REPRODUCTION
REVIEW

Focus on Meiosis

Cytoskeleton and cell cycle control during meiotic maturation of the mouse oocyte: integrating time and space

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Abstract

During meiotic maturation of mammalian oocytes, two successive divisions occur without an intermediate phase of DNA replication, so that haploid gametes are produced. Moreover, these two divisions are asymmetric, to ensure that most of the maternal stores are retained within the oocyte. This leads to the formation of daughter cells with different sizes: the large oocyte and the small polar bodies. All these events are dependent upon the dynamic changes in the organization of the oocyte cytoskeleton (microtubules and microfilaments) and are highly regulated in time and space. We review here the current knowledge of the interplay between the cytoskeleton and the cell cycle machinery in mouse oocytes, with an emphasis on the two major activities that control meiotic maturation in vertebrates, MPF (Maturation promoting factor) and CSF (Cytostatic factor).

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Introduction

In most animal species, sexual reproduction requires the fusion of two haploid gametes: the spermatozoon and the oocyte. Meiosis, which ensures the formation of these highly specialized cells, is a long process. In mammalian oocytes, the short period called meiotic maturation, which concludes meiosis, is absolutely crucial for the production of a functional gamete. During this period, the oocyte undergoes two cellular divisions without an intermediate phase of DNA replication. These divisions consist of a sequence of cellular events that are controlled by the cytoskeleton of the oocyte and are highly regulated in time (Fig. 1). Microtubules form the spindle and segregate homologous chromosomes during the first meiotic division (MI, a reductional division) and sister chromatids during the second division (MII, an equational division). In addition, spindle microtubules and actin microfilaments control the asymmetry of these meiotic divisions. Both divisions produce a small cell called the polar body and a large cell, the oocyte, which keeps its size and the entire maternal stores accumulated during oogenesis.

During the past few years, the understanding of the principles at play during the meiotic divisions in oocytes has been improved, mostly due to *in vivo* studies in mouse oocytes and *in vitro* analyses achieved in cytoplasmic egg extracts. In the light of these recent results, we review here our current knowledge on the mechanisms that control the organization of the cytoskeleton in time and space during meiotic maturation of mammalian oocytes.

Spindle assembly in the oocyte relies on chromosomes

Chromosomes control bipolar spindle assembly

During mitosis, spindle assembly is directed to a large extent by the centrosomes, the main sites of microtubule polymerization (Ou & Rattner 2004). At the onset of mitosis, the single centrosome has been duplicated and the two centrosomes separate and migrate to opposite sides of the nucleus. As a consequence, as soon as the nuclear envelope breaks down, growing microtubules emanating from both centrosomes interact with the chromosomes and become rapidly organized into a bipolar spindle. Oocytes lack centrosomes and the microtubules are polymerized at discrete sites in the cytoplasm called MTOCs (Microtubule organizing centers). In the mouse oocyte, where multiple MTOCs are dispersed in the cytoplasm,

