CSIRO PUBLISHING

Reproduction, Fertility and Development
http://dx.doi.org/10.1071/RD12242

Review

Molecular participants in regulation of the meiotic cell cycle in mammalian oocytes

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Abstract. Meiosis in oocytes consists of two consecutive asymmetric cell divisions, each completed by the extrusion of one set of chromosomes into a small polar body. First polar body (PBI) extrusion is triggered by the inactivation of cyclin-dependent kinase 1 (CDK1), following the degradation of its regulatory subunit cyclin B1 by the ubiquitin proteasome pathway. The present review covers the sequence of events leading to PBI extrusion, and compares them to the corresponding events in mitotic cell division. The latest findings regarding the contribution of ubiquitin chain topology, separase, securin, cyclin B1, CDK1, Polo-like kinase 1 and mitogen-activated protein kinase kinase 1/2 to the regulation of meiosis are discussed.

Additional keywords: CDK1, cyclin b1, MAPK, PLK1, polar body, proteasome, securin, separase.

Received 25 July 2012, accepted 30 October 2012, published online 4 December 2012

Meiosis

Meiosis is a particular example of cell division that occurs exclusively in gametes. Many of the effectors that take part in the execution of cell division are shared between meiosis and mitosis; however, there are several fundamental differences between them, with the meiotic division in oocytes having its own unique characteristics. The present review outlines major regulatory aspects of the first meiotic division in mammalian oocytes and compares them to their mitotic counterparts.

Meiosis is initiated during embryonic life, following a premeiotic replication stage of the primordial germ cells. Recombination between maternal and paternal chromosomes in the embryonic oocytes is followed by their arrest at the diplotene stage of the first prophase (Fig. 1), with homologous chromosomes attached at the sites of crossover, known as chiasmata (Fig. 2; Revenkova and Jessberger 2005). The meiotic arrest is removed only upon sexual maturity, when, at each reproductive cycle, a selected number of ovarian follicles responds to the surge of LH and the oocytes that reside in them resumes meiosis (Fig. 1; Edson *et al.* 2009). The period of time between birth and sexual maturity is dedicated to the physical growth of oocytes and only oocytes that reached their full size are capable of resuming meiosis (Sorensen and Wassarman 1976). The first morphological manifestation of the resumption of meiosis is the disassembly of the nuclear membrane, termed germinal vesicle breakdown (GVBD). Interestingly, oocytes removed from the ovarian follicle resume meiosis spontaneously (Pincus and Enzmann 1935; Edwards 1965). Oocytes that reinitiate meiosis progress to MI.

To become a germ cell containing half the number of chromosomes, meiotic cells undergo two consecutive cell divisions without an intervening S-phase. In oocytes, these divisions are asymmetric; each results in a large oocyte and a small polar body, a non-functional cell-like structure that degrades soon thereafter. Eventually, each germ cell gives rise to a single mature oocyte. During the first meiotic division, homologous chromosomes divide, extruding one set of chromosomes into the first polar body (PBI). Unlike somatic cells that exit mitosis and enter G_1, oocytes that have completed MI, arrest again at the metaphase of the second meiotic division (i.e. MII), and the extrusion of a second polar body (PBII) takes place only after fertilisation. Because meiotic divisions are asymmetric and take place in a much larger cell then the average somatic cell, they require additional steps of cellular organisation, such as spindle migration to the cortex. This, to some extent, may explain why, as opposed to the approximately 1-h long mitosis, meiosis takes longer and is extended over several hours (not taking into consideration prophase arrest, which may take decades in some mammals).

The meiotic spindle differs from the mitotic spindle because it lacks centrosomes, and is instead formed from microtubule organising centres (MTOC) that self-organise into a bipolar spindle (Schuh and Ellenberg 2007). In addition, at MI, sister kinetochores must organise side by side and orient as one functional unit to the same pole. This is different from MII and mitotic sister kinetochores, which oppose each other to allow segregation to different poles (Brar and Amon 2008; Holt and Jones 2009). Since meiosis involves two cell divisions, it

