REPRODUCTION
REVIEW

Regulation of dynamic events by microfilaments during oocyte maturation and fertilization

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Abstract

Actin filaments (microfilaments) regulate various dynamic events during oocyte meiotic maturation and fertilization. In most species, microfilaments are not required for germinal vesicle breakdown and meiotic spindle formation, but they mediate peripheral nucleus (chromosome) migration, cortical spindle anchorage, homologous chromosome separation, cortex development/maintenance, polarity establishment, and first polar body emission during oocyte maturation. Peripheral cortical granule migration is controlled by microfilaments, while mitochondria movement is mediated by microtubules. During fertilization, microfilaments are involved in sperm incorporation, spindle rotation (mouse), cortical granule exocytosis, second polar body emission and cleavage ring formation, but are not required for pronuclear apposition (except for the mouse). Many of the events are driven by the dynamic interactions between myosin and actin filaments whose polymerization is regulated by RhoA, Cdc42, Arp2/3 and other signaling molecules. Studies have also shown that oocyte cortex organization and polarity formation mediated by actin filaments are regulated by mitogen-activated protein kinase, myosin light-chain kinase, protein kinase C and its substrate p-MARKS as well as PAR proteins. The completion of several dynamic events, including homologous chromosome separation, spindle anchorage, spindle rotation, vesicle organelle transport and pronuclear apposition (mouse), requires interactions between microfilaments and microtubules, but determination of how the two systems of the cytoskeleton precisely cross-link, and which proteins link microfilaments to microtubules to perform functions in eggs, requires further studies. Finally, the meaning of microfilament-mediated oocyte polarity versus embryo polarity and embryo development in different species (*Drosophila*, *Xenopus* and mouse) is discussed.

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Introduction

Microfilaments, microtubules, and intermediate filaments are the three main cytoskeletal systems of vertebrate and many invertebrate cells. Actin is an abundant protein with well established roles in fundamental processes ranging from cell migration to membrane transport. The nucleation and functions of microfilaments have been extensively investigated. It is known that new actin filaments are formed by cutting of existing filaments or *de novo* by the action of specialized nucleating components. The most highly characterized nucleating component is the Arp (actin-related protein) 2/3 complex. Polymerization of actin is catalyzed by the Arp 2/3 complex, which is stimulated by various molecules such as cortactin and WASP/Scar family proteins (Gunst 2004, Vartiainen & Machesky 2004). Formins represent another family of proteins that nucleate actin filaments by modulating their elongation and protecting them from capping proteins (Evangelista *et al.* 2003, Harris & Higgs 2004, Zigmond 2004*a*). To maintain a steady state, filaments, facilitated by actin-depolymerizing factor (ADF)/cofilin, undergo depolymerization to limit the rate of new filament nucleation and elongation (Zigmond 2004*b*, Gohla *et al.* 2005).

It has been well established that eukaryotic cells require filamentous actin to maintain their shape and for migration, growth, polarization, organelle movement, endocytosis/exocytosis, replication and gene regulation, while relatively little is known about the roles of actin filaments in germ cells. However, new knowledge has been accumulating on the functions of the cytoskeleton in germ cell development, fertilization and early embryo development. The sperm head cortical cytoskeleton exhibits significant changes during the acrosome reaction, consistent with the concept of cytoskeletal proteins as highly dynamic structures participating actively in processes before fertilization (Dvoráková *et al.* 2005). The role of the actin cytoskeleton in sperm capacitation and acrosome

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reaction, and the related signal transduction pathways have recently been reviewed (Breitbart *et al.* 2005). Meiotic maturation in mammalian oocytes is a complex process that involves extensive rearrangement of microtubules and actin filaments (Roth & Hansen 2005), as well as other cytoskeleton-associated proteins providing the framework for various dynamic processes. Polymerization of nonfilamentous (G-) actin into filamentous (F-) actin is important for various aspects of oocyte development and fertilization. In this paper, we will summarize our understanding of the role of actin filaments in the control of dynamic events during oocyte meiotic maturation and fertilization.

Actin filaments are not required for germinal vesicle breakdown and meiotic spindle formation, but control chromatin movement during oocyte development

Oocytes resume first meiosis under the stimulation of hormone(s) or after release from an inhibitory environment. In both vertebrates and invertebrates, meiotic divisions in oocytes are typically asymmetric, resulting in the formation of a large oocyte and small polar bodies. The size difference between the daughter cells is usually a consequence of asymmetric positioning of the spindle before cytokinesis (Verlhac *et al.* 2000). In mouse oocytes, after germinal vesicle breakdown (GVBD), the meiotic spindle forms in the center of the cell and begins to migrate to the cortex just 2.5–3 h before polar body extrusion (Verlhac *et al.* 2000). In germinal vesicle (GV) stage mammalian oocytes, actin filaments are distributed as a relatively thick uniform area around the cell cortex and are also found near the GV. After GVBD, microfilaments are present in both the cortex and around the female chromatin (Kim *et al.* 1998, Wang *et al.* 2000). In mature mouse oocytes, the spindle is located peripherally beneath an actin cap (Longo & Chen 1985). When immature mouse oocytes are incubated in medium containing cytochalasin B or D, GVBD occurs and a meiotic spindle forms; however, the spindle is located in the cell center, and the formation of an actin-rich domain in the cortex and peripheral positioning of the spindle are blocked (Longo & Chen 1985, Verlhac *et al.* 2000, Liu *et al.* 2002, Calarco 2005). Jasplakinolide (JAS), which induces microfilament polymerization and stabilization, and exerts an effect opposite to the effects of cytochalasin B, also prevents spindle migration to the oocyte cortex (Terada *et al.* 2000). When mouse oocytes are incubated in colchicine or nocodazole to disrupt microtubule organization, chromosomes condense and become localized to the cortex (Longo & Chen 1985, Verlhac *et al.* 2000). Thus, GVBD and meiotic spindle formation are not regulated by microfilaments, but polarized movement of the chromosomes depends on a microfilament-mediated process in maturing mouse oocytes. Similar results have been obtained in various other species, including hamster (Terada *et al.* 1995), cattle (Kim *et al.* 2000), pig (Sun *et al.* 2001*a*) and human (Kim *et al.* 1998). Reports about the effects of microfilament disassembly on meiotic spindle formation and peripheral movement are contradictory in maturing *Xenopus* oocytes, and this question needs further clarification (Ryabova *et al.* 1986, Gard *et al.* 1995). It has been shown that formin-2, an actin polymerization modulator, is required for microtubule-independent chromatin positioning during mouse oocyte metaphase I (Leader *et al.* 2002). How the actin filaments exert their effect on chromatin migration is not yet known. Simerly *et al.* (1998) reported that microinjection of myosin IIB antibody blocked microfilament-directed chromosome scattering in colcemid-treated mature mouse oocytes, suggesting a role for myosin IIB in mediating chromosome-cortical actomyosin interactions. Verlhac *et al.* (2000) showed that the Mos-MAP kinase pathway controls the activity of the actin microfilament network, perhaps through myosin IIA in mouse oocytes.

