Int. J. Dev. Biol. 52: 201-217 (2008)
doi: 10.1387/ijdb.072337jk

THE INTERNATIONAL JOURNAL OF DEVELOPMENTAL BIOLOGY
www.intjdevbiol.com

On the transition from the meiotic to mitotic cell cycle during early mouse development

JACEK Z. KUBIAK[1,*], MARIA A. CIEMERYCH[2,3], ANNA HUPALOWSKA[2,5], MARTA SIKORA-POLACZEK[2,4] and ZBIGNIEW POLANSKI[4]

[1]*Institute of Genetics & Development, UMR 6061 CNRS, Mitosis & Meiosis Group, IFR 140 GFAS, University of Rennes 1, France,* [2]*Department of Embryology and* [3]*Department of Cytology, Institute of Zoology, University of Warsaw, Poland,* [4]*Department of Genetics & Evolution, Institute of Zoology, Jagiellonian University, Cracow, Poland and* [5]*Laboratory of Cell Biology, International Institute of Molecular and Cell Biology, Warsaw, Poland*

ABSTRACT Here, we outline the mechanisms involved in the regulation of cell divisions during oocyte maturation and early cleavages of the mouse embryo. Our interest is focused on the regulation of meiotic M-phases and the first embryonic mitoses that are differently tuned and are characterized by specifically modified mechanisms, some of which have been recently identified. The transitions between the M-phases during this period of development, as well as associated changes in their regulation, are of key importance for both the meiotic maturation of oocytes and the further development of the mammalian embryo. The mouse is an excellent model for studies of the cell cycle during oogenesis and early development. Nevertheless, a number of molecular mechanisms described here were discovered or confirmed during the study of other species and apply also to other mammals including humans.

KEY WORDS: *meiosis, mitosis, oocyte, embryo, MPF, CSF, spindle assembly checkpoint, Mad2, Rec8*

Cell cycle is often modified in different cell types during development and aging of the individual. These adaptations seem to be of a particular importance for the success of the early developmental program. The cell cycle must be modified during meiosis, when DNA replication is suppressed, during spermatogenesis in males and in oocytes undergoing the process of meiotic maturation in females. The oocyte enters the first meiotic prophase that can take several years. Then, in sexually mature females, the oocyte resumes meiosis and progress through two M-phases of the meiotic maturation, each of them differently regulated, to assure correct separation of maternal chromatin. Fertilized egg transits from meiotic to the mitotic cell cycle and resume DNA replication.

In the current review we will focus on M-phase modifications that are the subject of the research in our laboratories. We choose three examples of specific M-phase adjustments i.e. metaphase I, and metaphase II of oocyte meiotic divisions and the first mitosis of mouse embryo. We center our attention on the temporal regulation of metaphase I progression and the singularities of the spindle assembly checkpoint during this period, mechanisms of sustaining and exiting metaphase II-arrest, and finally the unique regulation of the first embryonic mitosis. The correct timing of events during these periods is of the key importance for the success of development. Therefore, it is carefully controlled and precisely measured by specific molecular mechanisms described in this paper.

The main M-phase regulatory mechanisms

Each meiotic as well as mitotic M-phase is initialized through activation of the M-phase Promoting Factor (MPF) (Masui, 2001). MPF is a complex of the enzymatic subunit CDK1 (Cyclin-Dependent Kinase 1) and the regulatory one - cyclin B (Fig. 1). Activation of MPF requires stoichiometric association of cyclin B with CDK1, and a series of phosphorylations (Thr-161 or equivalent) and Cdc25-mediated dephosphorylations (Thr 14/Tyr 15; Gautier *et al.*, 1990, Nurse, 1990). Inactivation of MPF requires CDK1 dissociation from cyclin B followed by the proteolytic

Abbreviations used in this paper: APC/C, anaphase promoting complex/cyclosome; CDK1, Cyclin-Dependent Kinase 1; CSF, cytostatic factor; GVBD, germinal vesicle breakdown; MPF, M-phase Promoting Factor; MI, metaphase I; MII, metaphase II; NEBD, nuclear envelopm breakdown; SAC, spindle assembly checkpoint.

