Human Reproduction Update, Vol.17, No.1 pp. 68–75, 2011
Advanced Access publication on September 9, 2010 doi:10.1093/humupd/dmq044

human reproduction update

Positioning to get out of meiosis: the asymmetry of division

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Submitted on June 1, 2010; resubmitted on August 5, 2010; accepted on August 11, 2010

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BACKGROUND: During meiosis, mammalian oocytes undergo two successive cell divisions without an intermediate replicative phase. This brief period, called 'meiotic maturation', is crucial for the formation of an egg capable of being fertilized and of generating viable and euploid offspring.

METHODS: We review our current knowledge of the cellular and molecular mechanisms that control asymmetry and appear to be shared between mammalian species, as well as the associated misfunctions that impair the formation of functional female gametes.

RESULTS AND CONCLUSIONS: The two successive divisions that comprise mammalian oogenesis are asymmetric. They lead to the formation of small polar bodies and the large and polarized egg. This asymmetry depends upon the dynamic organization of the oocyte cytoskeleton during both divisions. During meiosis I, microfilaments and associated molecules ensure the targeting of the microtubule spindle at the oocyte periphery. During meiosis II, they anchor the spindle under the plasma membrane. In parallel, the cortex overhanging the spindle is dramatically reorganized. Establishment and maintenance of this cortical domain are crucial for the completion of fertilization. Loss of this differentiated area is characteristic of ageing or low-quality gametes and associated with increased maternal age or post-ovulatory ageing.

Key words: oocyte / asymmetric divisions / cytoskeleton / pre- and post-ovulatory ageing

Introduction

During meiosis, mammalian oocytes undergo two successive cell divisions without an intermediate replicative phase. This brief period, called 'meiotic maturation', is crucial for the formation of an egg capable of being fertilized and of generating viable and euploid offspring. Each division must ensure accurate segregation of the maternal genome and highly asymmetric partition of the cytoplasm, so that a tiny polar body and a large oocyte are generated. The sequential events of these asymmetric divisions are tightly controlled by microtubule and microfilament cytoskeletons (Fig. 1). Upon entry into the first meiotic division (meiosis I), microtubules form a bipolar spindle. During meiosis I, microfilaments perform spindle positioning at the periphery of the oocyte and the extrusion of a first polar body in parallel with chromosome segregation. During second meiotic division (meiosis II), the microtubule spindle is positioned at the periphery of the oocyte until fertilization triggers the emission of the second polar body. In addition, eccentric positioning of the spindle is associated with local reorganization of actin cytoskeleton until fertilization. The mechanisms that coordinate microtubule and