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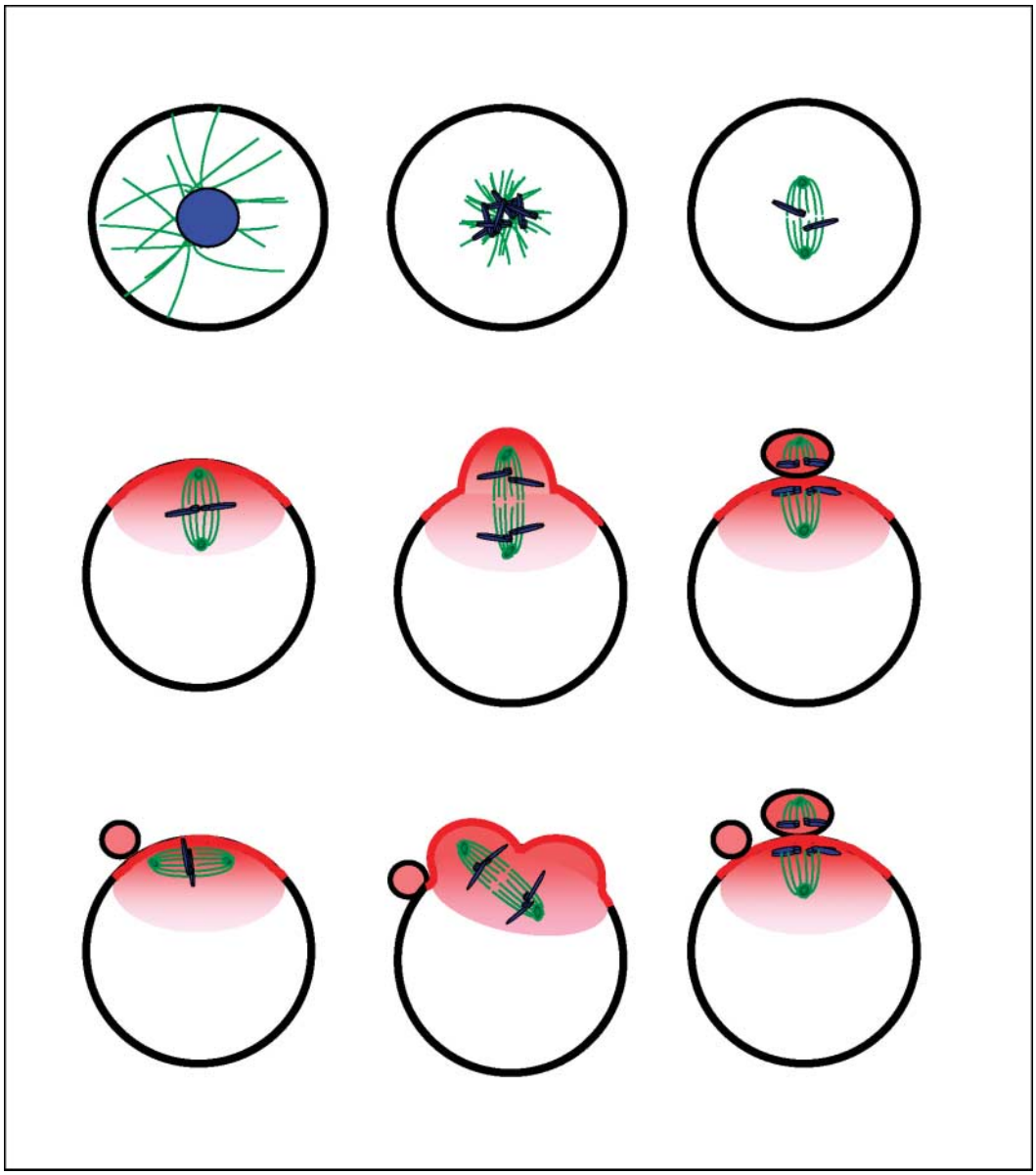
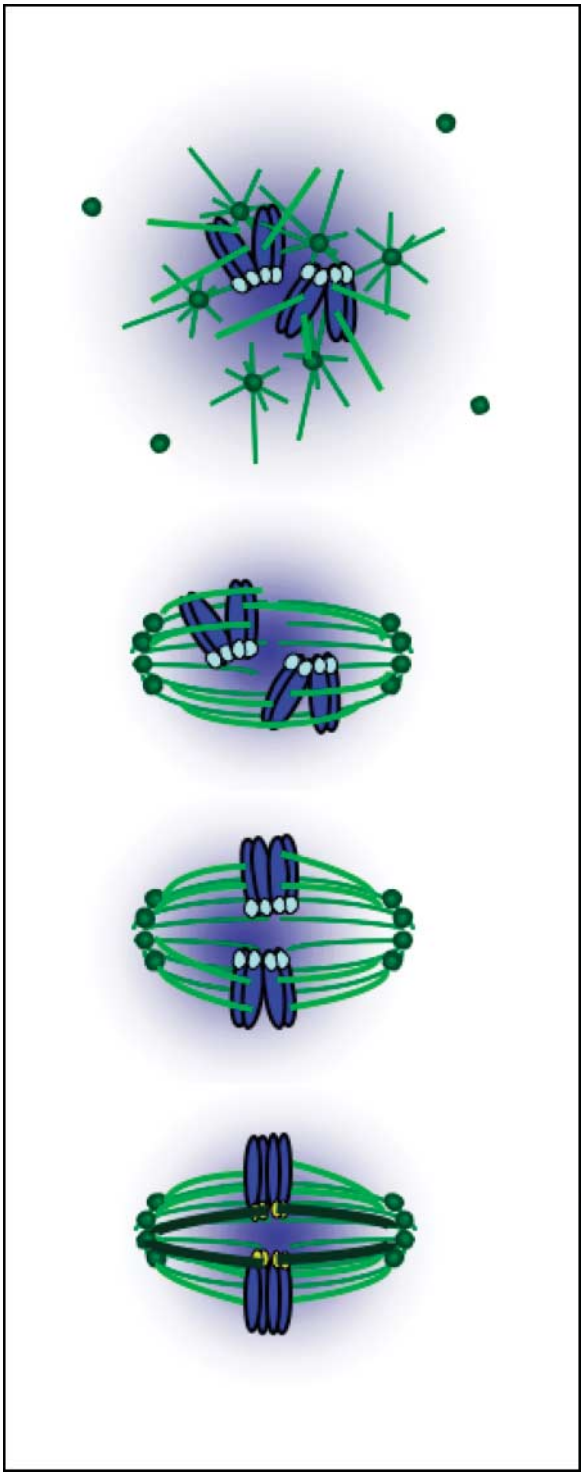


Figure 1 The cellular events of meiotic maturation in mouse oocytes. At GVBD (first row), microtubules (in green) polymerize radially around the mass of chromosomes and organize progressively into a bipolar spindle. At the end of MI (second row), the bipolar spindle migrates toward the oocyte cortex. Spindle migration induces the local reorganization of the cortex of the oocyte (red and pink area). The first polar body is extruded in the axis of spindle migration. During MII arrest (third row), the metaphase spindle is anchored under the plasma membrane. The cortex reorganization is maintained in the vicinity of the spindle. Fertilization or experimental activation of the oocyte triggers a 90° spindle rotation and the extrusion of the second polar body.

chromosomes take the lead in spindle assembly. At the onset of MI, just after GVBD (Germinal vesicle breakdown), MTOCs are preferentially activated and/or recruited in the vicinity of the chromosomes and microtubules are preferentially stabilized in this area. Randomly oriented growing microtubules are then progressively organized into a bipolar array around the chromosomes (Fig. 2).