It is well known that spindle microtubules force chromosome movement in opposite directions during mitosis and meiosis. In pig (Wang *et al.* 2000, Sun *et al.* 2001*a*) and mouse (Soewarto *et al.* 1995) oocytes cultured in medium containing cytochalasin B or D (5–10 μg/ml), the transition from meiosis (M)I to MII was inhibited. Cell-cycle progression was inhibited and most oocytes were arrested at the MI stage. Therefore, it appears that microfilaments are needed for microtubule functions, and the segregation of homologous chromosomes requires interaction between microtubules and microfilaments. In rat oocytes, significantly higher levels of associated actin were observed on metaphase I chromosomes (Funaki *et al.* 1995). However, when mouse oocytes were treated with low-dose (1 μg/ml) cytochalasin D, anaphase I entry (chromosome segregation) occurred, although cytokinesis was blocked (Kubiak *et al.* 1991, Verlhac *et al.* 2000). Therefore, the effect of high-dose cytochalasin D on chromosome separation may be aspecific. Whether the actin filaments interact with microtubules to ensure homologous chromosome segregation during first meiosis needs further clarification.

Actin filaments are required for peripheral spindle anchorage in *Xenopus* and sheep oocytes (Ryabova *et al.* 1986, Le Guen *et al.* 1989, Gard *et al.* 1995). Recently, it has been reported that myosin-10 (Myo10) plays a critical role in spindle formation and anchoring in *Xenopus* oocytes. Myo10 proteins are phosphoinositide-binding, actin-based motors. *X. laevis* Myo10 associates with microtubules *in vitro* and *in vivo*, and is concentrated at the point where the meiotic spindle contacts the F-actin-rich cortex. Myo10 showed striking colocalization with meiotic spindle microtubules, and F-actin also colocalized with the spindle, both in the cortical cap and the interior of the spindle. In eggs expressing dominant negative Myo10 or injected with Myo10 antibody, spindle microtubule assembly was severely impaired. Disruption of

Myo10 function also disrupted spindle-F-actin association and spindle anchoring (Weber *et al.* 2004). Although the disruption of microfilaments did not cause inward movement of chromosomes, the meiotic spindle was overlaid by F-actin in MII-stage pig oocytes (Sun *et al.* 2001*a*). It is possible that the actin cap anchors the spindle at the cortex by interacting with spindle microtubules.

Regulation of organelle movement and positioning by the cytoskeleton

Correct positioning and active movement of organelles are essential for oocyte growth, maturation and fertilization. During mammalian oocyte growth, organelles move to the cell cortex, forming an 'organelle zone', while organelles (except for cortical granules) move centrally during oocyte maturation, forming an 'organelle-free zone' at the cortex of a mature oocyte.

Mitochondria

Accumulation of active mitochondria in the peripheral cytoplasm and around the germinal vesicles is characteristic of fully grown pig oocytes collected from large follicles. Mitochondria accumulate in the perinuclear area during meiotic progression from GVBD to anaphase I stage. Larger mitochondrial foci are formed and moved to the inner cytoplasm in mature oocytes. After fertilization, mitochondria migrate to the peripronuclear region (Sun *et al.* 2001*b*). This mitochondrial translocation is mediated by microtubules, not by microfilaments, since disruption of microtubules, but not microfilaments, blocked mitochondria migration (Sun *et al.* 2001*b*). In mouse oocytes, a perinuclear accumulation of mitochondria characterizes the premetaphase I stage of reinitiated meiosis. A temporal, spatial and developmental relationship exists between the location of microtubule organizing centers and the progressive translocation of mitochondria to the nuclear region, indicating that mitochondrial translocations are mediated by microtubules (Van Blerkom 1991). Moreover, microfilament depolymerization by cytochalasin did not affect central migration of mitochondria (Calarco 2005). In contrast, it was found that, in larger, stage I *Xenopus* oocytes, a dense network of actin cables extends throughout the cytoplasm, linking the GV and mitochondrial mass to the cortical actin shell (Roeder & Gard 1994).