***Address correspondence to:** Jacek Z. Kubiak. Institute of Genetics & Development, UMR 6061 CNRS, Mitosis & Meiosis Group, IFR 140 GFAS, University of Rennes 1, Faculty of Medicine 2 Ave. Prof. Léon Bernard, 35043 Rennes cedex, France. Fax. 33-(0)2-2323-4478. e-mail: jacek.kubiak@univ-rennes1.fr

Published online: 14 February 2008

0214-6282/2008/$35.00

degradation of the latter. Dissociation of CDK1 and cyclin B is mediated by the proteasome (Nishiyama *et al.*, 2000). The complex is targeted to the proteasome via ubiquitin pathway due to the polyubiquitination of the cyclin B (tagging the protein with the ubiquitin chain) still within the MPF complex (Fig.1). Dissociation of cyclin B and CDK1 is mediated by the lid of the proteasome. CDK1, once dissociated from cyclin, becomes inactivated by dephosphorylation of Thr-161 and phosphorylation of Thr 14/ Tyr15. Simultaneously Cyclin B is deubiquitinated and degraded within the proetasome. Its neosyntesis and accumulation is necessary for the subsequent MPF activation before the next G2/M transition (Murray *et al.*, 1989). In general, MPF activity is transient and detectable only during M-phase, which in majority of somatic cells takes on average 40-50 min (Howell *et al.*, 2004; Jones *et al.*, 2004; Meraldi *et al.*, 2004). However, under certain conditions MPF activity can be stabilized for several hours and the way in which this stabilization can be achieved varies.

The potent MPF stabilizing factor operating in many cell types is spindle assembly checkpoint (SAC; Fig. 2) (Lew and Burke, 2003; Homer *et al.*, 2005a; Malmanche *et al.*, 2006). SAC becomes activated during prometaphase when chromosomes are not aligned in the spindle and emanate signals to the cytoplasm informing the checkpoint machinery that the kinetochores are not under proper tension. When all chromosomes become stably attached with spindle microtubules via kinetochores (thus fulfilling the key requirement for correct segregation), this signal is switched off, and the cell enters metaphase i.e. becomes competent to start the anaphase and complete the division. Thus, SAC activity prevents precocious inactivation of MPF which could result in abnormal separation of sister chromosomes (or chromatids). The main components of SAC are Mad2, Mad1, BUB1, and BUBR1 kinetochore proteins (Fig. 2) (Brady and Hardwick, 2000; Shah and Cleveland, 2000; Nasmyth, 2005). Mad2 is the factor responsible for binding Cdc20 that is an activator of anaphase promoting complex/cyclosome (APC/C) (Fang *et al.*, 1998; Yu, 2002). APC/C, a multiprotein complex, is a specific E3 ligase mediating poluybiqitination and targeting different mitotic proteins including cyclin B to degradation in the proteasome (Peters, 2002; Passmore, 2004; Fry and Yamano, 2006). The association of Cdc20 with Mad2 prevents APC/C activity and the SAC remains active. The dissociation of the complex triggers SAC inactivation. Recently it was documented that the complex of Mad2 with Cdc20 is regulated by the equilibrium between polyubiquitination and deubiquitination of Cdc20 (Reddy *et al.*, 2007; Stegmeier *et al.*, 2007). Thus, SAC inactivation is caused by the polyubiquitination of Cdc20 and does not require Cdc20 degradation, what explains its very rapid inactivation.

Another mechanism of MPF stabilization, described as cytostatic activity (CSF), functions specifically in oocytes that reached metaphase II of the second meiotic division (Fig. 3) (Masui, 2001; Tunquist and Maller, 2003; Schmidt *et al.*, 2006,). In these oocytes stabilization of MPF does not require SAC activity (Tsurumi *et al.*, 2004) and CSF alone is responsible for sustaining MPF activity and preventing anaphase onset. The molecular nature of CSF is complex. One of the first factors discovered to be involved in this activity are Mos/MEK/MAP (ERK1/ERK2) kinase i.e. MAP kinase pathway (Fig. 3) (Colledge *et al.*, 1994; Hashimoto *et al.*, 1994). Action of this pathway is vital for both proper functioning of meiotic spindle (via Doc1R and MISS; Lefebvre *et al.*, 2002; Terret *et al.*, 2003a) and for MPF stabilization (Brunet and Maro, 2005). Recently, the crucial role in the CSF activity has been attributed to a member of Emi protein family – Emi2 (Endogenous meiotic inhibitor 2), that has been shown to bind Cdc20, and in consequence, prevent APC/C activation and anaphase onset (Liu and Maller, 2005; Rauh *et al.*, 2005; Schmidt *et al.;* 2006, Shoji *et al.*, 2006). Details concerning CSF and its components are described in the next chapters.