The respective roles of chromosomes, microtubules and associated factors in meiotic spindle assembly have been enlightened by the study of the microtubules in mouse oocyte fragments lacking chromosomes, called cytoplasts (Brunet *et al.* 1998). In cytoplasts, microtubules assemble stable bipolar spindles. This observation indicates that in mouse oocytes, microtubules have the potential to polymerize and organize in the absence of chromosomes into bipolar structures due to the activities of motor proteins and MAPs (Microtubule associated proteins). This property plays an essential role in spindle formation in the mouse oocyte. Often, several bipolar spindles form in mouse

oocyte cytoplasts, demonstrating that chromosomes are necessary to restrain MTOCs activity and microtubule organization in their vicinity. This restriction is crucial to form a unique spindle in the large volume of the oocyte. Moreover, the size of the observed bipolar spindles can vary from one cytoplast to the next. This indicates that chromosomes are involved in the control of the size of the spindle, most probably through local activation of microtubule stabilizing factors. Recently, a central role of chromosomes in spindle assembly has been established in other experimental systems. This role is required for spindle assembly with or without centrosomes and is based on the activity of the small GTPase 'Ran' (Karsenti & Vernos 2001, Kalab *et al.* 2002, Zheng 2004). During M phase, Ran, bound to GTP (RanGTP) is concentrated in the vicinity of the chromosomes and locally activates various factors required for spindle formation (Hetzer *et al.* 2002, Caudron *et al.* 2005). This mechanism is most probably at play during the meiotic divisions of the mouse oocyte. Among the characterized effectors of RanGTP, TPX2 (Targeting protein for Xenopus kinesin-like protein 2) is responsible for a RanGTP dependant microtubule polymerization around the chromosomes (Wittmann *et al.* 2000, Gruss *et al.* 2001). TPX2 is expressed in the mouse oocyte and is associated to the spindle microtubules, as it is in other systems. TPX2 is likely involved in spindle formation in the mouse oocyte by supporting microtubule assembly in the vicinity of the chromosomes (J Dumont and M H Verlhac, personal communication). The other RanGTP effectors involved in spindle assembly by stabilizing microtubules or controlling microtubule organization have not been studied yet in mouse oocytes. DOC1R (Deleted oral cancer 1 related), initially identified in the mouse oocyte as a MAP kinase substrate, may be a novel RanGTP effector (Terret *et al.* 2003*a*). This protein interacts with importins (M E Terret and M H Verlhac, personal communication) as shown for other RanGTP effectors. It is localized on the spindle during the meiotic M phases. DOC1R depletion in the oocyte induces the formation of microtubule asters in the whole cytoplasm (Terret *et al.* 2003*a*). DOC1R may be required to restrain the RanGTP gradient in the vicinity of the chromosomes in the oocyte.



Chromatin controls metaphase plate formation

Once bipolar spindle assembly is achieved, chromosomes align on the spindle equator and form the metaphase plate. During mitosis, this alignment is monitored by the

Figure 2 Chromosomes monitor MI spindle assembly in mouse oocyte. Chromosomes (in blue) locally restrain the assembly and organization of microtubules. MTOCs (in dark green) are locally activated and RanGTP (blue gradient) triggers the polymerization of microtubules around the chromosomes (in light green). The microtubules progressively organize into a bipolar structure around the chromosomes. The kinetochores associated with the bivalents are inactive during most of the MI (light blue discs). As a consequence, bivalents migration toward the spindle equator is achieved by direct interactions between chromosome arms and spindle microtubules. At the end of MI, kinetochores are activated (yellow discs) and become able to anchor microtubules and form kinetochore fibers (in dark green): the complete formation of K-fiber correlates with a brief metaphase transition and triggers anaphase onset.

kinetochores, structures associated with the centromeres of both sister chromatids. Kinetochores capture, stabilize microtubules and form robust 'kinetochore fiber' (or K-fiber). When K-fibers connect both kinetochores of one chromosome to the opposite spindle poles, the chromosome is transported to the equator of the spindle (Biggins & Walczak 2003). In mouse oocytes, during the first meiotic M phase, bivalent chromosomes alignment on the metaphase plate involves alternative mechanisms. In contrast to the situation described in mitosis, the kinetochores associated with bivalents are not competent for anchoring and/or stabilizing microtubules during most of the first meiotic M phase. However, in the absence of K-fibers, bivalent chromosomes are nevertheless transported towards the equator of the spindle and maintained in this area for a few hours (Brunet *et al.* 1999). It has been shown that spindle microtubules exert pushing forces on the chromosome arms. These forces, also called 'polar wind', are mediated by microtubules motors associated with chromatin (Brunet & Vernos 2001). Two chromatin-associated motors Kif 4 and Kif 22, the respective homologs of the *Xenopus* kinesin-like proteins Xklp1 and Xkid, are likely required for bivalent congression in the oocyte. Xklp1 can anchor the microtubules to the chromosome arms by 'freezing' the dynamic properties of the microtubules contacting the arms (Vernos *et al.* 1995, Bringmann *et al.* 2004). Xkid is necessary for chromosome arms congression (Antonio *et al.* 2000, Funabiki & Murray 2000). Although in somatic cells the polar wind is not essential (Levesque & Compton 2001), it governs chromosome congression in the oocyte.

After this long prometaphase, the activation of the kinetochores triggers the formation of K-fibers leading to the accurate alignment of the chromosomes on the metaphase plate (Brunet *et al.* 1999). The mechanism leading to the late activation of the kinetochores in mouse oocytes remains elusive. Kinetochores may be submitted to a very slow and original maturation. Molecular components of the kinetochore including members of the SAC (Spindle assembly checkpoint) machinery (see below) or motors like the kinesin CENP-E are present on kinetochores just after GVBD. This suggests that kinetochore maturation is not regulated by the recruitment of kinetochore components but more likely by post-translational modifications of some of these factors. MI duration is determined by the kinetics of MPF (Maturation promoting factor) activity (see below). A high level of MPF activity, only reached late in MI, could induce post-translational modifications of kinetochore components leading to the setting up of K-fibers. In fact, K-fiber formation remains one of the 'black boxes' of Mitosis. Only a few proteins involved in this process have been characterized (Biggins & Walczak 2003) and their role and regulation during MI remain to be studied in oocytes.