Endoplasmic reticulum (ER) and Golgi complex

The control of translocation of other organelles is not well documented in oocytes. In sheep oocytes, large aggregates of Golgi complexes and ER cisternae are associated with microtubules (Crozet 1988). In *Xenopus* egg cytosol, membrane fractions, including Golgi stacks and rough ER, construct a membrane network, and this involves the extension of membrane tubules along microtubules by the action of microtubule-based motor proteins. Indeed, a close relationship was observed between microtubules and ER redistribution during *Drosophila* oogenesis (Bobinnec *et al.* 2003). However, it has been reported that redistribution of ER in the cytoplasm to the nuclear area is dependent on microfilaments in starfish eggs (Terasaki 1994). Spir, an actin organizer and essential regulator of *Drosophila* oocyte polarity, is targeted to intracellular membrane structures, providing a novel link between actin organization and intracellular transport (Kerkhoff *et al.* 2001). Recently, it has been shown that translocations of the cortical ER network to the poles of the zygote depend on both microfilament-driven cortical contractions and sperm aster-microtubule-driven translocations (Sardet *et al.* 2003, Prodon *et al.* 2005). The regulation of ER and Golgi complex migration during oocyte development and fertilization is not known in mammalian eggs.

Centrosomes

Centrosomes, which govern the organization of microtubules, undergo cell-cycle-dependent changes in compaction/decompaction and separation that are synchronized with DNA cell cycles (Schatten *et al.* 2000). In sea urchins, the sperm centrosome is introduced into the egg during fertilization. Sperm centrosome expansion and separation typically occur concurrently in the fertilized egg, and centrosomes condense into the two spindle poles at prometaphase. Microtubule inhibitors prevent centrosome expansion and separation, while microfilament inhibition by cytochalasin D prevents centrosome separation, but not expansion or compaction (Schatten *et al.* 1988). During mouse oocyte maturation, microfilament depolymerization by cytochalasin B did not affect central migration of microtubule organizing centers (MTOCs) devoid of centrioles (Calarco 2005). Thus, microfilaments regulate centrosome separation, but not centrosome movement, in the ooplasm.

Actin filaments participate in oocyte cortex formation

Actin filaments that are associated with the plasma membrane are important for generating cell-surface specialization areas, and also provide the driving force for remodeling cell structure. This is achieved through the regulation of actin-binding proteins. Ezrin, Radixin and Moesin (ERM) proteins are thought to be among the components forming a bridge between the actin cytoskeleton and the plasma membrane (PM). Dmoesin, the only member of the ERM family in *Drosophila*, is required during oogenesis to anchor microfilaments to the oocyte cortex (Polesello *et al.* 2002). Eggs of the freshwater oligochete *Tubifex* undergo a drastic reorganization of the cortical actin cytoskeleton during the metaphase of second meiosis. At the end of first meiosis, the egg cortex displays only scattered actin filaments and tiny dots of F-actin; during the following 90 min, cortical F-actin gradually increases

in amount, becomes organized into foci that are interlinked by actin bundles, and generates a geodesic dome-like organization. The reorganization of cortical actin during the metaphase of second meiosis requires activation of protein kinase C (PKC), which depends on increases in [Ca^{2+}]i (Shimizu 1997). Activation of the Arp2/3 complex by Cdc42 and other signaling molecules plays a central role in stimulating actin polymerization at the cell surface (Ma *et al.* 1998). It has been shown that Cdc42 is involved in the actin assembly in sea urchin eggs (Nishimura & Mabuchi 2003). Recently, it was shown that active RhoA and Cdc42 assemble a dynamic ring of actin filaments (F-actin) and myosin-2 around wounded sites of *Xenopus* oocytes (Benink & Bement 2005).

The mouse oocyte cortex possesses numerous actin filaments that emanate from the plasma membrane, forming a uniform layer of F-actin (Fig. 1A). The membrane anchorage sites of actin filaments are marked by electron dense material on the inner leaflet of the plasma membrane. The free ends of filaments emanating from the plasma membrane of oocytes intermesh to form a dense, cortical layer. With meiotic maturation, the distribution of cortical actin is changed (Longo 1987). Relatively dense uniform layers of F-actin are also observed in porcine, bovine and human oocyte cortices (Kim *et al.* 1998, 2000, Pickering *et al.* 1998, Sun *et al.* 2001*c*). The cortices of a number of mammalian eggs are not structurally homogeneous but are polarized to form cell polarity (Longo 1985). Little is known about the molecules interacting with actin filaments in the mammalian egg cortex or proteins that connect actin to the plasma membrane. Recently, immunostaining of Rho proteins showed that Rac1 and RhoB are present in the cortical ooplasm, but Cdc42 is absent in the mouse (Kumakiri *et al.* 2003).

Actin filaments are involved in oocyte polarity establishment

In *Drosophila* and *Xenopus*, mRNA determinants and proteins in the cortex might play a role in oocyte polarization and body-plan specification. In *Drosophila*, development of the embryonic germ cells depends on posterior transport and site-specific translation and anchoring of *oskar* (osk) mRNA and protein within the posterior subcortical region of the oocyte. Transport of osk mRNA is mediated by microtubules, while anchoring of osk gene products (proteins) at the posterior pole of the oocyte is microfilament-dependent (Jankovics *et al.* 2002). In *Xenopus* oocytes, cytoplasmic actin cables extend from the GV to the animal cortex, and form a three-dimensional network surrounding clusters of yolk platelets in the vegetal cytoplasm. Disruption of F-actin in stage VI oocytes by cytochalasin results in distortion and apparent rotation of the GV in the animal hemisphere, suggesting that actin plays a role in maintaining the polarized organization of amphibian oocytes (Roeder & Gard 1994). Microtubules are involved in the translocation of the message to the vegetal hemisphere, while cortical microfilaments are important to anchor mRNA at the cortex (Yisraeli *et al.* 1990).