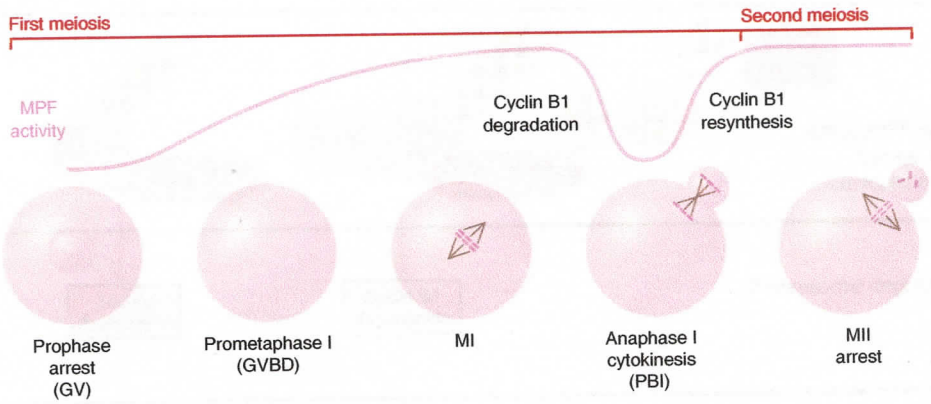

Fig. 1. Meiotic timeline associated with oscillations in cyclin-dependent kinase 1 (CDK1)/cyclin B1 (maturation-promoting factor (MPF)) activity. The times indicated relate to mouse meiosis and may vary between strains. Following LH stimulation or isolation from the ovarian follicle, MPF levels rise, inducing the resumption of meiosis, visually indicated by germinal vesicle breakdown (GVBD; also termed 'nuclear envelope breakdown'). Further increases in MPF levels mediate condensation of the chromosomes and formation of the MI spindle. A drop in MPF activity as a result of cyclin B1 proteasomal-dependent degradation induces extrusion of the first polar body (PBI), in which one set of homologes is expelled to a small polar body. The following resynthesis of cyclin B1 reactivates MPF, leading directly to the second meiosis. The oocyte then arrests at MII until fertilisation.

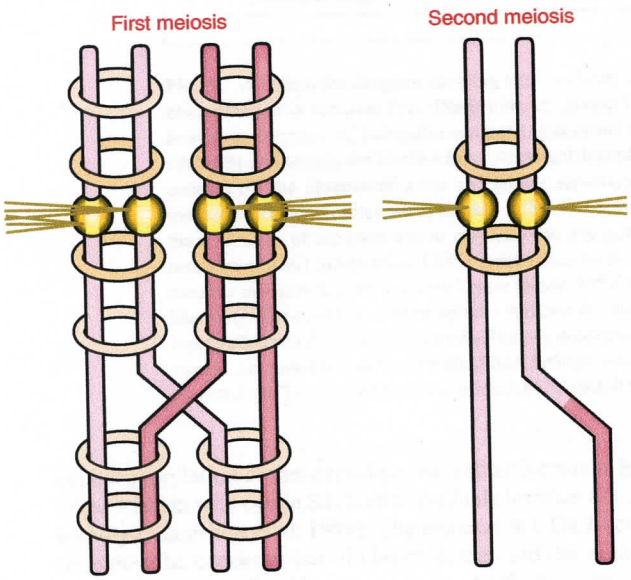

Fig. 2. A schematic diagram of chromosome conformation during meiosis I and meiosis II. At the prophase arrest of the first meiosis, homologous chromosomes, containing two sets of sister chromatids, are linked by chiasmata. Each set of sister chromatids is conjoined by cohesin complexes. During prometaphase and metaphase, spindle fibres attach to the sister kinetochores, which are mono-oriented, to facilitate separation of the two sisters to the same side at anaphase. Prior to the separation of homologues, the cohesin located on chromosome arms (light blue rings) is removed. The cohesin that resides adjacent to the centromeres (light brown rings) remains intact and forms the physical link between sister chromatids at the second meiosis (right). This remaining cohesin is removed before anaphase II. The kinetochores of sister chromatids now bi-orient in order to be pulled to two opposite directions by spindle fibres.

requires sequential loss of cohesion between chromosomes. Chromosome cohesion is maintained by a multi-subunit protein, cohesin. The first reductional division requires the removal of cohesin from chromosome arms, but not centromeres, to prevent precautious separation of sister chromatids at the first meiotic division (Brar and Amon 2008; Sakuno and Watanabe 2009). The need for specialised control of cohesin loss and the long time-course of the first meiosis are likely to contribute to its susceptibility to non-disjunction. Improper segregation of homologous chromosomes in the female gamete is a leading cause of aneuploidy and genetic malformations (Hunt 1998; Hassold and Hunt 2001).

These differences between meiosis and mitosis obviously require differential regulatory mechanisms throughout the cell cycle and, in particular, during spindle assembly and localisation. Although most our knowledge today is based on mitotic research, studies focused specifically on meiosis often find altered roles as well as specified functions for major cellular effectors.