As already mentioned, cyclin B degradation is crucial for the long term MPF inactivation. However, except cyclin B, numerous other proteins are degraded sequentially during the whole mitotic process (e.g. early mitotic proteins Nek2A, cyclin A), and their sequential degradation regulates the M-phase progression. Among

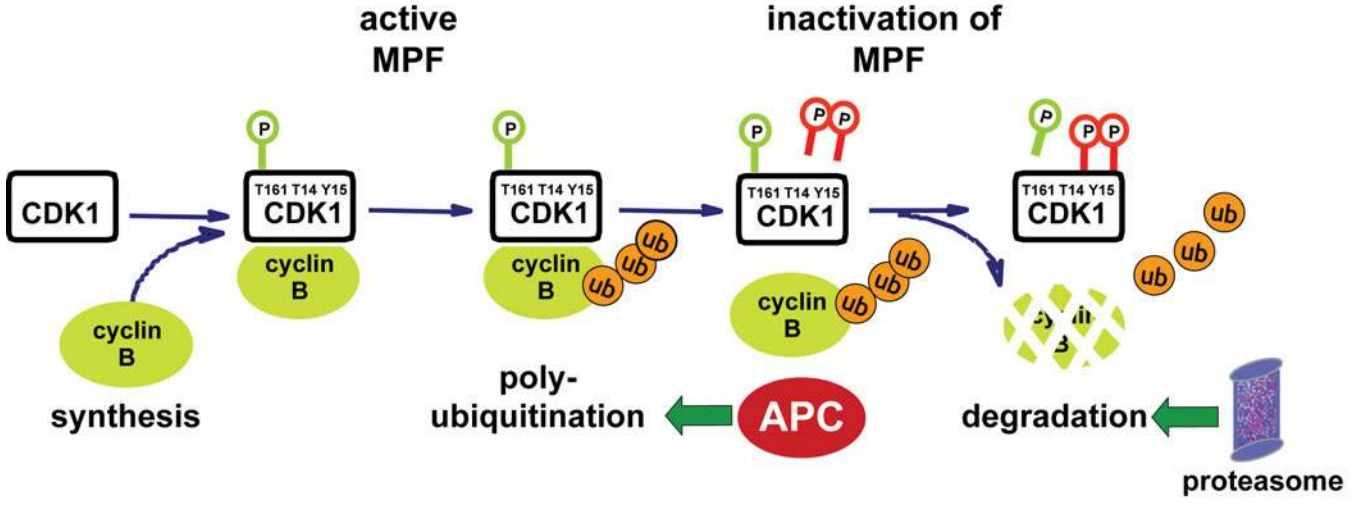

Fig. 1. Activation and inactivation of M-phase promoting factor (MPF) during the M phase. *Newly synthesized cyclin B associates with CDK1. The complex is phosphorylated on Thr-161, becomes active and provokes M-phase entry. At the end of M-phase, cyclin B becomes polyubiquitinated and targeted to the proteasome. The lid of the proteasome induces dissociation of polyubiquitinated cyclin B from CDK1 and its deubiquitination. CDK1 becomes inactive and it is phosphorylated on Thr-14 and Tyr-15, while cyclin B is degraded within the proteasome. Polyubiqitination of cyclin B is mediated by E3 ligase within the high molecular weight complex APC/C.*