Longo and Chen (1985) first described the polarity of mouse oocytes. They found that the cortex of eggs is not

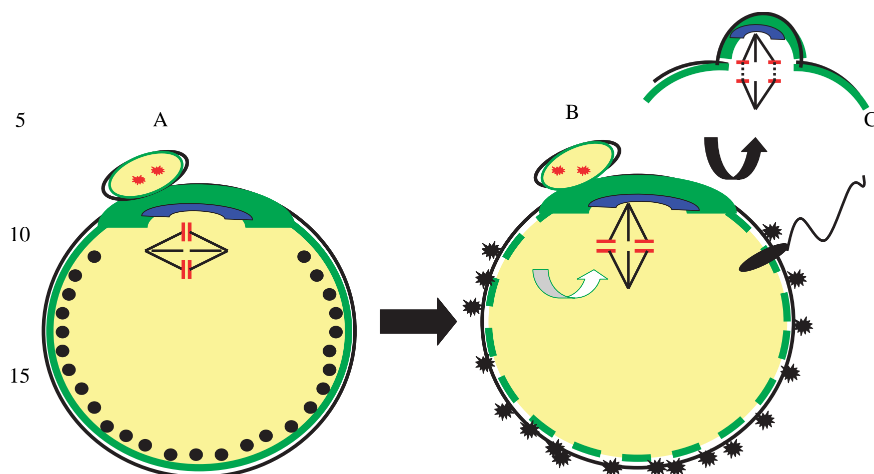

Figure 1 Diagram through the equatorial plane of mouse oocytes illustrating the dynamic events regulated by actin filaments during in vitro maturation and after sperm penetration. (A) Cortical granules and nucleus (spindle) migrate to the cell cortex mediated by microfilaments during oocyte maturation. In the MII stage, the oocyte meiotic spindle is anchored parallel to the plasma membrane by microfilaments, while anchorage of cortical granules is independent of microfilaments. The oocyte cortex is formed and oocyte polarity is established. Note that the meiotic spindle is overlaid by a thick actin cap (green) and PAR proteins (blue), and there is no distribution of cortical granules and microvilli in the animal pole. (B) After sperm penetration (away from animal pole), driven by microfilaments, the spindle rotates 90° to position itself vertically to the plasma membrane, and cortical granules are released. (C) After the oocyte enters anaphase/telophase, the completion of polar body emission requires the action of actin filaments (green). PAR proteins (blue) contribute to eccentric spindle positioning, oocyte cortex polarization and polar body emission site determination.

structurally homogeneous but is polarized. When induced to undergo maturation, the actin cytoskeleton undergoes rearrangement to bring about polarization, which is required for asymmetric division. The meiotic spindles form in the center of immature oocytes and then move peripherally. Coincident with the cortical localization of the meiotic spindle is the formation of an area devoid of microvilli, that is, there are loss of microvilli and thickening of the actin layer associated with this region of the egg cortex. The MII mouse oocyte is transformed into a highly polarized egg, characterized by an actin cap and cortical granule-free domain (CGFD) overlying the meiotic spindle that is in close proximity to the cortex (Deng *et al.* 2003) (Fig. 1A), and ER clusters restricted to the cortex of the vegetal hemisphere (FitzHarris *et al.* 2003). Spindle movements are often related to interactions between the cell cortex and the spindle asters. The spindles of mammalian oocytes are, however, typically devoid of astral microtubules, which normally connect the spindle to the cortex. In mouse oocytes, the spindle migrates along its long axis, but the choice of its direction of migration is the consequence of a slightly off-center positioning of the GV rather than the consequence of a predefined cortical site that could influence its migration. In mos$^{-/-}$ mouse oocytes, the spindle forms centrally but does not migrate. In these oocytes, a compensation mechanism exists: the spindle elongates during anaphase, and the pole closest to the cortex moves while the other remains in place. Thus, polarity and asymmetric division are established either after migration of the spindle to the cortex in wild-type oocytes, or after elongation, without migration, of the first meiotic spindle in mos$^{-/-}$ oocytes (Verlhac *et al.* 2000).

In pig oocytes, as in mouse oocytes, a cortical granule-free domain (CGFD) (not as evident as in mouse oocytes) and an actin-thickening area were observed over the MII spindle of a mature oocyte, and actin filaments retain the chromatin at the proper position at the oocyte cortex (Kim *et al.* 1996*a*, Sun *et al.* 2001*c*). In MII-stage horse oocytes, the bulk of the microfilaments remain within the oocyte cortex, and an actin-rich plaque was found to overlie the spindle (Tremoleda *et al.* 2001). Oocyte polarity was also observed in bovine and human oocytes (Kim *et al.* 1998, 2000). Carroll *et al.* (2004) found that the mammalian unfertilized egg is polarized, with the meiotic spindle located in the cortex of the animal pole and clusters of endoplasmic reticulum in the vegetal hemisphere. Functionally, this cortical polarity may be related to the restriction of sperm–egg interaction and fusion. Sperm does not penetrate the oocytes in the microvilli-free area of the animal pole. Polarity is also involved in the dynamic changes of the egg cortex during fertilization, including polar body formation and fertilization cone development.

We are now beginning to understand the polarization mechanisms. The chromosomes, which are located beneath the cortex, play an important role in the formation of the cortical actin domain. If metaphase II chromosomes are artificially dispersed, by nocodazole treatment, each group of chromosomes will induce the formation of a domain rich in microfilaments and poor in microvilli in mouse oocytes (Verlhac *et al.* 2000). The CGFD and actin-rich domains are also observed over chromosome masses of nocodazole-treated pig oocytes (Sun *et al.* 2001*c*). It is now well known that the presence of chromosomes, but not the integrated meiotic spindle, initiates this cortical reorganization. Cortical polarization is altered after spindle disassembly by colcemid: the scattered meiotic chromosomes initiate myosin IIA, microfilaments assemble in the vicinity of each chromosome mass (Simerly *et al.* 1998), and the chromosome mass induces a focal accumulation of subcortical actin in mouse oocytes (Maro *et al.* 1986). Microinjected sperm chromatin in the cortical region also induces polarity formation, and this is blocked by inhibitors of microfilament polymerization or disassembly. Active mitogen-activated protein kinase (MAPK), which becomes enriched in the region of chromatin, is required for cortical reorganization, as was shown by microinjecting sperm chromatin that failed to induce cortical reorganization in Mos$^{-/-}$ eggs, which lack MAPK activity. Myosin light-chain kinase (MLCK), which can be directly phosphorylated and activated by MAPK, also appears to be involved, because the MLCK inhibitors ML-7 and peptide 18 prevent sperm chromatin-induced cortical reorganization (Deng *et al.* 2005).