Meiotic control by cyclin-dependent kinase 1 and the ubiquitin–proteasome pathway

Cyclin-dependent kinase 1 (CDK1) is a key regulator of meiosis. The activity of CDK1 depends on its phosphorylation status, as well as its hetero dimerisation with the regulatory subunit cyclin B1. This dimer is also known as maturation-promoting factor (MPF; Masui and Markert 1971) In fully grown oocytes arrested at prophase I and with an intact germinal vesicle (GV), CDK1 is inhibited by phosphorylation at Thr^{14} and Tyr^{15} (Nurse 1990; Morgan 1995; Lew and Kornbluth 1996). This phosphorylation is maintained by the contradicting activity of two kinases, Myt1 and Wee1b (also known as wee2), and the phosphatase Cdc25B (Lincoln *et al.* 2002; Han *et al.* 2005; Oh *et al.* 2010; Ruiz *et al.* 2010). Upon resumption of meiosis,

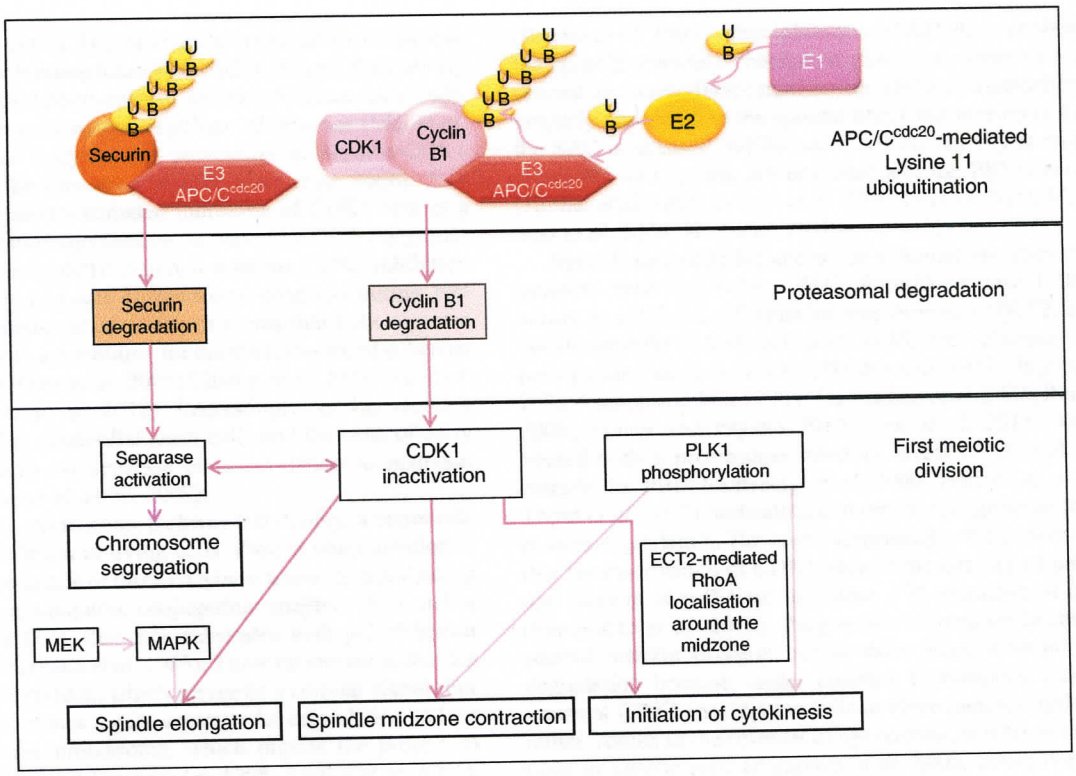

Fig. 3. A schematic diagram showing the regulatory events leading to the first meiotic division in mouse oocytes: protein ubiquitination is initiated first. Predominantly lysine 11-liked ubiquitin chains are assembled by an array of E1 (ubiquitin activating enzyme), E2 (ubiquitin conjugating enzyme) and E3 (ubiquitin ligase) ubiquitin ligases. As a result, proteins such as cyclin B1 and securin are labelled for proteasomal degradation. Securin degradation activates separase, which, in turn, cleaves the cohesin off the chromatids arms and allows separation of homologous chromosomes. Cyclin B1 degradation leads to the inactivation of cyclin-dependent kinase 1 (CDK1). Because active CDK1 inhibits separase, CDK1 inactivation also contributes to the activation of separase and to chromosome segregation. In parallel, released separase binds CDK1–cyclinB1 complexes, promoting CDK1 inactivation. CDK1 inactivation leads to a cascade of events. In anaphase, the spindle is elongated, a process that requires mitogen-activated protein kinase kinase (MEK)/mitogen-activated protein kinase (MAPK) activity in addition to CDK1 inactivity. The contraction of the spindle midzone is assisted by Polo-like kinase 1 (PLK1) phosphorylation. The combined CDK1 inactivation and PLK1 activity also facilitates cytokinesis: PLK1 promotes the localisation of RhoA at the cortex and later as a ring around the central part of the spindle. RhoA activity, which also requires the guanine exchange factor epithelial cell-transforming protein 2 (ECT2), facilitates cytokinesis. APC/C, anaphase-promoting complex/cyclosome; cdc20, cell division cycle protein 20.