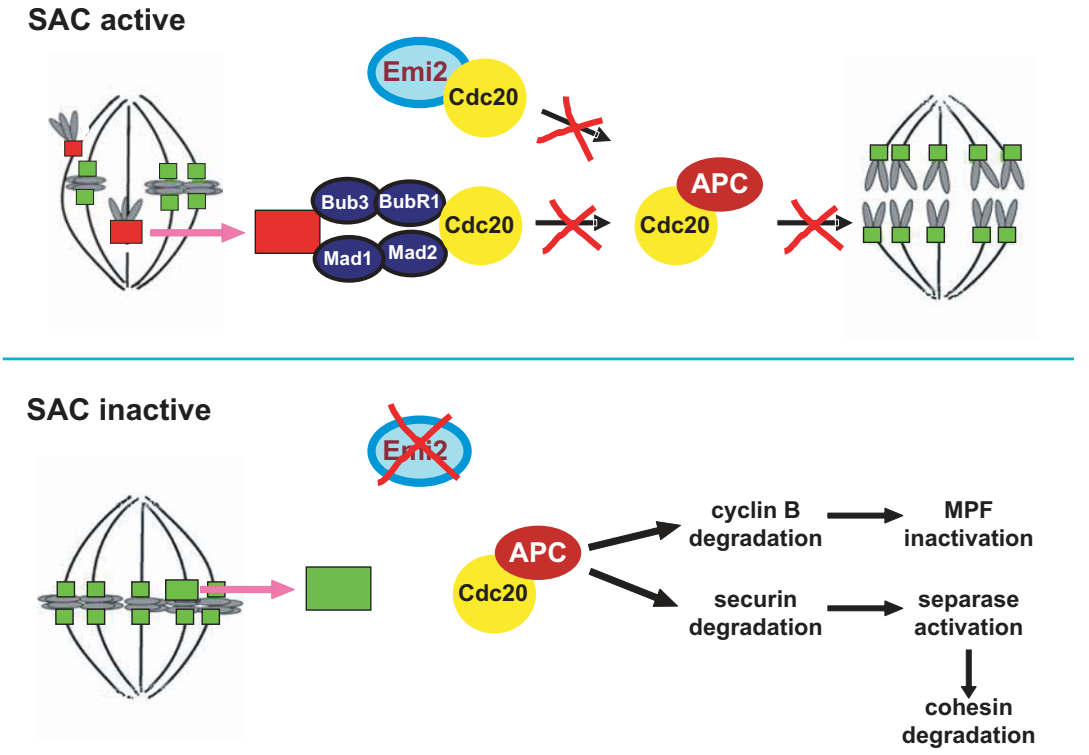

Fig. 2. Spindle Assembly Checkpoint (SAC) and anaphase promoting complex/cyclosome (APC/C) regulation. (SAC active) *Activation of APC/C in prometaphase is prevented even by a single kinetochore associated with checkpoint proteins Mad1, Mad2, Bub3, BubR1, that bind and inactivate the APC/C activator Cdc20. This mechanism is responsible for prolongation of prometaphase of the first meiotic division in the mouse oocyte. In metaphase II-arrested oocytes, the function of active SAC is replaced by Emi2 that saturates Cdc20 and prevents APC/C activation.* **(SAC inactive)** *Checkpoint proteins are released to the cytoplasm from kinetochores stably bound to spindle microtubules. Cdc20 becomes free, binds to APC/C allowing cyclin B degradation and MPF inactivation and in parallel securin degradation and the anaphase onset. During the second meiotic division in the oocyte, degradation of Emi2 provokes Cdc20 release and APC/C activation.*

these proteins are also cohesins which act as a 'glue' holding together homologous chromosomes during the first meiotic division or two chromatids during second meiotic division or mitosis (Haering and Nasmyth, 2003; Uhlmann, 2004). Rec8 is a crucial cohesin involved in the binding of homologous chromosomes in meiotically dividing oocytes and spermatocytes (Fig. 4) (Lee *et al.*, 2003; Prieto *et al.*, 2004; Lee *et al.*, 2006b). Its degradation catalyzed by separase is a prerequisite step for the proper chromosome segregation (Siomos *et al.*, 2001; Terret *et al.*, 2003b; Uhlmann, 2003). However, during the M-phase, until the moment of APC/C activation, separase remains inactive due to its binding to specific inhibitor – securin (Herbert *et al.*, 2003; Pines, 2006). Securin degradation occurs via Cdc20 - APC/C mediated manner (Hagting *et al.*, 2002). Thus, during oocyte meiotic maturation the release of Cdc20 from its inhibitors allows activation of APC/C leading to the degradation of securin, activation of separase and in consequence Rec8 proteolysis (Herbert *et al.*, 2003; Terret *et al.*, 2003b; Homer *et al.*, 2005b). Importantly upon the first meiotic division Rec8 localized at centromeres is protected from degradation by shugoshin what secures cohesion of sister chromatids (Watanabe and Kitajima, 2005). Finally, during the second meiotic division, this portion of Rec8 is also degraded (Fig. 4). MPF inactivation and cohesin degradation are separate events mediated, simultaneously by APC/C. Therefore, proper chromosomes separation, which is the main goal of each M-phase, is achieved only when well ordered sequence of events is respected. Modifications of meiotic and mitotic events, described below, enable such a fine control to be achieved with high precision during each specific M-phase.

