -2). During pronuclear formation, microtubule foci aggregated to each other and form disarrayed microtubule network (B-3). The maternal centrosomal material seems to be concentrated toward the pronucleus and becomes associated with the nuclear envelops (B-4). (Kim et al., 1994; Kim et al., 1995e).