dephosphorylation of these residues, as well as increased levels of association with cyclin B1, lead to gradual elevation of CDK1 activity (Karaiskou *et al.* 1999). The increase in CDK1 activity promotes the condensation of chromosomes and the organisation of the MI spindle (Hampl and Eppig 1995; Winston 1997; Brunet *et al.* 1999; Schuh and Ellenberg 2007). Conversely, the MI to anaphase I transition and the subsequent extrusion of the PBI require inactivation of CDK1. It is well established that CDK1 activity corresponds to cellular levels of cyclin B1, which drop as a result of its proteasomal degradation prior to cell division (Fig. 1; Masui and Clarke 1979; Murray *et al.* 1989; Glotzer *et al.* 1991). Unlike in somatic cells, the cyclin B1 pool in oocytes does not degrade completely upon the completion of the first meiotic division. Cyclin B1 re-accumulates following PBI extrusion (Ledan *et al.* 2001), leading to the reactivation of CDK1 and the progression to MII (Masui and Clarke 1979; Hashimoto and Kishimoto 1988; Choi *et al.* 1991).

Proteasomal degradation of an additional protein, securin, is essential for correct segregation of homologous chromosomes at anaphase I (Hagting *et al.* 2002; Holt and Jones 2009; Jones 2011). Securin is the inhibitor of separase, a peptidase that cleaves the oocyte-specific subunit of cohesion, rec8, on chromosome arms. This cleavage resolves the chiasmata, and subsequently allows the segregation of homologous chromosomes (Terret *et al.* 2003; Kudo *et al.* 2006; Fig. 3). The cohesin at the centromeres remains intact at the first meiotic division, ensuring that sister chromatids stay attached until MII (Fig. 2; Zou *et al.* 1999; Herbert *et al.* 2003; Terret *et al.* 2003; Kudo *et al.* 2006, 2009).

It was recently shown that separase also takes an active part in attenuating CDK1 activity. Separase phosphorylation and subsequent binding to the CDK1–cyclin B1 complex inhibits the action of both separase and CDK1 (Stemmann *et al.* 2001; Gorr *et al.* 2005; Huang *et al.* 2005; Holland and Taylor 2006;

Stemmann *et al.* 2006). This interaction is mutually exclusive to securin–separase binding (Gorr *et al.* 2005). Indeed, the viability and relatively mild phenotype of securin-deficient mice indicates the existence of another regulator of separase (Mei *et al.* 2001). In mouse oocytes, the prevention of separse-CDK1 binding blocks the formation of PBI (Gorr *et al.* 2006). This suggests that separase-mediated inhibition of CDK1 acts as a secondary regulatory mechanism, on top of cyclin B1 degradation, to ensure correct cell division. In addition, CDK1 inhibition of separase may attenuate chromosome cohesion during PBI extrusion (Pomerantz *et al.* 2012). It seems that both separase and CDK1 activity are required for the maintenance of cohesion in meiotic cells (Gorr *et al.* 2006; Chiang *et al.* 2011; Xu *et al.* 2011; Pomerantz *et al.* 2012). Interestingly, it was recently demonstrated that primordial germ cells and the cells of early embryos rely only on separase phosphorylation to maintain cohesion (Huang *et al.* 2008, 2009).