The positioning of meiotic spindles is known to involve actin filaments. The evolutionarily partitioning defective (PAR) proteins (microtubule regulatory proteins) have been demonstrated to play an important role in cell polarity in many cell types. Recent studies investigated the role of PAR proteins in establishing mouse oocyte polarity. PAR proteins are found on meiotic spindles before the MII stage, accumulate at the cortex of the animal pole during spindle migration, and are concentrated within a subdomain of the polarized actin cap (Vinot *et al.* 2004, Duncan *et al.* 2005, Moore & Zernicka-Goetz 2005) (Fig. 1). After mouse oocyte germinal vesicle breakdown, PAR-1 is localized on meiotic spindles in mouse oocytes (Moore & Zernicka-Goetz 2005), and PAR-3 surrounds the condensing chromosomes and associates with the meiotic spindles. Prior to emission of the first and second polar bodies, PAR-3 is located within a central subdomain of the polarized actin cap, which overlies the spindle. This cortical PAR-3 localization depends on intact microfilaments. These results suggest a role for PAR-3 in establishing polarity in the egg and in defining the future site of polar body emission (Duncan *et al.* 2005). Two PAR-6-related proteins have distinct polarized distributions in mouse oocytes. mPARD6a is first localized on the spindle and then accumulates at the pole nearest the cortex during spindle migration. In the absence of microtubules, the chromosomes still migrate to the cortex, and mPARD6a is found in association with the chromosomes, facing the cortex. The other protein, mPARD6b, is found on spindle microtubules until entry into MII and is relocalized to the cortex at the animal pole during metaphase II

arrest (Vinot *et al.* 2004). All these results suggest that PAR proteins are involved in establishment of mouse oocyte polarity and asymmetric meiotic division.

The roles of actin filaments in cortical granule movement, anchoring and exocytosis

Cortical granules (CGs) are secretory vesicles of the egg that play a fundamental role in preventing polyspermy at fertilization. During oocyte maturation, CGs become localized beneath the plasma membrane, forming a compact monolayer and, upon fertilization, undergo Ca^{2+}-dependent exocytosis.

CG migration

In sea urchin oocytes, CG translocation requires association with microfilaments, but not microtubules. Shortly after GVBD, CGs attach to microfilaments and translocate to the cell surface. Maturation-promoting factor (MPF) activation stimulates vesicle association with microfilaments, and is a key regulatory step in the coordinated translocation of CGs to the egg cortex (Wessel *et al.* 2002). Rho association with the CGs is a critical regulatory step in their translocation to the egg cortex. Inhibition of Rho blocks CG translocation, and microfilaments undergo a significant disorganization. In *Xenopus*, cortical flow of CGs is directed toward areas of localized contraction of the cortical F-actin cytoskeleton, but is suppressed by microtubules (Benink *et al.* 2000).

In mouse oocytes, the peripheral migration of CGs to the periphery is driven by microfilaments (Fig. 1A), and this process is blocked by treatment of oocytes with cytochalasin D, but not with nocodazole or colchicines (Connors *et al.* 1998). The same mechanism of CG migration also exists in pig oocytes. During maturation of pig oocytes, cortical granules and microfilaments become localized to the cell cortex (Sun *et al.* 2001*c*), and actin filaments drive CG movement to the cortex (Kim *et al.* 1996*b*, Sun *et al.* 2001*a*).

CG anchorage

Once the CGs are localized beneath the oolemma, their anchorage to the cortex is independent of either microfilaments or microtubules; they remain at the cortex after treatment of metaphase II-arrested eggs with either microfilament inhibitors or microtubule inhibitors in mouse and pig oocytes; that is, there is neither inward movement nor precocious exocytosis (Connors *et al.* 1998, Sun *et al.* 2001*a*). In addition, it has been shown that the anchorage of CGs at the cortex is a Rho-independent process in mouse oocytes (Covian-Nares *et al.* 2004).

CG exocytosis

Sperm–egg fusion induces increased intracellular free calcium concentration ($[Ca^{2+}]i$) and exocytosis of CGs. Ca^{2+} is released from intracellular stores and activates PKC, leading to CG exocytosis (Sun 2003). New studies suggest that CG release in mature eggs is dependent on calcium-dependent proteins like those in somatic cells required to undergo calcium-regulated exocytosis (Abbott & Ducibella 2001), and calmodulin-dependent kinase II (CaMKII) may act as a switch in the transduction of the calcium signal (Sun 2003). The role of actin filaments in the exocytosis of cortical granules is unclear. In *Xenopus*, the actin cytoskeleton maintains exocytosing cortical granules as discrete invaginated compartments, such that when actin is disrupted, they collapse into the plasma membrane. Invaginated, exocytosing cortical granule compartments are directly retrieved from the plasma membrane by F-actin coats that assemble at their surface. These dynamic F-actin coats appear to drive closure of the exocytic fusion pores and ultimately compress the cortical granule compartments (Sokac *et al.* 2003). In mouse, the microfilament inhibitor cytochalasin B blocked sperm-induced exocytosis (Tahara *et al.* 1996), and JAS was also found to prevent cortical granule exocytosis after artificial activation (Terada *et al.* 2000), suggesting that actin filaments participate in CG exocytosis. In contrast, neither microfilaments nor microtubules are involved in CG exocytosis during pig oocyte activation (Sun *et al.* 2001*a*). Furthermore, the actin cytoskeleton in the cortex is thought to be a physical barrier to CG exocytosis. A decrease in F-actin was detected upon fertilization and upon parthenogenetic activation of rat eggs. Exposing the eggs to drugs that cause either polymerization or depolymerization of actin (JAS and cytochalasin D respectively) did not prevent CG exocytosis. In addition, cytochalasin D increased the percentage of eggs undergoing complete CG exocytosis induced by PKC activator (Eliyahu *et al.* 2005). In support of this hypothesis, the initial cortical release of Ca^{2+} promoted by sperm may be due to depolymerization of actin in starfish (Lim *et al.* 2002). The involvement of actin filaments in CG movement, anchorage and exocytosis in other mammalian species and the related mechanisms need clarification.