The asymmetry of the oocyte divisions depends on chromosomes

Spindle motility depends on interactions between actin and the chromosomes

Both meiotic divisions of the mouse oocyte are asymmetric. They produce a small cell called the polar body and the oocyte, which conserves its original size. Such asymmetry is ensured by the positioning of the spindle in the periphery of the large oocyte. The MI spindle generally forms in the center of the oocyte and migrates toward its periphery (Longo & Chen 1985, Maro & Verlhac 2002). The first polar body is extruded in the axis of the migration. The MII spindle forms in the periphery of the oocyte and is maintained under the plasma membrane during the metaphase arrest. Fertilization or experimental activation triggers spindle rotation and the extrusion of a second polar body (Maro & Verlhac 2002, Maro *et al.* 1984). Our knowledge on the mechanisms of asymmetric divisions stems from investigations on mitotic cells (Betschinger & Knoblich 2004) where spindle positioning depends on interactions between the cell cortex and 'astral' microtubules that connect the spindle poles to the cell cortex (Cowan & Hyman 2004). Oocyte lack centrosomes and the spindles lack in turn astral microtubules: alternative mechanisms must be at play to position the spindle within the oocyte. In mouse oocytes, spindle migration and anchoring require actin microfilaments but not microtubules (Longo & Chen 1985, Maro *et al.* 1986, Van Blerkom & Bell 1986, Verlhac *et al.* 2000*a*, Leader *et al.* 2002, Maro & Verlhac 2002), in contrast to what was described in oocytes from other species (Weber *et al.* 2004, Yang *et al.* 2005). In mouse oocytes, these processes rely on original interactions between the microfilaments and the chromosomes themselves (Maro *et al.* 1986, Van Blerkom & Bell 1986, Verlhac *et al.* 2000*a*, Maro & Verlhac 2002), but the molecular basis of such interactions are so far unknown. First, actin microfilaments organization and dynamics in the oocyte remains to be characterized. Are actin-associated motors (myosins) required? A possible role of myosin II in polar body formation has been proposed (Simerly *et al.* 1998) but the mechanisms involved remain to be elucidated. What are the components linking chromosomes to the actin network? PARD6A, a member of the PAR family (PARtitioning defective; Ahringer 2003) may be involved in this process. During MI, PARD6A is concentrated on the spindle half that leads the migration. Upon microtubule depolymerization it concentrates on the surface of the chromosomes oriented toward the cortex (Vinot *et al.* 2004). It may be part of a multi-protein complex coupling chromosomes, microtubules and actin microfilaments and support spindle motility and anchoring.

Chromosomes control the cortical reorganization of the mouse oocyte

In mouse oocytes, the eccentric position of the spindle is associated with a local reorganization of the oocyte cortex (Fig. 1). This cortical domain appears during spindle migration and is maintained over the spindle during MII. Reorganization is marked by a local loss of microvilli (Johnson *et al.* 1975), an accumulation of actin microfilaments under the plasma membrane (Maro *et al.* 1984, Longo & Chen 1985) and the exclusion of cortical granules (Deng *et al.* 2005). The function of this process is unclear. It may serve to generate a restriction domain for the assembly of the contractile actin ring and cytokinesis in order to minimize the size of the polar body. The cortical reorganization depends on the actin network, on the chromosomes but not on microtubules (Maro *et al.* 1986, Van Blerkom & Bell 1986, Verlhac *et al.* 2000*a*, Maro & Verlhac 2002). In addition, it does not require physical interactions between the chromosomes and the cortex (Maro & Verlhac 2002). Thus, the chromosomes themselves trigger this reorganization by an 'at distance' effect. The molecular mechanisms at play are so far unexplored.

In conclusion, the asymmetry of the mouse oocyte divisions depends an original role of the chromosomes on the organization of the actin network. Direct interactions between chromosomes and actin govern spindle positioning. In addition, chromosomes mediate cortical actin reorganization by an 'at distance' effect. The similarity with the role of chromosomes in microtubule organization in the oocyte is striking. As far as microtubules are concerned, chromatin associated motors mediate physical interactions between the chromosomes and the spindle microtubules. In addition, chromosomes control in their vicinity the activation of factors required for spindle assembly (Karsenti & Vernos 2001, Kalab *et al.* 2002, Zheng 2004). On the basis of these similarities, we propose that in mouse and more generally in mammalian oocytes, chromosomes act as a 'territory landmark' to organize both microtubules and actin microfilaments within the large cytoplasm. This spatial control is essential to achieve the two asymmetric meiotic divisions that lead to the formation of a functional gamete.

Cyclin B coordinates meiotic maturation

During the cell cycle, M-phase is controlled through the activation and inactivation of the MPF (Masui & Markert 1971), composed of a kinase, $p34^{cdk1}$ and its regulatory sub-unit, cyclin B (Lohka *et al.* 1988, Doree & Hunt 2002). The modulation of cyclin concentration by synthesis and degradation is of central importance for the control of MPF activity (Murray & Kirschner 1989). The mitotic cyclins are synthesized throughout the cell cycle and destroyed during a short period at the metaphase–anaphase transition (Evans *et al.* 1983) by the 6 ubiquitin pathway (Glotzer *et al.* 1991, Hershko *et al.* 1991). In the oocyte, all the cellular events taking place during meiotic maturation and leading to the two asymmetric cell divisions have to be ordered in a timely manner. The control of the timing of meiotic maturation most likely depends on cyclin B. Changes in cyclin B levels, through changes in MPF activity, regulate not only the timing of the cell cycle phases during meiosis but also the orderly events leading to the formation of functional meiotic spindles and asymmetric divisions.