The ubiquitin–proteasome pathway (UPP) plays a major role in protein degradation in living cells. Protein ubiquitination is facilitated by a cascade of three enzymes: a ubiquitin activating enzyme (E1), a ubiquitin conjugating enzyme (E2) and a ubiquitin ligase (E3), that tags substrates with polyubiquitin chains (Fig. 3; Hershko *et al.* 1983). These chains are linked via a specific lysine residue, which serves as a cellular signal. For example, certain chains tag the substrate for degradation and are recognised by the proteasome, which digests the protein to peptides (Hershko and Ciechanover 1998; Köhler *et al.* 2001). Of the seven lysines (K) of ubiquitin, the most well-studied residue known to mediate proteasomal degradation in cells is K48 (Busch and Goldknopf 1981; Hershko *et al.* 1981; Hershko *et al.* 1983). The turnover of mitotic and meiotic proteins is mediated by the E3 ubiquitin ligase anaphase-promoting complex/cyclosome (APC/C; Sudakin *et al.* 1995). Recent studies have shown that APC/C can assemble different types of ubiquitin chains *in vitro*. Moreover, a different residue, namely K11, is the one that mediates proteasomal degradation during mitosis (Chau *et al.* 1989; Hershko and Ciechanover 1998; Kirkpatrick *et al.* 2006; Jin *et al.* 2008; Garnett *et al.* 2009; Williamson *et al.* 2009; Wu *et al.* 2010). This was recently affirmed in the meiotic first division (Pomerantz *et al.* 2012).

The APC/C is a multisubunit complex, the activity of which is regulated by binding of activators and inhibitors (Peters 2006). Switching its coactivator between cell division cycle protein 20 (Cdc20) and Cdc20 homolog 1 (Cdh1) confers substrate specificity. APC/C^{Cdh1} is active at prophase I, maintaining low levels of cyclin B1 and thus preventing premature activation of CDK1 (Peters 2006; Reis *et al.* 2007; Bassermann *et al.* 2008). At MI, the coactivator is switched and APC/C^{Cdc20} targets cyclin B1 and securin for degradation to allow PBI extrusion (Fig. 3; Reis *et al.* 2007). The loss of CDK1 activity causes another shift to APC/C^{Cdh1}, which also targets cyclin B1 and securin in addition to other substrates, including Cdc20, for degradation (Peters 2006). Specifically in oocytes, APC/C^{Cdh1} also acts in prometaphase I to keep CDK1 activation gradual, thus controlling the kinetics of anaphase onset (Reis *et al.* 2006).

To prevent premature anaphase and segregation errors, APC/C^{Cdc20} activity is inhibited at MI until proper binding of chromosome kinetochores to the spindle fibres is achieved (Brunet *et al.* 1999). This inhibition of APC/C is conferred by the spindle assembly checkpoint (SAC), an array of proteins serving as a surveillance mechanism. Once all kinetochores are properly connected to the spindle fibres and tension is formed, the SAC is released, inhibition of APC/C activity is removed, cyclin B1 and securin are degraded and the PBI is extruded. (Homer *et al.* 2005; Leland *et al.* 2009; Li *et al.* 2009; McGuinness *et al.* 2009; Wei *et al.* 2010).

Apart from cyclin B1 and securin, numerous other meiotic proteins, such as Cdc20, Cdc25, Polo-like kinase 1 (PLK1), aurora A, epithelial cell-transforming protein 2 (ECT2) and the oocyte-specific MAPK activator MOS, are substrates of the proteasome (Ishida *et al.* 1993; Baldin *et al.* 1997; Hagting *et al.* 2002; Lindon and Pines 2004; Jeganathan *et al.* 2006; Reis *et al.* 2006; Young and Pagano 2010; Liot *et al.* 2011). Oocytes treated with a proteasome inhibitor arrest at MI and fail to extrude the PBI (Josefsberg *et al.* 2000; Herbert *et al.* 2003; Terret *et al.* 2003), indicating that meiotic progression requires protein degradation. However, surprisingly, it has been shown that the inactivation of CDK1 even in the absence of proteasomal activity is sufficient to induce PBI extrusion in oocytes (Pomerantz *et al.* 2012). Progression of meiosis to reach the second meiotic division cannot take place without protein degradation because, under constant proteasomal inhibition, transient CDK1 inactivation fails to drive meiosis further and, rather, results in the reversal of cytokinesis, similar to observations in mitotic cells (Potapova *et al.* 2006, 2009; Pomerantz *et al.* 2012).

PLK1 in mitosis and meiosis

Despite its key role, CDK1 is not the only kinase that acts throughout meiosis. Several cellular kinases, such as PLK1 and mitogen-activated protein kinase kinase (MEK) 1/2, are known to function in meiosis (Schindler 2011); however, their roles are significantly better characterised in mitosis.