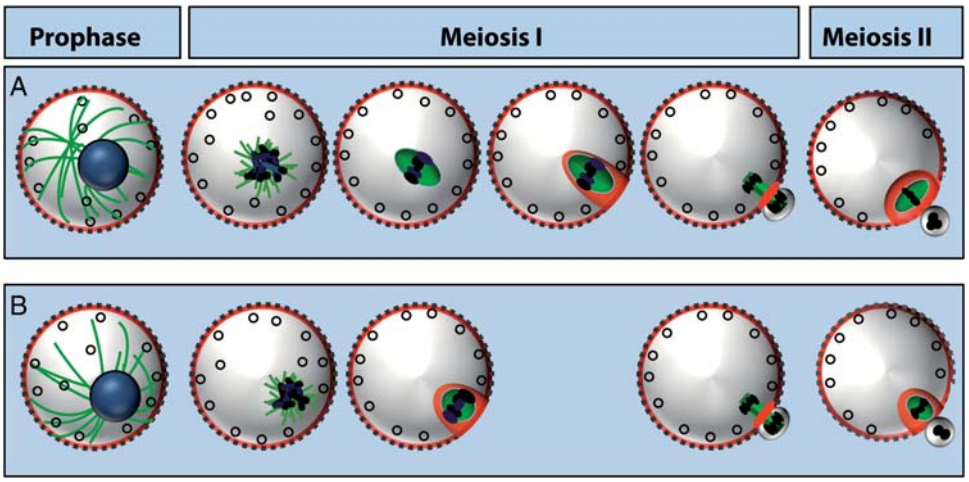

Figure 1 Establishment of asymmetry in mammalian oocytes. The cellular events of meiotic divisions are schematized using mouse (**A**) and human (**B**) oocytes as paradigms. Actin appears in red, microtubules in green and chromosomes in blue. Cortical granules are depicted as small grey discs and microvilli as dark indentations on the oocyte surface. At meiosis I onset, the germinal vesicle envelope breaks down and microtubules form a bipolar spindle around the chromosomes. Targeting of the cortical granules to the cortex is achieved. At the end of meiosis I, the spindle migrates to the cortex and cortical reorganization is initiated. After first polar body extrusion, oocyte enters meiosis II and spindle rapidly forms below the first polar body.

microfilament networks, from meiosis resumption until fertilization, have been studied for decades in numerous mammalian species. However, the multiplicity and availability of functional analysis tools, coupled to high-resolution imaging in live maturing oocytes have recently enabled a better understanding of these mechanisms and their importance in the genesis of functional gametes. In the light of recent and exciting results, here we review our current knowledge of these mechanisms that appear to be mostly shared among mammalian species.

Methods

The Pubmed database was systematically searched for studies related to cytoskeleton organization, polar body formation and fertilization in mammalian or vertebrate oocytes.

Results

Biological relevance of asymmetric divisions in mammalian oocytes

It is generally accepted that one of the purposes of the formation of small polar bodies and a large oocyte is to maintain the stores accumulated during the growth phase in the egg. Various critical maternal effectors are stored for use after the oocyte–embryo transition (Wu *et al*., 2003; Tong *et al*., 2009; Zheng and Dean 2009). However, maximizing the volume of the oocyte cytoplasm may not be a strict prerequisite, as suggested by experiments performed in mice, where *in vitro* development to 2-cell embryo is not impaired upon removal of increasing volumes (up to half of the total volume) of oocyte cytoplasm (Cui *et al*., 2005). To clarify this point, it would be necessary to analyse the long-term developmental potential of the resulting zygotes.

Minimizing the size of the polar body is, in contrast, essential to avoid any competition between two daughter cells, similar in size, for fertilization. Owing to its reduced surface and absence of microvillosities, the polar body is incapable of sperm binding (Motosugi *et al*., 2006) and cannot be fertilized (Fisk *et al*., 1996). One biological function of the asymmetry is to generate, within the zona pellucida only 1-cell able to bind to sperm, be fertilized and give rise to an embryo: the oocyte.

A second consequence of such asymmetric division is the formation of a 'polarized' egg. The *Drosophila* egg is a paradigm of polarization, in the sense that the asymmetric organization of its cytoplasm and cortex is associated with asymmetric distribution of developmental cues, mainly mRNA, which defines future embryonic patterning (Riechmann and Ephrussi, 2001). In mammals, there is compelling evidence that the axial development in the embryo or fetus cannot be related to egg organization (Johnson, 2009). Polarization in mammalian oocytes therefore has an attenuated meaning and refers to anisotropic organization of the cell. The oocyte exhibits a highly differentiated and restricted cortical domain, which contains the chromosomes, in contrast to the rest of the oocyte lacks microvilli, and defines the site of emission of the second polar body (Fig. 1). Establishment and maintenance of this domain are crucial for the completion of fertilization. Absence of polarization, marked by the loss of the differentiated area, is characteristic of ageing or low-quality gametes. Since microvilli promote interaction with sperm (Runge *et al*., 2007), the differentiated domain of the oocyte lacks any sperm-binding capacity (Hiiragi and Solter, 2004; Motosugi *et al*., 2006). At fertilization, the emission of the second polar body and sperm entry thus occur in separated areas. In mammals, oocyte polarization can be considered to be a security mechanism, ensuring the formation of distinct cortical domains that physically

separate incorporation of the paternal genome into the cell from the elimination of a set of maternal chromosomes in the polar body.