Cyclin B metabolism controls the timing of meiotic maturation

MPF is activated at GVBD (Fig. 3) and increases until it reaches a plateau at the end of the first meiotic M-phase (Choi *et al.* 1991, Verlhac *et al.* 1994). A transient decline in MPF activity takes place during the transition between meiosis I and meiosis II. MPF is reactivated rapidly to enter meiosis II and is maintained at a high level during the metaphase II arrest.

The immature oocyte contains only a small amount of cyclin B, just enough to induce entry into the first meiotic M-phase (Hampl & Eppig 1995, Winston 1997,

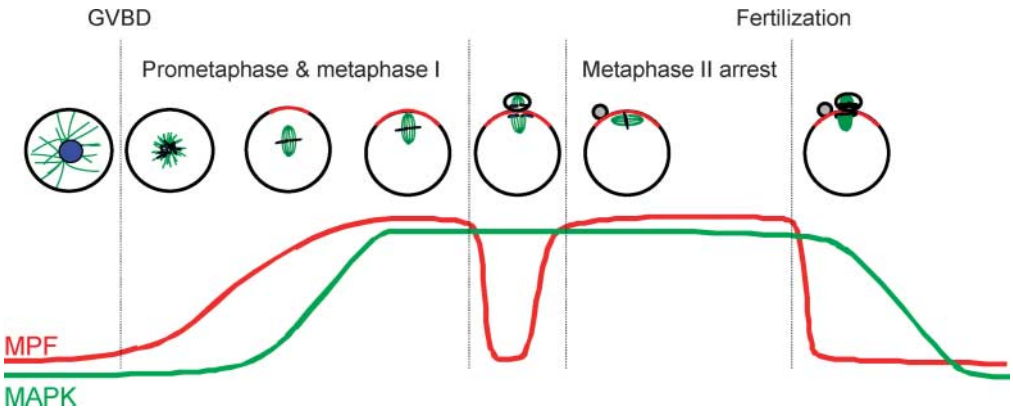


Figure 3 MPF and MAPK activities during meiotic maturation in mouse oocytes. MPF activity appears as a red line and MAPK as a green line. The different steps of meiotic maturation are schematized as in Figure 1.

Hashimoto & Kishimoto 1988, Ledan *et al.* 2001). It enters the germinal vesicle just prior to GVBD (Marangos & Carroll 2004). After GVBD, the level of synthesis of cyclin B increases progressively, reaching its maximum at the end of the first meiotic M phase, and the newly synthesized protein becomes associated immediately with the $p34^{cdk1}$ kinase to form an active complex (Hampl & Eppig 1995, Winston 1997, Ledan *et al.* 2001). Cyclin B degradation is required for polar body extrusion (Ledan *et al.* 2001, Herbert *et al.* 2003, Terret *et al.* 2003*b*). Thus, MPF activity is regulated by a translation-dependent mechanism that determines the level of cyclin synthesis.

The role of cyclin B1 synthesis in the control of the duration of meiotic maturation was demonstrated using two strains of mice, CBA/Kw and KE, which differ greatly in the timing of meiotic maturation (Polanski *et al.* 1998). KE oocytes take approximately 3–4 hours longer than CBA/Kw oocytes to extrude the first polar body. The rate of cyclin B1 synthesis during prometaphase I is higher in CBA/Kw than in KE oocytes although the overall level of protein synthesis and the amount of cyclin B1 messenger RNA are identical in both strains, suggesting that cyclin B1 translation is controlled differently in these two strains (Polanski *et al.* 1998). Among the different mechanisms that control the expression of maternal mRNAs, polyadenylation has been implicated in cyclin B1 translation in Xenopus and mouse oocytes (Barkoff *et al.* 2000, de Moor & Richter 1999, Ledan *et al.* 2001, Tay *et al.* 2000). Finally, increasing cyclin B1 synthesis in KE oocytes speeds up first polar body extrusion (Ledan *et al.* 2001, Polanski *et al.* 1998).

The formation of the first meiotic spindle is regulated by cyclin B levels

During MI, the formation of a functional spindle is a very slow process. These kinetics correlate with the progressive increase in MPF activity (Polanski *et al.* 1998, Fig. 3). The MPF activity required for GVBD (sufficient for entry into M-phase) only allows the formation of a single aster of microtubules around the condensed chromosomes (Fig. 4). A first threshold in MPF activity is then required to organize the microtubules into a bipolar structure. In contrast, the further migration of the chromosomes toward the vicinity of the spindle equator does not depend on changes in MPF level. A second threshold in MPF activity is required at the end of MI for the activation of the kinetochores (Polanski *et al.* 1998, Brunet *et al.* 1999), it allows the capture and stabilization of microtubules by the kinetochores and the further assembly of robust K-fibers. The setting up of the whole set of K-fibers is rapidly followed by anaphase onset. These data indicate that MPF activity controls the formation of a functional spindle in the oocyte. MPF may also indirectly control the position of the spindle. Like kinetochore activation, spindle migration is only initiated once MPF activity has reached a high level. Thus, MPF may control the activity of proteins associated to the microfilaments (Satterwhite *et al.* 1992) and in turn induce spindle migration: the mechanisms and the molecules involved remain to be investigated.