PLK1 is a well-conserved Ser/Thr protein kinase that has multiple roles in mitosis (Petronczki *et al.* 2008). PLK1 acts largely by binding to phosphorylated substrates via the N-terminal Ser/Thr kinase domain and the C-terminal polo box domain (PBD). This site also confers self-inhibition. In addition, PLK1 activity is controlled by phosphorylation on its T-loop region within the kinase domain (Jang *et al.* 2002; Elia *et al.* 2003). This structure enables PLK1 to interact and cooperate with other mitotic kinases (e.g. aurora A and CDK1), phosphatases (e.g. CDC25 and CDC14) and numerous substrates to accomplish its multiple roles during cell division (Yuan *et al.* 2011). PLK1 participates in the mitotic CDK1 auto-amplification loop, regulates spindle formation and promotes mitotic cohesin removal (Abrieu *et al.* 1998; Alexandru *et al.* 2001; Hornig and Uhlmann 2004; Sumara *et al.* 2004; Yuan *et al.* 2007). PLK1 is activated by aurora A kinase at the centrosomes during the mitotic G_2–M transition (Macůrek *et al.* 2008; Seki *et al.* 2008). At prometaphase and metaphase, PLK1 controls aurora B enrichment and activation at the inner kinetochores, which modulates the sensing and correction of mitotic misorientation of sister kinetochores (Chu *et al.* 2011; Salimian *et al.* 2011). Aurora B also controls meiotic kinetochore orientation. This is not trivial, because

meiosis I requires mono- rather than bi-orientation of the kinetochores. Although it has been shown recently that the protein complex monopolin, the Ark1 protein and the chiasmata itself facilitate conversion of kinetochore configuration to the desired mono-orientation, the role, if any, of PLK1 in this meiotic adaptation remains unknown (Hauf *et al.* 2007; Monje-Casas *et al.* 2007; Sakuno *et al.* 2011).

In addition, PLK1 was recently found to facilitate the initial stage of mitotic cytokinesis (Brennan *et al.* 2007; Lénárt *et al.* 2007; Santamaria *et al.* 2007; Wolfe *et al.* 2009). Cytokinesis consists of a series of four stages: (1) specification of the cleavage plane; (2) ingression of the cleavage furrow; (3) midbody formation; and (4) abscission (Normand and King 2010). Specification of the cleavage plane is regulated by the small GTPase RhoA (Piekny *et al.* 2005; Li *et al.* 2010) assisted by the guanine nucleotide exchange factor ECT2 and the Rac GTPase activating protein 1(GAP) Cyk-4. Rho A cycles between an active GTP-bound form and an inactive GDP-bound form (Niiya *et al.* 2006). ECT2 is phosphorylated by PLK1 and is then recruited to the central spindle complex centralspindlin (comprised of Cyk-4 and mitotic kinesin-like protein 1 (MKLP1)), subsequently activating RhoA. The association with centralspindlin is required to restrict the activity of ECT2, and consequently RhoA, to a narrow zone, essential for the following step of cytokinesis: cleavage furrow assembly and contractile ring formation (Yuce *et al.* 2005). Ingression of the actomyosin contractile ring is facilitated by the activity of the myosin II motor. During the following third stage of cytokinesis, the actomyosin ring interacts with the central spindle (also called the midzone), the area where central microtubules overlap as chromosomes are pulled to the opposite poles. Proteins that concentrate at the central part of the spindle at telophase form the midbody and facilitate these interactions. In addition to trafficking and fusion proteins, midbody proteins are required for abscission, the sealing of the cell membrane.