A significant aspect of egg polarization, often neglected, concerns the asymmetry of the perivitelline space. The sperm preferentially enters in the polar body half of *in vivo* fertilized mouse eggs, and not the differentiated area, as previously mentioned (Hiiragi and Solter, 2004). This geometry has been demonstrated to depend on the asymmetry of the perivitelline space associated with the emission of the first polar body. Once emitted, the first polar body does not immediately degenerate. It remains in the space between the oocyte and the zona pellucida, and expands the perivitelline space in its vicinity. Motosugi *et al*. (2006) have shown that this expanded space around the polar body increases the probability that the sperm is present in the polar body half before binding. These data show that physical constraints linked to polar body extrusion influence the geometry of fertilization. They also highlight the importance of minimizing oocyte manipulations prior *in vitro* fertilization, since manipulations are known to displace the polar body from above the meiosis II spindle (Baca and Zamboni, 1967; Hewitson *et al*., 1999; Hardarson *et al*., 2000).

Cytological events of meiotic divisions in mammalian oocytes

Towards the periphery: spindle migration and anchoring

Asymmetry in mammalian oocytes relies on the targeting and maintenance of meiotic spindles at the cell periphery. The sequence of morphological events that leads to these asymmetric divisions has been documented in various mammalian species (Fig. 1). During recent years, the development of time-lapse microscopy in live oocytes, mainly using the mouse oocyte as an experimental model, has been critical to better understanding this process at cellular and molecular levels.

In the mouse oocyte, the germinal vesicle relocates from the periphery toward the central area of the oocyte during the growth phase. Nevertheless, the germinal vesicle remains on average slightly eccentric at the end of prophase (Albertini and Barrett, 2004; Brunet and Maro, 2007). At meiosis I onset, the germinal vesicle envelope breaks down, chromosomes condense and microtubules reorganize progressively around them into a bipolar spindle (Schuh and Ellenberg, 2007). At the end of meiosis I, the spindle migrates to the oocyte cortex. Migration occurs along the spindle axis and is directed towards the closest cortical domain of the oocyte, so that the distance covered is eventually minimized (Verlhac *et al*., 2000; Fig. 1A). Once migration is achieved, anaphase and extrusion of the first polar body are initiated. The axis of the bipolar spindle is randomly established at meiosis I onset (Verlhac *et al*., 2000). In addition, displacement of the spindle, once migration has been initiated, to a distant cortical domain results in the emission of the first polar body in this domain (Schuh and Ellenberg, 2008). These data clearly show that the site of polar body extrusion is not predetermined. After the first polar body has been extruded, the oocyte enters meiosis II and a bipolar spindle rapidly forms below the first polar body (Dumont *et al*., 2007a; Brunet *et al*., 2008). The spindle remains anchored parallel to the plasma membrane during the metaphase II arrest. Upon anaphase triggering by fertilization or experimental activation, a 90° rotation of the spindle allows the extrusion of the second polar body (Zhu *et al*., 2003; Fig. 1A).

All these events are mainly conserved in most mammalian species and are identical in rabbits (Yan *et al*., 2006). Differences observed are mostly due to variations in the position of the germinal vesicle at prophase I. In human oocytes, the position of the germinal vesicle varies from slightly (Goud *et al*., 2000; Nogueira *et al*., 2003) to significantly (Sathananthan *et al*., 2006) eccentric (Fig. 1B). In cow, pig or horse, the germinal vesicle is generally eccentric. As a consequence, the radial migration of the meiosis I spindle is shortened (Kim *et al*., 1996a, b, c; Kim *et al*., 1998; Lee *et al*., 2000; Tremoleda *et al*., 2001; Combelles *et al*., 2003; Li *et al*., 2005). During meiosis II, in these species as well as in humans, the spindle appears to be perpendicularly anchored to the cortex (Kim *et al*., 1996a, b, c; Sathananthan, 1997; Hewitson *et al*., 1999; Lee *et al*., 2000; Li *et al*., 2005; Fig. 1B).

Cortical reorganization, polar body formation and egg polarization

The cortex of mammalian oocytes displays uniform morphology during prophase I and the beginning of meiosis I. The surface of the oocyte is homogeneously covered by microvilli. Small vesicles called cortical granules, derived from the Golgi, are distributed evenly in the cortical cytoplasm. Targeting of these granules takes place during the prophase arrest, at the end of growth phase, and is achieved after meiosis I onset (Kim *et al*., 1996a, b, c; Connors *et al*., 1998; Carneiro *et al*., 2002; Liu *et al*., 2005; Fig. 1).