Coupling time and space

In mitosis, a quality control mechanism called the spindle assembly checkpoint ensures accurate chromosome

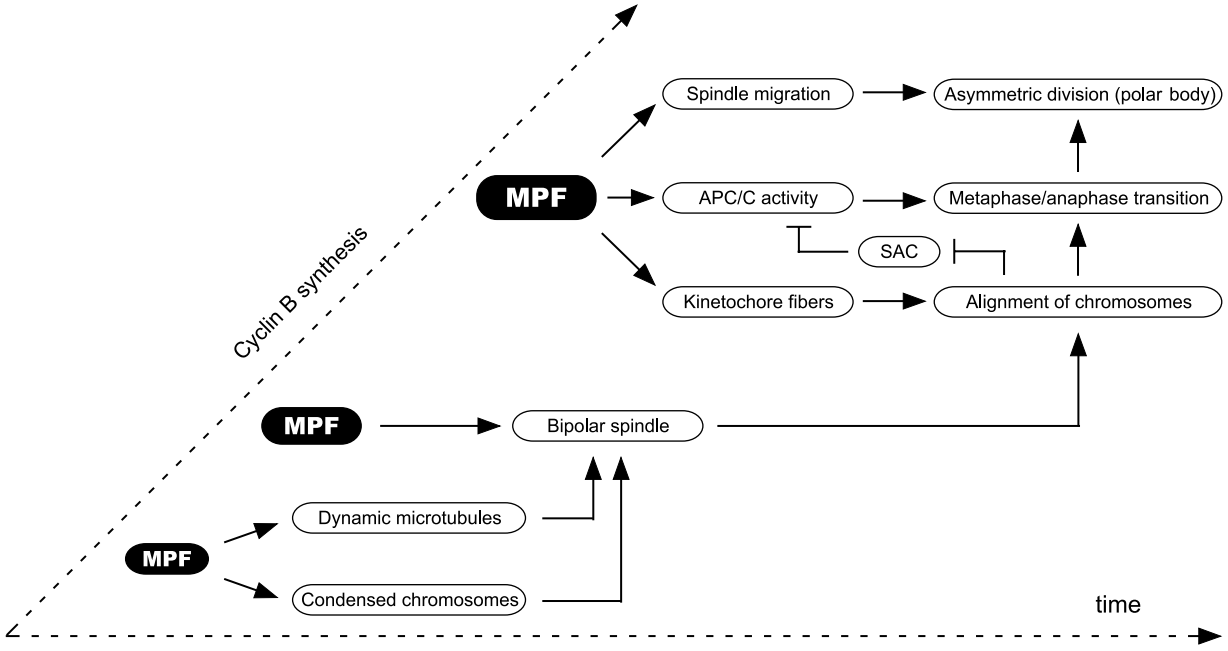


Figure 4 Cyclin B synthesis controls the timing of meiotic maturation through the level of MPF activity.

segregation by delaying anaphase onset until all the chromosomes are correctly attached to the spindle through their kinetochores. The spindle checkpoint prevents anaphase by inhibiting the anaphase promoting complex/cyclosome (APC/C), an E3 ubiquitin ligase whose activity is required for cyclin B and securin degradation. The APC/C is therefore required for anaphase onset and exit from mitosis (Taylor *et al.* 2004). This checkpoint depends on the activity of Bub (Budding uninhibited by benzimidazole) and Mad (Mitotic arrest-deficient) kinetochore proteins (Hoyt *et al.* 1991, Li & Murray 1991). Until recently it was generally accepted that mammalian female meiotic divisions are error prone because they lack a functional spindle checkpoint (in humans, an estimated 20% of concepti have an abnormal chromosomal content as a consequence of errors occurring during female meiosis I). It has been now clearly established that the activity of the APC/C is required for meiotic progression and that the spindle assembly checkpoint is active in mouse oocytes (Brunet *et al.* 2003) through the checkpoint components Bub1 (Tsurumi *et al.* 2004) and Mad2 (Wassmann *et al.* 2003, Tsurumi *et al.* 2004, Homer *et al.* 2005).

As expected, inactivation the spindle assembly checkpoint in mouse oocytes accelerates progression through MI. However, only a 2–3 hours shortening of the first meiotic M-phase takes place (Tsurumi *et al.* 2004, Homer *et al.* 2005), suggesting that the APC/C is inactive during most of the first meiotic M-phase. Thus, activation of the APC/C would only occur when MPF activity has reached the second threshold level, also required for activation of the kinetochores (Polanski *et al.* 1998, Brunet *et al.* 1999). The spindle assembly checkpoint does not control the timing of the first meiotic M-phase but rather delays the metaphase–anaphase transition until the spindle microtubules are attached to the kinetochores and the chromosomes are properly aligned on the metaphase plate (Fig. 4).

Since the metaphase–anaphase transition takes place when the spindle has reached the oocyte cortex (Verlhac *et al.* 2000*a*), one may wonder whether a checkpoint exists to monitor the position of the spindle. Such a checkpoint exists in budding yeasts: the mitotic exit network (MEN) verifies the correct positioning of one spindle pole in the newly formed bud (D'Amours & Amon 2004). This is unlikely to be the case in mouse oocytes: in $mos^{-/-}$ oocytes while the spindle does not migrate, the metaphase–anaphase transition still happens at the right time (Verlhac *et al.* 1996, Verlhac *et al.* 2000*a*) leading to the formation of large polar bodies. Large polar bodies were also observed when meiotic maturation is accelerated by inactivation of the spindle assembly checkpoint (Homer *et al.* 2005).