The first meiotic M-phase in oocytes: cyclin B-associated machinery governs meiotic timing

Like the M-phases in other cell types, the first meiotic M-phase is promoted by high activity of MPF. However, unlike in other cells the first meiotic M-phase in mammalian oocytes is particularly long lasting 6-10 hours (Polanski, 1997a; Polanski, 1997b). It is obvious that such prolonged M-phase may be associated with unique and complex events which have to occur to ensure proper progression through meiosis. It seems that special control of metabolism of cyclin B, the regulatory subunit of MPF, may help to extend the duration of the first meiotic M-phase (MI). In mitotic cells the bulk of cyclin B is accumulated and complexed with CDK1 at late G2 phase which results in the fast establishment of M-phase upon complex activation (Pines and Hunter, 1991; Jackman *et al.*, 2003). Recently, cyclin A2, probably associated with both CDK1 and CDK2, was found to participate in this process in HeLa cells (Gong *et al.*, 2007). In the mouse oocyte the situation is different since only a limited pool of cyclin B is present at the onset of meiotic maturation and no information about a potential role of cyclin A2 despite its presence in oocytes is available (Winston *et al.*, 2000).

The pool of cyclin B complexed with CDK1 constitutes so called pre-MPF (Fig. 1), and is maintained stable in prophase oocyte through Emi1-dependent inhibition of APC/C (Reis *et al.*, 2006; Marangos *et al.*, 2007). The first stimulus to resume meiotic maturation comes through Cdc25-dependent dephosphorylation of pre-MPF (Hoffmann *et al.*, 1993), leading to its activation and the increase in the MPF activity to the level sufficient to induce

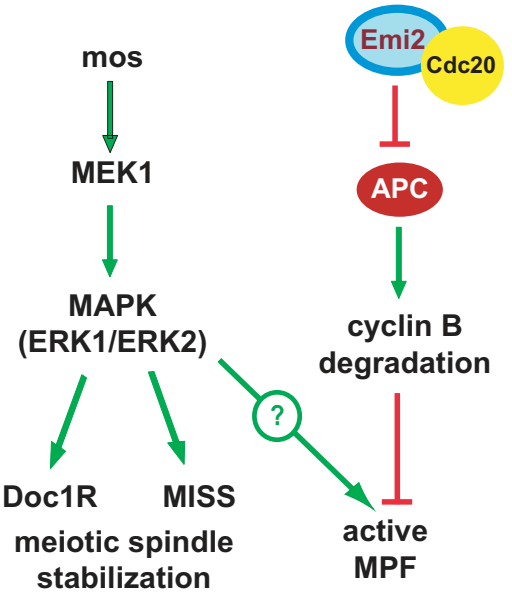

Fig. 3. Cytostatic factor (CSF) activity in metaphase II-arrested mouse oocytes. *MAP kinase pathway is involved in meiotic spindle stabilization via phosphorylation of the spindle proteins Doc1R and MISS and independently, via an unknown mechanism, influences MPF activity. Emi2 binds Cdc20 and prevents APC/C activation, thus blocking cyclin B degradation and MPF inactivation.*