During meiosis I, as the spindle comes close to the periphery, the overhanging cortex is dramatically reorganized. This process is conserved among mammalian species. Microvilli disassemble and filaments accumulate under the plasma membrane to form a so-called actin cap (Longo and Chen, 1984; Tremoleda *et al*., 2001). In parallel, the density of cortical granules strongly decreases in this area, either by premature exocytosis or redistribution in the cytoplasm (Ducibella *et al*., 1988; Carneiro *et al*., 2002; Ferreira *et al*., 2009; Fig. 1). Cortical reorganization may be critical for the formation and shaping of the polar body. In mitosis, the cleavage plane of the cell is defined by the equator of the metaphase spindle which serves to position the cytokinetic furrow (Glotzer, 2009). This is not the case in oocytes where anaphase and polar body emission are initiated in parallel. The spindle undergoing anaphase protrudes in the expanding polar body. Cortical reorganization, by locally modifying the physical properties of the cortex, may favour spindle protrusion and restrict the area in which the polar body can be emitted. Interestingly, the anaphase I spindle does not elongate, in contrast to mitotic spindle (Verlhac *et al*., 2000). This feature probably contributes to minimizing the polar body size. Unfortunately, such mechanisms remain hypothetical and the physical basis of polar body formation remains to be investigated.

During meiosis II, the spindle reforms and becomes anchored in the periphery of the oocyte. The cortical area overhanging the spindle keeps remains modified, conferring polarized characteristics to the egg (Fig. 2).

Molecular basis of asymmetry in mammalian oocytes

The underlying processes and factors of asymmetry in mammalian oocytes are summarized in Table I.

Mechanisms of spindle positioning

Asymmetric divisions in most experimental models rely on accurate positioning of the spindle within the cell. Positioning is achieved through physical interactions between spindle poles and cell cortex. Specialized spindle microtubules, named 'astral' microtubules, polymerize from the poles to the cortex, where they anchor the spindle on cortical cues (Cowan and Hyman, 2004). Mammalian, and more generally vertebrate, oocytes lack centrosomes and astral microtubules (for a review, see Sathananthan *et al.*, 2006). Alternative mechanisms are at play to position the meiotic spindle in the periphery of the cell. These mechanisms have recently started to be unravelled mostly due to several functional analyses performed on mouse oocytes.

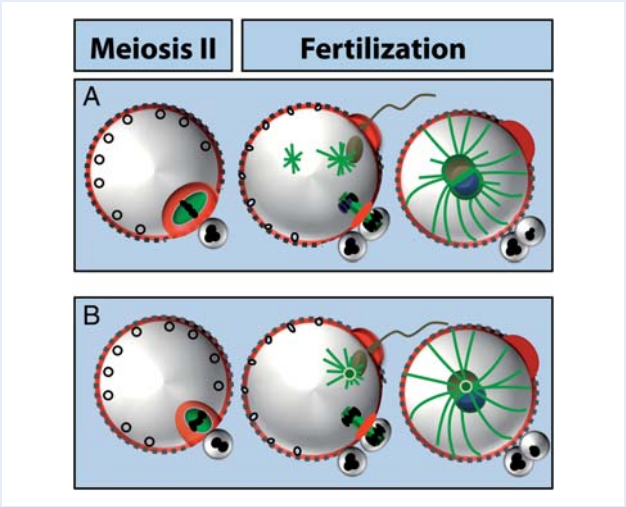

Figure 2 Fertilization cancels egg asymmetry. Maternal and paternal chromatins are schematized in dark blue and brown, respectively. Centrosome appears as a yellow ring. The metaphase II arrested oocyte is highly asymmetric. The spindle remains anchored in the periphery of the oocyte with the surrounding cortex reorganized. Fertilization triggers the emission of the second polar body and cortical granules exocytosis. Male and female pronuclei progressively form. Progressive microtubule reorganization around the pronuclei in mouse (**A**) or sperm aster assembly in human (**B**) induce the progressive migration and apposition of the pronuclei at the centre of the zygote.