Actin filaments are involved in polar body emission

It is well known that animal cells use a contractile ring that is associated with the plasma membrane to create a cleavage furrow that partitions the cell into two daughter cells. The contractile ring is a network of actin and myosin filaments, and the motor activity of myosin translocates actin filaments to drive its constriction (Glotzer 2005). Actin filaments in the contractile ring often appear in parallel bundles. In mitotic cells, contractile ring assembly is directed by the RhoA guanosine triphosphatase (GTPase), which activates myosin and actin filament assembly (Glotzer 2005). Accumulating evidence shows that meiotic oocytes adopt similar mechanisms for releasing the first and second polar bodies during maturation and fertilization (Fig. 1C). Human oocytes in late telophase

showed the presence of a concentrated ring of actin in the cleavage furrow between the oocyte and the second polar body (Pickering *et al.* 1998). The RhoA kinase (ROCK) specific inhibitor Y-27632 and microinjection of RhoA antibody caused abnormal microfilament organization and blocked first and second polar body extrusion in mouse oocytes (Zhong *et al.* 2005). When actin filaments are disrupted, polar body emission is blocked in mouse (Maro *et al.* 1984), hamster (Terada *et al.* 1995) and sheep (Le Guen *et al.* 1989).

During sperm incorporation, both myosin II isotypes concentrate in the second polar body cleavage furrow of mouse eggs (Simerly *et al.* 1998), and this may indicate its role in polar body emission through interaction with actin. P-MARCKS (myristoylated alanine-rich C-kinase substrate) is a major substrate for PKC. It is enriched in the periphery of the actin cap overlying the MI or MII spindle to form a ring-shaped subdomain. Because phosphorylation of MARCKS modulates its actin cross-linking function, this localization suggests that p-MARCKS functions as part of the contractile apparatus during polar body emission. p-MARCKS phosphorylation may be regulated by an atypical isoform of PKC (Michaut *et al.* 2005).

Regulation of sperm incorporation by microfilaments

In many species, fusion between sperm and the oocyte occurs at the tip of microvilli, and the actin filaments in the microvilli may participate in sperm–egg binding and fusion. In sea urchin, the elongating microvilli swell to form the fertilization cone to engulf the entering spermatozoon (Schatten & Schatten 1980). The polymerization of actin beneath the plasma membrane of the fertilization cone and inhibition of sperm incorporation by cytochalasin B or D are observed in eggs of zebrafish and sea urchin (Schatten & Schatten 1981, Hart *et al.* 1992). An extensive recruitment of polymerized actin into the site of sperm incorporation occurs, resulting in the formation of the fertilization cone (Cline *et al.* 1983). In starfish, it was reported that cytochalasin B inhibits sperm incorporation, fertilization cone formation, and actin filament organization (Kyozuka & Osanai 1988), whereas another report indicated that the development of the fertilization cone/actin filament complex is not essential for incorporation of the sperm, while the actin-filament-containing structure is necessary for moving sperm through the outer egg coats (Kyozuka & Osanai 1994).

The need of microfilaments for sperm incorporation is somewhat inconclusive in mammals. In the mouse, it was reported that the sperm head is incorporated in the presence of the microfilament inhibitor latrunculin A, indicating an absence of microfilament activity at this stage (Schatten & Schatten 1986), while a recent report showed that Rho protein(s) regulating actin-based cytoskeletal reorganization is involved in the events leading to sperm incorporation (Kumakiri *et al.* 2003). Another report indicated that the microfilament modulator JAS inhibited sperm incorporation into mouse eggs (Terada *et al.* 2000). Recently, it was shown that the actin microfilament-disrupting drugs cytochalasin B, JAS and latrunculin B resulted in a decrease in the percentage of eggs fertilized and average number of sperm fused per mouse egg; however, the same group found that treatment with another microfilament inhibitor, cytochalasin D, resulted in an increase in the average number of sperm fused per egg and percentage of polyspermic eggs (McAvey *et al.* 2002). Preincubation of hamster eggs with cytochalasin D and washing prior to addition of spermatozoa had no effect on penetration of guinea pig and human sperm (Rogers *et al.* 1989). In pig and cattle, sperm incorporation is mediated by microfilaments, as cytochalasin B treatment inhibited fertilization (Sutovsky *et al.* 1996, Sun *et al.* 2001*a*), while cytochalasin D does not prevent sperm fusion and incorporation in sheep (Le Guen *et al.* 1989). The difference in results may be explained by the different microfilament disruptors used to treat the eggs before insemination or different manipulating procedures. It will be important to clarify this basic biologic question in future studies.