nuclear envelope breakdown (also described as Germinal Vesicle Breakdown, GVBD) and chromatin condensation (Fig. 5). Thus, the oocyte enters M-phase already at this initial phase of meiotic maturation; however it is still a long way to establish conditions of a full M-phase. Resumption of meiotic maturation through pre-MPF activation results in crucial events ensuring further progression of the M-phase. The induction of a massive synthesis of cyclin B (Hampl and Eppig, 1995, Winston, 1997) is controlled by nuclear factor(s) released into the cytoplasm upon nuclear envelope disassembly play important role in this process (Hoffmann *et al.,* 2006). Either inhibition of the protein synthesis (Hampl and Eppig, 1995; Polanski *et al.*, 1998) or removal of such nuclear factor (Hoffmann *et al.*, 2006) results in the lack of cyclin B accumulation and in the arrest of MPF activity at the intermediate level. This shows a biphasic control of MPF activity with the initial phase independent of cyclin B synthesis relying on activation of pre-MPF, and the second one requiring cyclin B synthesis to induce full MPF activity necessary to achieve complete M-phase characteristics. Additionally, protein synthesis inhibition results in anomalies of spindle formation and chromosome condensation. Such anomalies are also observed when oocytes mature in the absence of the nuclear factor(s) and cannot be repaired by restoration of normal level of MPF activity through exogenous expression of cyclin B (Polanski *et al.*, 2005). Clearly, such spindle and chromatin aberrations are not linked directly to poor cyclin B synthesis but rather reflect the inhibition of the synthesis of other cell cycle regulating proteins. Since nuclear factor(s) appear to stimulate cyclin B translation through its mRNA 3'UTR dependent control mechanism (Hoffmann *et al.,* 2006) it is possible that a similar mechanism could initiate translation of other proteins crucial for spindle assembly, for example Cdc6 (Anger *et al.*, 2005), and chromosome condensation. Such mechanism might couple the intensive translation of cyclin B (and possibly other proteins) with the initiation of meiotic maturation, thus, securing that appropriate synthesis of necessary proteins occurs at the right time (Fig. 5).

Regardless of other proteins translated during meiotic maturation cyclin B synthesis seems to play the key role in determining the duration of the first meiotic M-phase. Striking differences in the level of cyclin B synthesis and in corresponding MPF activity were reported in mouse oocytes differing in the length of the first