Spindle migration and anchoring have been known for a long time to depend on actin microfilaments. Pharmacological poisons of microfilaments inhibit both processes (Longo and Chen, 1985; Maro *et al.*, 1986). More precisely, it has been shown that spindle migration relies on the activity of one specific actin filament nucleator: FORMIN2 (Leader *et al.*, 2002; Dumont *et al.*, 2007b). However, it is only recently that the actin network supporting spindle motility could be visualized in live maturing oocytes, enabling a better understanding of the processes at play (Azoury *et al.*, 2008; Schuh and Ellenberg, 2008). Prior to migration, a fine network of microfilaments is present in the whole cytoplasm. This network is dynamic and constantly remodelled. When spindle migration is initiated, the network becomes denser around the spindle and towards the cortex. Microfilaments run along spindle microtubules forming a tight sheath. This structure follows, or more probably leads, migration until anaphase (Azoury *et al.*, 2008; Schuh and Ellenberg, 2008).

Upon microtubule depolymerization, chromosomes still migrate to the cortex (Longo and Chen, 1985; Maro *et al.*, 1986) via a dense microfilament meshwork (Azoury *et al.*, 2008). In addition, bipolar spindles, formed upon the ablation of the chromosomes, are localized to the periphery of the oocyte (Brunet *et al.*, 1998). Taken together, these data indicate that both chromosomes and microtubules (or microtubule organizing centres) can interact directly with actin microfilaments and contribute to spindle positioning.

Oocytes exhibit numerous small Golgi stacks that are homogeneously dispersed in the cytoplasm. Complete Golgi disorganization upon specific drug treatment was shown to fully inhibit spindle migration (Wang *et al.*, 2008). This observation suggests that individual Golgi stacks may participate in spindle migration by locally anchoring individual microfilaments and promoting a step-by-step translocation of the spindle.

Forces that drive spindle migration rely on microfilament dynamics (Li *et al.*, 2008) and on the activity of actin-associated motors called myosins. Myosin 10 is essential in spindle cortical anchoring in *Xenopus laevis* oocyte, where it functions as a microtubule–microfilament linker (Weber *et al.*, 2004). The myosin 10 transcript is relatively abundant in mouse oocytes (Evsikov *et al.*, 2006), and this motor may

Table I Asymmetry in mammalian oocytes: underlying processes and factors.

Process		Factors	References
Spindle positioning	Spindle robustness[1]	CDC42	Na and Zernicka-Goetz (2006)
	Spindle migration	aPKC	Page Baluch *et al.* (2004)
	Spindle anchoring	FYN	McGinnis *et al.* (2009), Luo *et al.* (2009)
		FORMIN2	Leader *et al.* (2002), Dumont *et al.* (2007b)
		MOS/MAP kinase	Choi *et al.* (1996), Verlhac *et al.* (2000), Phillips *et al.* (2002)
		MLCK	Schuh and Ellenberg (2008)
		Myosin 10	Weber *et al.* (2004)
		Myosin 2	Simerly *et al.* (1998)
Cortical reorganization	Loss of microvilli	RAN	Deng *et al.* (2007)
	Cortical granules exclusion	RAC	Halet and Carroll (2007)
	Actin cap assembly	FYN	Luo *et al.* (2009)

[1]Spindle integrity and robustness are obvious prerequisites for migration and anchoring. Spindle assembly mechanisms are not discussed here, for a complementary review see Dumont and Brunet (2010).

link spindle microtubules to the actin network. In addition, the activated form of myosin 2 concentrates at the meiotic spindle poles (Simerly *et al*., 1998; Schuh and Ellenberg, 2008). This motor may participate in generating pulling forces specifically at the poles during migration. Various myosins most probably generate the forces that drive spindle movement, by pulling both on spindle microtubules and spindle poles.