Actin filaments control the meiotic spindle rotation after egg activation

In most mammals, the metaphase II meiotic spindle is perpendicular to the egg surface, but in mature mouse oocytes, the second meiotic spindle lies parallel to the plasma membrane. After sperm penetration, the spindle rotates 90° to the vertical position so that the second polar body can be extruded into the perivitelline space (Fig. 1B). The rotation of the meiotic spindle is controlled by microfilaments. When oocytes are treated with cytochalasin B or D, spindle rotation is inhibited. Consequently, the oocyte forms an extra female pronucleus in the ooplasm instead of extruding a second polar body (Zhu *et al.* 2003). By using Pol-Scope (Cambridge Research & Instrumentation, Boston, MA, USA), Navarro *et al.* (2005) also observed the inhibition of spindle rotation by cytochalasin D in mouse oocytes. Actin filaments are also required for meiotic spindle rotation in *Xenopus* oocytes. Bipolar spindles are observed in cytochalasin B-treated oocytes; however, rotation of the MI and MII spindles into an orientation orthogonal to the oocyte surface is inhibited by cytochalasin B (Gard *et al.* 1995). When the mouse oocyte metaphase spindle is dispersed with nocodazole, small clusters of chromosomes are redistributed around the egg cortex in a microfilament-dependent process. Each cluster has the capacity, after removal from nocodazole, to organize a spindle that rotates to yield a polar body (Maro *et al.* 1986). A small GTPase, RhoA, which regulates microfilament organization, also regulates spindle rotation (Zhong *et al.* 2005). Unlike at first meiosis, inhibition of actin filaments immediately after egg

activation by cytochalsin B or D or inhibition of RhoA does not affect sister chromatid separation, and two pronuclei form; however, second polar body emission is blocked in mouse and pig oocytes (Landa & Hajkova 1990, Boediono *et al.* 1995, Zhu *et al.* 2003, Zhong *et al.* 2005). The intriguing finding that microfilaments regulate homologous chromosome segregation, but not sister chromatid separation, needs clarification.

In mitotic cells that undergo 90° spindle rotation, there is increasing evidence that close cooperation between cortical filamentous actin and astral microtubules is indispensable for successful spindle rotation. In recent years, the dynactin complex has emerged as the key agent to mediate actin/microtubule interactions at the cortex (Schaerer-Brodbeck & Riezman 2000). The mechanisms controlling meiotic spindle rotation in mouse oocytes require further investigation.

Regulation of pronuclear apposition by cytoskeletal components

After sperm penetration, a haploid male pronucleus and a female pronucleus form and appose in the center of the egg to restore diploidy of the zygote. The migration of the sperm and egg nuclei during sea urchin fertilization depends on microtubules that are organized into a radial monastral array, the sperm aster (Schatten & Schatten 1986). The movement of the female pronucleus toward the male pronucleus is mediated by microtubules of the sperm aster directed toward their slower growing, attached or ‘minus’ ends. Reinsch and Karsenti (1997) confirmed the mechanism of nuclear movement *in vitro* by showing that synthetic nuclei assemble in cytoplasmic extracts from interphase *Xenopus* eggs moving along microtubules toward their minus ends. During fertilization in sea urchin, porcine, bovine and human fertilized eggs, the sperm introduces the centrosome into the egg, and microtubules nucleated by centrosomes cause the union of male and female pronuclei. The microfilament inhibitor cytochalasin B affects neither pronuclear formation nor movement (Van Blerkom *et al.* 1995, Sutovsky *et al.* 1996, Kim *et al.* 1997, Sun *et al.* 2001*a*).

Mouse fertilization is a special case in which centrosomes are maternally inherited. Microtubules are organized by numerous egg cytoplasmic sites, and microtubule activity is required during pronuclear apposition in the mouse egg (Schatten & Schatten 1981, 1986). Pronuclear apposition also requires microfilaments in fertilized mouse oocytes (Schatten & Schatten 1981, Maro *et al.* 1984, Terada *et al.* 2000). Thus, both microtubules and microfilaments are required for pronuclear apposition in the mouse.

Actin filaments are required for contractile ring formation during cleavage

In fertilized eggs of sea urchin and sand dollar, accumulation of the contractile ring microfilaments at the equatorial cell cortex was first noticed at the beginning of telophase (shortly before furrow formation), and the accumulated microfilaments were organized into parallel bundles as furrowing progressed. The bundles were eventually fused into a tightly packed filament belt (Mabuchi 1994). In *Xenopus* eggs, actin filaments (F-actin) and myosin II (myosin) assemble to form the contractile ring. F-actin patches grow rapidly in the furrow region. These patches then align in tandem, elongate and fuse with each other to form short F-actin bundles. The short bundles then form long F-actin bundles that compose the contractile ring. Myosin II assembles as spots at the growing end by way of the cortical movement. F-actin accumulates at the position of the myosin spot a little later than the F-actin patches. The myosin spots and F-actin patches are then simultaneously reorganized to form the contractile ring bundles (Noguchi & Mabuchi 2001, Noguchi *et al.* 2001). Astral microtubules also play an important role in controlling the formation of the contractile ring in *Xenopus* eggs (Takayama *et al.* 2002).

During cytokinesis in the mouse, the cleavage furrow staining of actin is more intensive than the rest of the egg cortex, and microfilament inhibitors block cleavage (Schatten *et al.* 1986, Schatten & Schatten 1986). Myosin IIA isoform accumulates in the mitotic cleavage furrow (Simerly *et al.* 1998), and RhoA plays essential roles in the formation of the actin filaments and the cleavage furrow (Zhong *et al.* 2005). A microfilament-rich cleavage furrow was also observed in fertilized pig eggs, in which actin filaments are required for cleavage (Kim *et al.* 1997). Two-cell, bovine embryos were arrested when exposed to heat shock that caused disruption of microtubules and microfilaments (Rivera *et al.* 2004).

The role of actin filaments in the control of various dynamic events during oocyte meiotic maturation and fertilization in several representative animals is summarized in Tables 1 and 2.

Oocyte polarity versus embryo polarity and embryo development in different species

Embryo development depends on the establishment of polarity that defines the axial characteristics of the body. Depending on the species, developmental axes are set up either before or after fertilization. In most organisms, the egg possesses a primary animal-vegetal (A/V) axis acquired during oogenesis, and this axis helps establish the embryonic axes (Sardet *et al.* 2004). In addition, the oocyte polarization determines where the development ‘determinants’ and germ plasm will be located in the egg, and therefore the blastomere fate (Rongo & Lehmann 1996).