Thus cyclin B levels, through the regulation of MPF activity, seems to synchronize the different events leading to the formation of the polar body (Fig. 4): setting up of the K-fibers (required for the final alignment of the chromosomes on the metaphase plate), activating the APC/C (required for chromosome separation and exit from the first meiotic M-phase) and spindle migration (required for asymmetric division). The time required for the formation of kinetochore fibers and the subsequent alignment of the pairs of homologous chromosome will allow spindle migration before the inactivation of the spindle assembly checkpoint leading to the metaphase–anaphase transition.

The peculiar case of the metaphase II spindle

In contrast to the first meiotic division, the entry into the second one is similar to mitosis: MPF activity increases rapidly and the spindle forms quickly. Moreover, the meiosis II chromosomes are identical to mitotic chromosomes, composed of sister chromatids with active kinetochores. However, the oocyte arrests at metaphase for many hours until fertilization, with the chromosomes perfectly aligned on the metaphase plate and high MPF activity. This metaphase arrest is maintained through an activity called the Cytostatic factor (CSF; Masui & Markert 1971).

The presence of CSF was demonstrated in vertebrate oocytes by transferring cytoplasm from a metaphase II arrested oocyte into cleaving frog (Masui & Markert 1971) and mouse (Masui & Markert 1971, Kubiak *et al.* 1993) embryos, leading to a cell cycle arrest in mitosis. CSF activity requires the activation of the Mos–MAP kinase pathway (Sagata *et al.* 1989, Haccard *et al.* 1993, Colledge *et al.* 1994, Hashimoto *et al.* 1994, Verlhac *et al.* 1996). The signaling pathway emerging from the Xenopus work performed mainly in oocyte extracts looks like a linear track, from Mos synthesis to the APC/C inhibitor Mad2 (Tunquist & Maller 2003). The Mos pathway in mouse oocytes (Fig. 5), emerging from *in vivo* studies performed using $Mos^{-/-}$ oocytes, is more complex (Verlhac *et al.* 1996, Verlhac *et al.* 2000*b*, Lefebvre *et al.* 2002, Terret *et al.* 2003*a*, Dumont *et al.* 2005). Although it was thought that the only requirement to induce a proper metaphase arrest was to maintain a high MPF activity, recent work demonstrated that the organization of the spindle has to be maintained by specific mechanisms (Fig. 5).

Keeping a metaphase spindle

Metaphase is a transient state of mitosis. In CSF arrested oocytes, it can last for many hours or even days. While spindle microtubules turn over rapidly, as in mitosis, the meiotic spindle remains as a stable structure during the arrest (de Pennart *et al.* 1988, Gorbsky *et al.* 1990), with chromosomes perfectly aligned on the equator of the spindle (Brunet *et al.* 1999). Specific mechanisms and components are required to maintain such a stable structure (Lefebvre *et al.* 2002, Terret *et al.* 2003*a*). MISS (MAP kinase-interacting and spindle-stabilizing protein) and DOC1R are two MAP kinase substrates associated with the spindle in metaphase II arrested oocytes (Lefebvre *et al.* 2002, Terret *et al.* 2003*a*). DOC1R accumulates during meiotic maturation while MISS is only present during MII. DOC1R depletion leads to the formation of elongated spindles enriched in astral microtubules with numerous

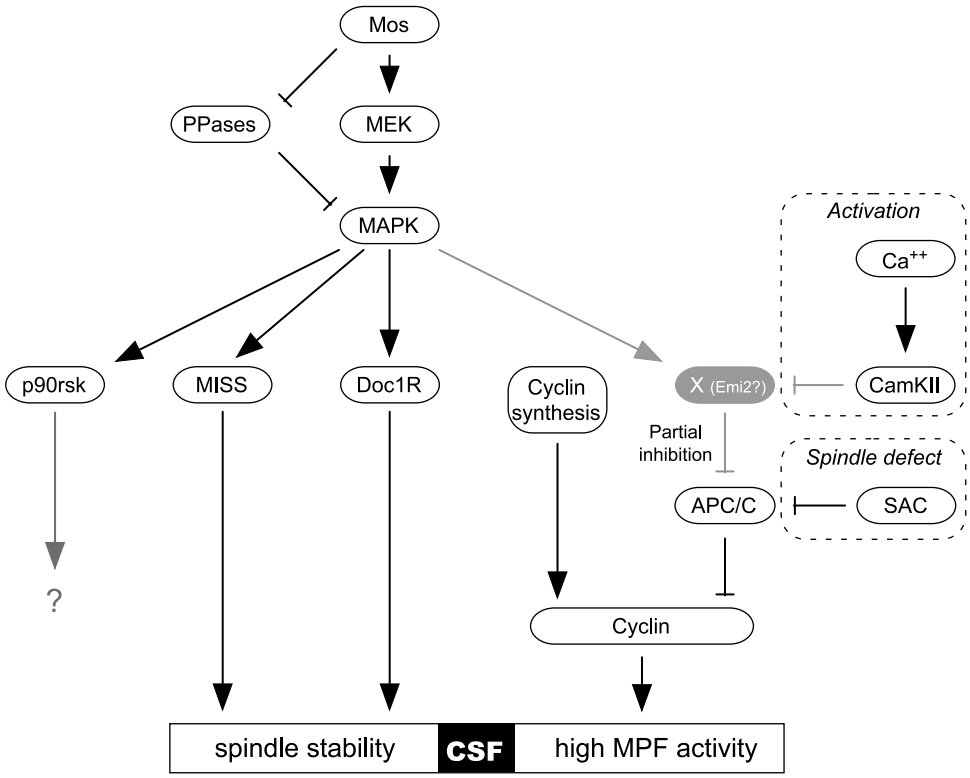


Figure 5 The metaphase II arrest in mouse oocytes. The components and interactions shown in grey remain to be discovered.

asters of microtubules in the cytoplasm during MII. Oocytes depleted for MISS show disorganized MII spindles often lacking one pole, or have long microtubules emanating from both poles and cytoplasmic asters. In both cases, the MII spindle forms normally but become later disorganized indicating a role for both proteins in the maintenance of the spindle structure during the arrest. MISS and DOC1R are regulated by multiple phosphorylations, through the activity of MAP kinase and other kinases, likely MPF. Thus, cooperation between MPF and the MAP kinase pathway (leading to CSF activity) is at play to maintain the spindle structure when the metaphase state is highly prolonged.