Myosins are activated by the myosin light chain kinase (MLCK). MLCK inhibition disrupts spindle migration (Simerly *et al*., 1998; Schuh and Ellenberg, 2008). MLCK is a substrate of mitogen-activated protein (MAP) kinase (Morrison *et al*., 1996), whose activity depends in turn on the product of the c-mos proto-oncogene. The Moloney sarcoma oncogene (MOS)/MAP kinase pathway is a central regulator of the meiotic cell cycle (reviewed in Brunet and Maro, 2005). It is also essential for spindle positioning. The absence of spindle migration has initially been observed in oocytes of *mos* knockout mice (Choi *et al*., 1996; Verlhac *et al*., 2000). Inhibition of other members of the pathway yields a similar phenotype (Phillips *et al*., 2002). Therefore, the MOS/MAP kinase pathway, via MLCK, most likely controls the activation of myosins involved in spindle movement. Interestingly, spindle migration is a late event of meiosis I and MAP kinase activity increases progressively after germinal vesicle breaks down, in mouse (Verlhac *et al*., 1994), pig (Inoue *et al*., 1995) and bovine (Fissore *et al*., 1996) oocytes. Kinetics of MAP kinase activation may determine the specific timing of meiosis I spindle takeoff in each mammalian species.

Reorganizing the cortex

As for spindle migration and anchoring, the actomyosin cytoskeleton is central to cortical reorganization. This process is based on the local remodelling of the actin cytoskeleton. Microfilaments accumulate under the plasma membrane and form the so-called actin cap. The exact organization of microfilaments within the cap remains, as yet, unknown.

Local loss of microvilli most probably relies on the dismantling of their actin backbone. Microfilaments control cortical granule redistribution under the plasma membrane (Connors *et al*., 1998; Sun *et al*., 2001). In addition, myosin 2 is enriched in the actin cap of various mammalian oocytes (Simerly *et al*., 1998; Hewitson *et al*., 1999).

Chromosomes are the second key player in this process. In the absence of microtubules, chromosomes still migrate and conserve the capacity to induce cortical reorganization (Maro and Verlhac, 2002). In addition, artificial chromatin-coated beads injected in meiosis II oocytes can induce cortical reorganization (Deng *et al*., 2007). Such reorganization occurs in the absence of any physical interactions between the beads and the cortex. The extent of the newly formed actin cap depends on the amount of injected chromatin beads. It also depends on the beads-to-cortex distance, remote beads failing to induce any cortical reorganization. Taken together, these data demonstrate that chromosomes themselves trigger cortical reorganization by an 'at a distance' effect.

It is now established that this effect depends on the small GTPase, RAN. The GTP-bound and active from of RAN (RAN-GTP) is sequestered in the nucleus during interphase and becomes distributed as a gradient around chromosomes during mitosis, due to localization of its specific GTP exchange factor, RCC1, on chromatin. Within this RAN-GTP gradient, specific factors required for microtubule assembly are activated and promote spindle formation (Caudron *et al*., 2005). Akin to mitosis, the RAN-GTP gradient is generated around the chromosomes in mouse oocytes (Dumont *et al*., 2007a). Alterations to this gradient can abolish cortical reorganization around chromosomes or chromatin beads (Deng *et al*., 2007) demonstrating the priming role for RAN-GTP in this process. This finding also sheds light on the close relation between chromosome migration and cortical reorganization. In mouse oocytes, the RAN-GTP gradient being centred on chromosomes is located far from the cortex at the onset of meiosis I. Chromosomes must be translocated towards the periphery at the end of meiosis I, for cortical reorganization to be initiated by RAN-GTP.

Downstream of RAN, a possible player is a second small GTPase, ras-related C3 botulinum toxin substrate (RAC). RAC is localized uniformly in the cortex of the oocyte, while its active GTP-bound form is only found in the reorganized area. Activation of RAC strictly depends on the chromatin vicinity, suggesting a possible role for the RAN-GTP gradient in this process. Inhibition of RAC activity impairs spindle anchoring, and leads to both detachment of the spindle and to the loss of the actin cap in meiosis II oocytes. Extrusion of the second polar body is eventually inhibited (Halet and Carroll, 2007). Interestingly RAC has been shown to regulate the actin-myosin 2 complex in various biological processes (Bement *et al*., 2006). We can therefore speculate that RAC-GTP, under the control of the RAN-GTP gradient, locally monitors actomyosin activity to induce cortical reorganization.