Some of the mitotic functions of PLK1 have been shown in meiosis; for example, involvement in the activation of cdc25 and auto-amplification of CDK1 upon resumption of meiosis, as well as facilitating MI spindle assembly (Karaiskou *et al.* 1999; Tong *et al.* 2002). In prophase-arrested oocytes, PLK1 localises to the GV and associates with the spindle poles and chromosomes at MI. During anaphase I, PLK1 translocates to the spindle midzone (Wianny *et al.* 1998; Pahlavan *et al.* 2000; Tong *et al.* 2002; Otsuki *et al.* 2009). Several recent studies have indicated conserved regulation of cytokinesis between mitosis and meiosis. It was shown that PLK1 activity mediates cytokinesis via RhoA localisation in mouse oocytes undergoing first meiosis (Fig. 3; Pomerantz *et al.* 2012). Moreover, depletion of ECT2 from mouse oocytes abolishes RhoA translocation and prevents PBI extrusion (Elbaz *et al.* 2010). Therefore, PLK1 regulation of cytokinesis seems to depend on RhoA and ECT2, as it does in mitosis (Yuce *et al.* 2005). PLK1 activity is also required for contraction of the anaphase I spindle (Pomerantz *et al.* 2012). Although the involvement of PLK1 in both meiotic cytokinesis and spindle contraction required CDK1 activity, they are likely to be separate pathways, because unsuccessful cytokinesis in ECT2-depleted oocytes does not interfere with chromosomal meiotic progression. Homologous chromosome division was not perturbed since the MII spindle was correctly formed. In some cases, the two sets of chromosomes (both entrapped in the oocyte due to cytokinesis failure) have formed two MII spindles in the oocytes (Elbaz *et al.* 2010). PLK1 is known to cooperate with CDK1 in mitotic cytokinesis. CDK1 initially 'primes' a substrate by phosphorylation, creating a docking site for PLK1, which, in turn, binds the phosphorylated substrates via its PBD domain. Alternatively, PLK1 can self-prime its substrates, creating its own docking sites or a site for a third party. This the case with Cyk-4, which is phosphorylated by PLK1 to create a docking site for ECT2 (Burkard *et al.* 2009; Wolfe *et al.* 2009). In addition to MKLP1/kif23, Cyk4 is a component of the centralspindlin complex. These proteins are all mitotic targets of PLK1, required for cytokinesis, midbody formation and microtubule bundling and motility (Mishima *et al.* 2002; Pavicic-Kaltenbrunner *et al.* 2007; Hutterer *et al.* 2009). Because the meiotic phenotype of PLK1 inactivation during anaphase is slightly different from the mitotic one (Brennan *et al.* 2007; Pomerantz *et al.* 2012), it would be very interesting to examine whether similar interactions take place in meiosis.

PLK1 may interact and phosphorylate the APC/C, thereby attenuating its activity (Kotani *et al.* 1998; Daum *et al.* 2000; Golan *et al.* 2002). In addition, it was suggested that PLK1 may activate APC/C^{Cdh1} indirectly through phosphorylation and activation of the phosphatase CDC14A. By doing so, PLK1 promotes the proteasome-dependent degradation of the APC/C^{Cdh1} substrates CDC20 and aurora A (Yuan *et al.* 2007). PLK1 is itself a target of APC/C^{Cdh1} and its degradation is a prerequisite to abscission, the final step of mitotic cytokinesis (Lindon and Pines 2004). The abscission factor centrosomal protein 55 kDa (Cep55) is phosphorylated by PLK1, which prevents association of Cep55 with the anaphase spindle. This inhibition is removed upon PLK1 degradation, which is initiated as the switch from APC/C^{Cdc20} to APC/C^{Cdh1} takes place, towards mitotic exit. The addition of the proteasome inhibitor MG132 prevents PLK1 degradation and subsequent Cep55 recruitment to the midbody, slowing down abscission (Lindon and Pines 2004; Bastos and Barr 2010). There are several known mitotic substrates of PLK1 that potentially take part in meiosis as well. One example is the Golgi protein Nir1. CDK1 priming of Nir1 allows its dissociation from the Golgi and subsequent PLK1 phosphorylation allows its localisation to the midbody, where it acts to promote the completion of cytokinesis (Litvak *et al.* 2004). Similarly, the association of the SAC protein bubR1 with the kinetochores and stabilisation of mitotic kinetochore–microtubular interaction depends on PLK1 phosphorylation (Elowe *et al.* 2007; Matsumura *et al.* 2007; Wong and Fang 2007). Clearly, many of the meiotic roles of PLK1 are yet to be discovered, and further research is required to unveil the complete picture of PLK1 involvement in meiotic division.

Mitogen-activated protein kinase

The mitogen-activated protein kinase (MAPK) pathway is a well-known transducer of extracellular and intracellular stimuli consisting of MAPK kinase kinase (MAP3K), MEK and extracellular signal-regulated kinase (ERK). In oocytes, the

MAPK pathway operates during the resumption of meiosis (which corresponds to the G_2 to M transition), under MOS, an oocyte-specific MAP3K that activates the downstream MEK1/2 and ERK1/2 (Shaul and Seger 2007). This mode of activation, which does not include a tyrosine receptor (which responds to an extracellular stimulus), is unique to meiosis. In somatic cells, this pathway acts predominantly at the G_1 to S transition and regulates transcription, inducing a cellular response. In oocytes undergoing meiosis, transcription is suppressed and the MOS–MEK1/2–ERK1/2 pathway affects downstream substrates that are transcription independent (Verlhac *et al.* 1996; Dupré *et al.* 2011). It was found that in addition to activating MEK1/2 phosphorylation, MOS activates MAPK in mouse oocytes by inhibiting a phosphatase (Verlhac *et al.* 2000). MAPK activity increases shortly after GVBD, and both MEK1/2 and ERK1/2 are constitutively phosphorylated and active until after fertilisation (Verlhac *et al.* 1994; Yu *et al.* 2007).