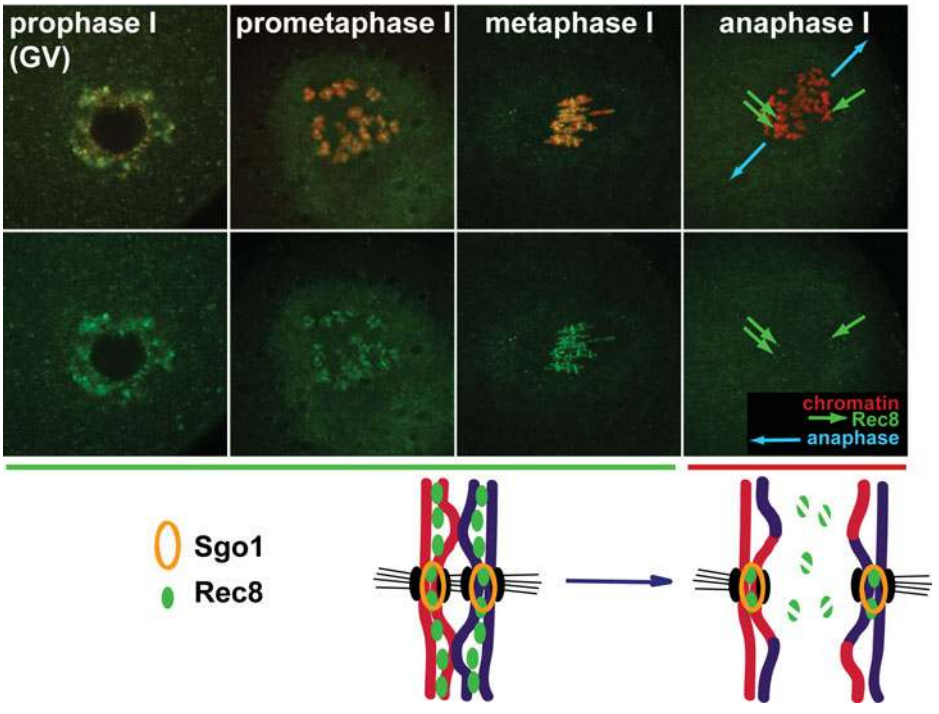

Fig. 4. Meiotic cohesin Rec8 in mouse oocytes. *In the prophase I oocyte (GV stage), prometaphase I and metaphase of the first meiotic division Rec8 assures cohesion of homologous chromosomes. It co-localizes with shugoshin (Sgo1) in the centromere region. During anaphase of the first meiotic division, Rec8 localized so far along chromosome arms becomes degraded, allowing separation of homologous chromosomes. Sgo1 prevents degradation of Rec8 localized within the centomeres assuring the stable binding of chromatids. Green arrows show centromeric localization of Rec8 during the first meiotic anaphase. Blue arrows indicate direction of anaphase movement of homologous chromosomes.*

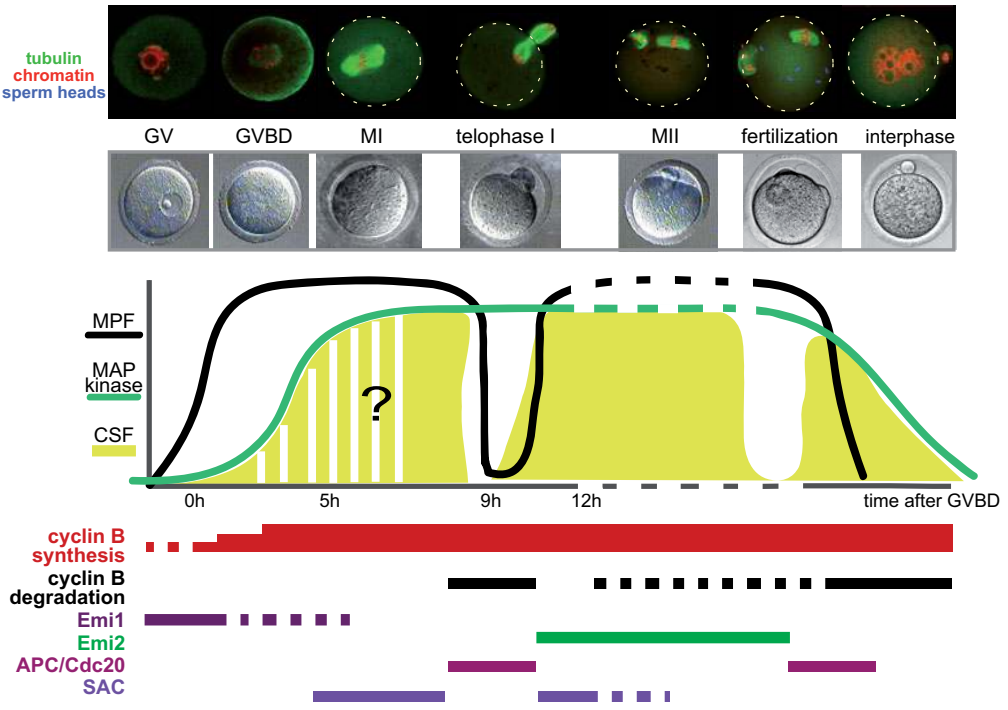

Fig. 5. Control of the cell cycle during meiotic maturation of mouse oocyte. **(Upper lane)** *Microtubules (green) and chromatin (red) in maturing oocytes.* **(Lower lane)** *Morphology of maturing oocytes, MPF (black), MAP kinase (green) and CSF activity (yellow) during oocyte maturation.* **(Bottom)** *Cyclin B synthesis (red) augments during MI; cyclin B is massively degraded at anaphase I, during MII-arrest degradation is kept moderate (equilibrated with cyclin B synthesis). Cyclin B is massively degraded again upon fertilization and anaphase II of meiosis; Emi 1 is degraded upon GVBD. Emi2 is synthesized and accumulated in MII-arrested oocytes and degraded upon fertilization; APC/C-Cdc20 is activated during each meiotic transition (MI/MII and MII/interphase); it remains partially active to assure cyclin B turnover during M II-arrest (not shown); SAC is active before the MI/MII transition, and following entry into MII-arrest.*