Src tyrosine kinases are other known regulators of the actin cytoskeleton. The role of one member of this family, FYN, in cortical reorganization has been documented (Luo *et al*., 2009). Nevertheless, since FYN is primarily required for spindle integrity and cell cycle progression (McGinnis *et al*., 2009), the effects on cortical reorganization may not be direct. The mechanisms at play remain to be fully characterized.

PAR proteins: the usual suspects

In numerous model systems, asymmetric divisions are controlled by the PAR proteins (for *PARtitioning defective*). PAR3 and PAR6 form a complex with the atypical protein kinase C (aPKC), which is required for spindle positioning and distribution of developmental cues (Ahringer, 2003). PAR3, aPKC and two PAR6 homologues, PAR6A and PAR6B, are expressed in mouse oocytes. In addition, their localization varies during meiotic maturation (Page Baluch *et al*., 2004; Vinot *et al*., 2004; Duncan *et al*., 2005). aPKC and PAR6A are found on the spindle and at spindle poles during both meiosis I and II. At the end of meiosis I, PAR6A appears to be enriched on the spindle pole facing the closest cortex (Page Baluch *et al*., 2004; Vinot *et al*., 2004). PAR3 and PAR6B are associated with the meiosis I spindle. They both switch to the actin cap, PAR3 relocating before polar body extrusion and PAR6B at meiosis II (Vinot *et al*., 2004; Duncan *et al*., 2005). These PAR proteins are thus never stably associated in the oocyte, in contrast to what has been described in other experimental models. Nevertheless, based on their dynamic localization, hypothetical roles have been proposed for these proteins in the formation of the polar body or polarization of the oocyte. Unfortunately, their functions have not been so far analysed in mammalian oocytes, except for aPKC which is known to be required for meiotic spindle stability (Page Baluch *et al*., 2004). Investigations in

mouse oocytes on CDC42, an important regulator of the PAR complex, have shown a similar role in spindle integrity. Upon inactivation of CDC42, the meiosis I spindle elongates, and cohesion of the poles is lost. First polar body extrusion is eventually impaired (Na and Zernicka-Goetz, 2006). Alterations in egg polarization, like PAR6B mislocalization, are also observed. However, these former effects may result from the primary disorganization of the microtubule structure. Spindle integrity and cortical reorganization appear to be intermingled, and altering one often affects the other (Halet and Carroll, 2007; Luo *et al*., 2009). In conclusion, it is not yet clear whether the PAR complex plays a direct role in oocyte asymmetry or whether it participates in more upstream events like the formation and maintenance of a robust microtubule spindle.

Pre- and post-ovulatory ageing associate with a loss of asymmetry

Egg asymmetry relies on constant and dynamic remodelling of the actin cytoskeleton. This steady state has to be maintained during the metaphase arrest of meiosis II until fertilization occurs. A protracted delay in fertilization is associated with ageing of oocytes. This process, called post-ovulatory ageing is similar in mouse, rat, pig and humans. Upon prolonged metaphase arrest, the actin cap becomes progressively thinner. In parallel, the spindle moves towards the centre of the cell (Webb *et al*., 1986). The whole surface of the oocyte becomes uniform with reduced and homogenous density of microvilli (Peluso *et al*., 1980; Kim *et al*., 1996a, b, c; Sathananthan, 1997; Diaz and Esponda, 2004) and cortical granules are progressively internalized (Diaz and Esponda, 2004).

Maternal age disrupts egg asymmetry in the same way (Sathananthan, 1997; Diaz and Esponda, 2004; Pan *et al*., 2008), suggesting that similar mechanisms are altered. Large-scale analyses of maternal age-associated changes in oocyte gene expression have been performed (Hamatani *et al*., 2004; Steuerwald *et al*., 2007; Pan *et al*., 2008). Myosin 10 (Hamatani *et al*., 2004) and RAN (Pan *et al*., 2008) belong to the factors that are down-regulated with maternal age. Consistently, alterations to the RAN-GTP gradient induce symmetric division of the egg (Dumont *et al*., 2007a). Data generated in these studies will be useful in the future to unravel the molecular basis of age-associated loss of asymmetry.