The well-established function of MOS is the maintenance of MII arrest. This function was discovered in female MOS-knockout mice, which are infertile owing to parthenogenetic activation of their oocytes (Colledge *et al.* 1994; Hashimoto *et al.* 1994). In addition, the MAPK pathway is known to participate in the assembly and translocation of the first metaphase spindle. During early stages of meiosis, MAPK regulates spindle formation and positioning (Araki *et al.* 1996; Verlhac *et al.* 2000; Lefebvre *et al.* 2002; Tong *et al.* 2003; Lin *et al.* 2010). Both MEK1/2 and ERK1/2 are phosphorylated during PBI extrusion (Verlhac *et al.* 1994; Yu *et al.* 2007). Phosphorylated ERK and MEK1/2 are localised to the spindle poles at metaphase and migrate to the spindle midzone at anaphase. In addition, ERK is associated with the contractile ring in pig, but not mouse, oocytes (Lee *et al.* 2000; Hatch and 2001; Xiong *et al.* 2007; Yu *et al.* 2007). In addition, the MAPK pathway affects microtubule dynamics (Verlhac *et al.* 1996; Yu *et al.* 2007). MEK signalling is required for microtubule nucleation, and it is colocalised with the nucleation and spindle organisation proteins γ-tubulin, nuclear mitotic apparatus (NuMA) and dynactin at the meiotic and mitotic spindle poles. Prevention of these interactions, as well as of MEK activity, resulted in spindle defects (Echeverri *et al.* 1996; Xiong *et al.* 2007; Yu *et al.* 2007; Sun *et al.* 2008; Colello *et al.* 2011). Studies in MOS-knockout mice and in oocytes treated with the MEK1/2 inhibitor UO126 revealed that the MOS–MAPK pathway affects spindle translocation to the cortex. Specifically, spindle elongation was observed, compensating for the repositioning failure (Verlhac *et al.* 2000; Tong *et al.* 2003; Brunet and Verlhac 2011). Interestingly, a different outcome is observed when MEK1/2 and CDK1 are inhibited simultaneously in oocytes undergoing anaphase. Under these conditions, spindles of oocytes treated with UO126 were shortened and failed to contract at the midzone (Pomerantz *et al.* 2012). This phenotype is more likely to be associated with alterations in microtubule dynamics rather then spindle translocation, because the spindles were already localised at the cortex. It was previously suggested that microtubules become dynamic in the absence of both CDK1 and MEK1/2 activity (Gotoh *et al.* 1991; Verlhac *et al.* 1996).

Metaphase spindle migration in oocytes is facilitated by actin filaments, which form a dynamic structure encapsulating the spindle (Dumont *et al.* 2007; Azoury *et al.* 2008). The formation of this structure is facilitated by formin-2 and the small GTPases Rac and CDC42 (for reviews, see Azoury *et al.* 2009; Kwon and Lim 2011). A large polar body and elongated spindle phenotype was observed following the knock down of the *cis*-Golgi protein GM-130, which colocalises with phosphorylated (p-) MEK at the spindle poles. Depletion of GM-130 impaired p-MEK and γ-tubulin accumulation, whereas inhibition of MEK brought about a mislocalisation of GM-130 (Zhang *et al.* 2011). These studies clearly link MEK activity and actin-mediated spindle translocation; however, further research is needed to elucidate the full pathway.

Conclusion and perspectives

Oocytes initiate their meiotic division during embryonic development and arrest at the diplotene of the first prophase. Fully grown oocytes that reinitiate meiosis progress through the first telophase to the second metaphase, at which point they arrest again until fertilisation. This very unique protracted process is also characterised by two asymmetric cell divisions, thus resulting in the production of a single haploid gamete. The first meiotic division, during which the homologous chromosomes segregate, is particularly error prone and any misstep along this way may lead to aneuploidy and genetic malformations. The large body of information presently accumulated regarding the different components that participate in this process and their mode of action is impressive. Nevertheless, there is a pressing need for future research that will deepen the level of our understanding of the complexity of events that ensure the completion of meiosis in a faithful manner. This knowledge may be applied to prevent genetic malformations and infertility.

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