Keeping MPF high

Again, cyclin metabolism plays a key role during the metaphase II arrest (Fig. 5). It is supported throughout the continuous balanced synthesis and degradation of cyclin (Kubiak *et al.* 1993). The equilibrium between these two processes is dependent upon CSF that slows down degradation (Kubiak *et al.* 1993) and the continuous synthesis of cyclin B that is maintained at the highest level (Winston 1997). After first polar body extrusion cyclin degradation stops (Ledan *et al.* 2001) and the APC/C is only reactivated upon entry into the second meiotic M-phase by the high level of MPF. Cyclin degradation can only take place in the meiosis II oocyte once spindle formation has been completed and the chromosomes aligned on the metaphase plate, thus removing the inhibitory effect of the spindle assembly checkpoint. During the CSF arrest, the SAC is inactive, but it can be reactivated when spindle organization is perturbed, leading to a complete inhibition of the cyclin degradation pathway (Kubiak *et al.* 1993, Winston *et al.* 1995, Winston 1997). It was proposed in *Xenopus* that the CSF arrest was mediated through the activity of p90rsk (Bhatt & Ferrell 1999, Gross *et al.* 1999) and the spindle assembly checkpoint proteins Bub 1 and Mad 2 (Tunquist *et al.* 2002, Tunquist *et al.* 2003), downstream of the Mos–MAP kinase pathway. However, in mouse oocytes, it was demonstrated that neither p90rsk (a characterized MAP kinase substrate in mouse oocytes; Kalab *et al.* 1996), nor Bub1 or Mad2 are required for the metaphase II arrest (Tsurumi *et al.* 2004, Dumont *et al.* 2005). Thus, the APC/C inhibitor responsible for the maintenance of a high level of MPF activity during the metaphase II arrest remains to be identified. The most likely candidate is Emi2/Xerp1 (Liu & Maller 2005, Rauh *et al.* 2005, Schmidt *et al.* 2005, Tung *et al.* 2005), although it does not seem to be regulated by the Mos–MAP kinase pathway in *Xenopus* egg extracts (Schmidt *et al.* 2005). Emi2/Xerp1 is a target of CamKII, a kinase that is transiently activated by Ca^{++} at fertilization and mediates CSF inactivation (Lorca *et al.* 1993, Winston and Maro 1995).

Thus, the oocyte during the metaphase II arrest is in a very dynamic state, with highly dynamic spindle microtubules keeping all the chromosomes perfectly aligned on the metaphase plate (Brunet *et al.* 1999), with a stable

level of MPF dependent upon the constant synthesis of cyclin B counterbalanced by regulated degradation (Kubiak *et al.* 1993). These equilibriums are regulated by downstream targets of the Mos–MAP kinase pathway, some of them remaining to be identified.

Conclusion

The production of functional female gametes is essential for the propagation of all mammalian species. It is dependent to a large extent on the dynamic organization of the oocyte cytoskeleton during the two successive meiotic divisions. Defects in the cytoskeleton organization during these divisions can first lead to chromosome segregation errors with dramatic consequences. In humans, it is estimated that 15–20% of oocytes display chromosome abnormalities linked to segregation errors (Pellestor *et al.* 2005). Moreover, at least 5% of all pregnancies are aneuploid as a result of such errors in oocytes, that strongly correlate with increased maternal age (Hassold & Hunt 2001). The recent demonstration of the existence of a functional spindle checkpoint in mammalian oocytes is essential. It implies that in addition to spindle checkpoint deficiencies (intensively studied in mitotic systems), other uncharacterized mechanisms contribute to the high frequency of missegregations in the mammalian oocyte. The elucidation of the chromosome-dependant mechanisms controlling microtubule and actin networks organization in the oocyte may be of a great importance to identify the cellular and molecular basis of aneuploidy.

In addition, the formation of a mature oocyte also relies on microtubule and actin microfilament-dependent processes. Anomalies in any of these processes can prevent the production of competent oocytes and lead to fertility problems. A striking example is the female sterility of *formin 2* knockout mice, *formin 2* encodes an actin-polymerizing protein involved in spindle migration in the oocyte (Leader *et al.* 2002). Similarly, cytoskeleton-dependent asymmetry of the meiotic division maintains the maternal stores accumulated during oogenesis in the oocyte (Matzuk *et al.* 2002). Loss of asymmetry in the meiotic division, and more generally disorganization of the oocyte cytoskeleton are characteristics of ageing or low-quality gametes (Webb *et al.* 1986, Diaz & Esponda 2004).

Understanding all the dynamic processes involved in the formation of a mammalian oocyte competent for fertilization is a major goal for reproductive biologists. Some of the principles at play during the meiotic divisions of the mammalian oocytes are just starting to be understood, but the mechanisms remains to be elucidated at the cellular and molecular levels. The mouse oocyte, which allows the combination of molecular cell biology with genetics, appears more and more as the system to investigate these questions.

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