Loss of asymmetry is deleterious for successful fertilization. In theory, internalized cortical granules cannot be swiftly recruited to the plasma membrane. Upon fertilization, their exocytosis in the perivitelline space and the consequent polyspermy block are compromised. Such oocytes exhibit increased rates of spontaneous activation. Upon experimental activation, they undergo cleavage or fragmentation (Webb *et al*., 1986; Kim *et al*., 1996a, b, c). A clear asymmetric morphology of the egg therefore appears to be a reliable criterion of gamete quality. Moreover, meiosis resumption may define a strict window of time during which fertilization can proceed. Once this window closed, oocyte architecture undergoes irreversible alterations enabling the genesis of a viable embryo.

Fertilization cancels egg asymmetry

Meiotic maturation leads to the progressive setting-up of asymmetry. Fertilization appears as a reverse process and induces the return to a symmetrical organization of the cell, male and female pronuclei being apposed in the centre of the zygote (Fig. 2) and the first mitotic division eventually generating two equal blastomeres (Wu *et al*., 1996; Louvet-Vallee *et al*., 2005).

This process of 'symmetrization' relies mostly on a predominant role of microtubules in the organization of the zygote. In mammals, with the exception of rodents, the sperm centriole persists after fertilization and becomes the main microtubule organizing centre in the zygote (for a review, see Schatten, 1994). It gives rise to a large aster of microtubules associated with sperm chromatin. This aster, called sperm aster, progressively enlarges in the whole cytoplasm. It serves to position progressively both male and female pronuclei at the centre of the cell (Longo, 1976; Simerly *et al*., 1995). In rodents, upon sperm entry the centriole disassembles. Microtubules are nucleated in the cytoplasm and organize in the tight vicinity of pronuclear envelopes. Growing microtubules connected to both pronuclei progressively fill the cytoplasm and promote, through a self-organizing process, pronuclei translocation towards the cell centre. A single aster emanating from the two apposed pronuclei is eventually observed (Schatten *et al*., 1985) as in non-rodent mammals. In myoblasts, microtubule-nucleating factors are concentrated to the outer surface of the nucleus. Microtubule assembly is restricted at the nuclear surface (Bugnard *et al*., 2005; Fant *et al*., 2009), the nucleus acting as a large microtubule organizing centre. The similar process may occur in rodent eggs. Despite morphological differences, microtubule structures emanating from the pronuclei in the rodents may be functionally identical to centriolar sperm asters observed in other mammals.

In monkey and bovine oocytes, pronuclei centring depends on dynein activity (Payne *et al*., 2003a, b). Dynein is a molecular motor which transports macromolecules or organelles on microtubules towards their (−) extremities. This activity is required for nucleus positioning in various and either centriolar or acentriolar model systems (for a review, see Reinsch and Gonczy, 1998). These observations strongly suggest that universal and dynein-dependant mechanisms ensure pronuclei centring and zygote 'symmetrization' in all mammalian species.

Concluding remarks

In mammals, the asymmetry of the meiotic divisions is essential for the formation of a functional female gamete. It ensures the generation of tiny polar bodies and a large oocyte, so that only the oocyte retains the ability to be fertilized. It leads to an asymmetric organization (or polarization) of the egg, which determines the geometry and the success of fertilization. Loss of asymmetry is a mark of low-quality oocytes and a signature of pre- and post-ovulatory ageing. Dissecting the mechanisms that control this asymmetry is therefore a major goal for cell and reproductive biologists. Recently, large scales and comparative transcriptomic analysis have been performed on mammalian oocytes. In parallel, high-resolution imaging in live maturing oocytes has been successfully developed. We believe that such complementary approaches will enable us to better understand the molecular mechanisms that are altered with ageing, and also to extend the analyses to a large variety of mammalian oocytes.

Acknowledgements

We thank M.E. Terret for critical reading of the manuscript.

Funding

S.B. is an INSERM fellow. This work was supported by grants from the *Ligue Nationale Contre le Cancer* (EL/2009/LNCC/MHV) and from the *Agence Nationale pour la Recherche* (ANR08-BLAN-0136-01 to M.H.V.).

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