Two mechanisms are employed to establish cell polarity: one is the cytoskeleton-oriented polar transport and the other is the capture and concentration of polar cell markers by the actin-rich cortex (Baum 2002).

Table 1 Involvement of actin filaments in various nuclear dynamic events during oocyte maturation, fertilization and early cleavage.

	Events								
Species	GVBD	Spindle formation	Chromation (spindle) movement	Spindle anchorage	Polar body emission	Sperm incorporation	Spindle rotation	Pronuclear apposition	Cleavage
Xenopus	NA	ND	ND	+	+	+	+	−	+
Mouse	−	−	+	ND	+	ND	+	+	+
Pig	−	−	+	ND	+	+	NA	−	+
Cattle	−	−	+	NA	+	+	NA	−	+
Human	−	−	+	NA	+	NA	NA	−	NA

GVBD, germinal vesicle breakdown; NA, not available; ND, not determined (contradictory reports).

The establishment of oocyte polarity, especially the anchoring of GV and spindle as well as the anchoring of 'determinants', depends on microfilaments. The *Drosophila* oocyte is a highly polarized cell that contains a large number of localized mRNAs and proteins. The precocious localizations of these 'determinants' before maturation and fertilization determine the future antero-posterior and dorsoventral polarities of the embryo (Sardet *et al.* 2002). During oogenesis, microtubules direct the transport of osk mRNA to the end of the oocyte, and actin filaments play a secondary role in osk mRNA anchorage, which ensures oocyte polarity and defines the future posterior pole of the embryo (for review, see Baum 2002, Albertini & Barrett 2004). PAR proteins have been shown to be crucial for oocyte determination and polarization in *Drosophila* (Wodarz 2002).

The division of the *Xenopus* oocyte cortex into structurally and functionally distinct animal pole and vegetal pole during oogenesis involves interactions between different cytoskeletal filament systems. As mentioned above, microtubules are involved in the translocation of the message to the vegetal hemisphere and microfilaments are important in anchoring the vegetal message at the cortex (Yisraeli *et al.* 1989). The network of microfilaments in the animal cortex is thicker and more contractile than that in the vegetal cortex (Sardet *et al.* 2002). The primary A/V oocyte polarity created by anchoring mRNAs in the vegetal cortex is the basis of early embryo organization (Alarcon & Elinson 2001). These specific maternal mRNAs accumulated in the vegetal cortex specify the development of the endoderm and mesoderm, as well as the dorsal-anterior development 'determinants' and germ plasm. The animal cortex becomes specialized for the events associated with sperm entry, cortical granule exocytosis, and polarized cortical contraction during fertilization (for review, see Chang *et al.* 1999). Unlike in *Drosophila*, fertilization will bring about the redistribution of the vegetal cortex 'determinants' (the remodeling of the A/V axis) to yield the embryonic axes in *X. laevis* (Chang *et al.* 1999, Sardet *et al.* 2004).

Mammalian oocytes also have distinct A/V polarity, with the meiotic spindle located in the cortex of the animal pole and clusters of ER in the vegetable pole (Carroll *et al.* 2004). Recent evidence supports the notion that mammals retain axis-orienting mechanisms that contribute to oocyte polarity. Axis specification in relation to the oocyte cortex, eccentric positioning of GV, anchoring of GV and spindle, and patterning of follicle cell/oocyte attachment are proposed as conserved mammalian oogenesis features that may be important to the survival and development of preimplantation embryos (for review, see Albertini & Barrett 2004). However, unlike in lower animals, it is commonly accepted that there are no mosaically distributed developmental determinants in mammalian zygotes and early embryos (Louvet-Vallee *et al.* 2005). Whether oocyte polarity determines cleavage pattern is still controversial. Although there is evidence that the site of the second meiotic division and the sperm entry point may affect the first cleavage pattern (Piotrowska & Zernicka-Goetz 2001, Plusa *et al.* 2002),

Table 2 Involvement of actin filaments in various cytoplasmic dynamic events during oocyte development and fertilization.

	Events					
Species	Mitochondria movement	Cortex formation	Polarity development	CG migration	CG anchorage	CG exocytosis
Drosophila	+	+	+	NA	NA	NA
Xenopus	+	+	+	+	NA	+
Mouse	−	+	+	+	−	+
Pig	−	+	+	+	−	−
Cattle	NA	+	+	NA	NA	NA
Human	NA	+	+	NA	NA	NA

CG, cortical granule; NA, not available.

and that specification of embryonic axes begins before cleavage in mouse development (Gardner 2001), recent video microscopy showed that the cleavage planes are oriented randomly in two-cell mouse embryos (Louvet-Vallee *et al.* 2005).

Concluding remarks

In conclusion, microfilaments mediate peripheral migration of the meiotic apparatus (chromosomes), eccentric movement of cortical granules, organization/maintenance of cortices, anchorage of spindle and message determinants at the cortex, establishment of polarity, and emission of the first polar body during oocyte maturation. Microfilaments are also involved in sperm incorporation, spindle rotation (mouse), cortical granule exocytosis and second polar body emission during fertilization. The completion of several dynamic events, including homologous chromosome separation, spindle anchorage, spindle rotation, vesicle and organelle transport, and pronuclear apposition (mouse), requires interaction between microfilaments and microtubules in oocytes, but further study is needed to determine how the two cytoskeleton systems precisely cross-link and which proteins link microfilaments to microtubules to perform the specific functions. Finally, the meaning of microfilament-mediated oocyte polarity versus embryo polarity and embryo development in different species (*Drosophila*, *Xenopus* and mouse) was discussed.

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