meiotic M-phase. The oocytes with long M-phase showed clear biphasic profile of the increase of MPF activity correlated with lower level of cyclin B synthesis, whereas the oocytes with short M-phase reached the full MPF activity quickly and in association with high level of cyclin B synthesis (Polanski *et al.*, 1998). Moreover, a mild overexpression of cyclin B in 'slow' oocytes resulted in M-phase shortening (Polanski *et al.*, 1998). This is consistent with the well documented dependence between APC/C and MPF (Cohen-Fix and Koshland, 1997) suggesting that in oocytes in which MPF activity increases slowly (as a consequence of slow accumulation of cyclin B) APC/C is activated later. Accordingly, the retarded activation of APC/C would delay the destruction of securins and cyclin B, the events marking the end of the first meiotic M-phase. On the other hand, fast increase of MPF activity seems to result in fast activation of APC/C and thus earlier destruction of its targets. Our recent results show that in the oocytes in which both CSF and SAC are disrupted the periodic changes in cyclin B level still occur, thus, suggesting the possibility of regulation via an oscillator driven by simple feedback between MPF and APC/C activity (Hoffmann, Kubiak and Polanski, in preparation). Therefore, the high level of cyclin B synthesis correlates with shortening of the first meiotic M-phase likely due to earlier activation of APC/C (Polanski *et al.*, 1998; Marangos and Carroll, 2004). However, this dependence may be easily reversed since the strong overexpression of cyclin B saturates APC/C what leads to the hampering of efficient degradation of its excess. This in turn results in a metaphase arrest (Ledan *et al.*, 2001; Marangos and Carroll, 2004). All these data put cyclin B translation in the centre of the control of meiotic timing. This notion becomes especially clear in the light of observations that cyclin B synthesis during meiotic maturation leads to immediate formation of active MPF, probably with no need of any posttranslational modifications (Hampl and Eppig, 1995).

Another main mechanism involved in the control of the timing of the first meiotic anaphase is the SAC. Requirement for stable connection between kinetochores and the spindle for the anaphase signaling or disappearance of SAC proteins from the kinetochores of the first meiotic spindle before anaphase suggested SAC function in oocyte meiosis (Brunet *et al.*, 1999; Brunet *et al.*, 2003). These data were recently confirmed by interfering with Mad2 and Bub1 proteins, the key elements of the SAC pathway (Wassmann *et al.*, 2003; Tsurumi *et al.*, 2004; Homer *et al.*, 2005c; Yin *et al.*, 2006; Wang *et al.*, 2007). Knocking down Mad2 (Tsurumi *et al.*, 2004; Homer *et al.*, 2005c; Wang *et al.*, 2007) or Bub1 (Tsurumi *et al.*, 2004; Yin *et al.*, 2006) resulted in premature induction of the first meiotic anaphase and elevated aneuploidy providing direct proof for SAC function during the first meiotic division of the mouse oocyte. Interestingly, upon SAC inactivation the first meiotic division is accelerated for only 2-3 hours still leaving 5-7 hours period of the M-phase (Tsurumi *et al.*, 2004; Homer *et al.*, 2005c). It seems, however, that even upon SAC disruption a period necessary to the extrusion of first polar body is not shorter than 5 hours, since the degradation of APC/C inhibitor Emi1 initiated at the M-phase entry may require about 3 hours (Marangos *et al.,* 2006) and the proteolysis of APC targets takes at least two more hours as revealed by the kinetics of cyclin B-GFP and securin-GFP degradation in mouse oocytes (Ledan *et al.,* 2001; Hyslop *et al.,* 2004; Homer *et al.,* 2005b; Homer *et al.,* 2005c; Hoffmann, Kubiak and Polanski, in preparation).

Surprisingly, SAC does not detect some chromosomal anomalies in female mouse meiosis (LeMaire-Adkins *et al.*, 1997). Moreover, the trisomies in humans resulting from meiotic errors are 8 times more frequent for the female meiosis than for the male one (Hassold *et al.*, 1993). These data suggest that SAC in mammalian oocytes may not be as efficient as in other cell types. On the other hand, even a single unattached bivalent is capable

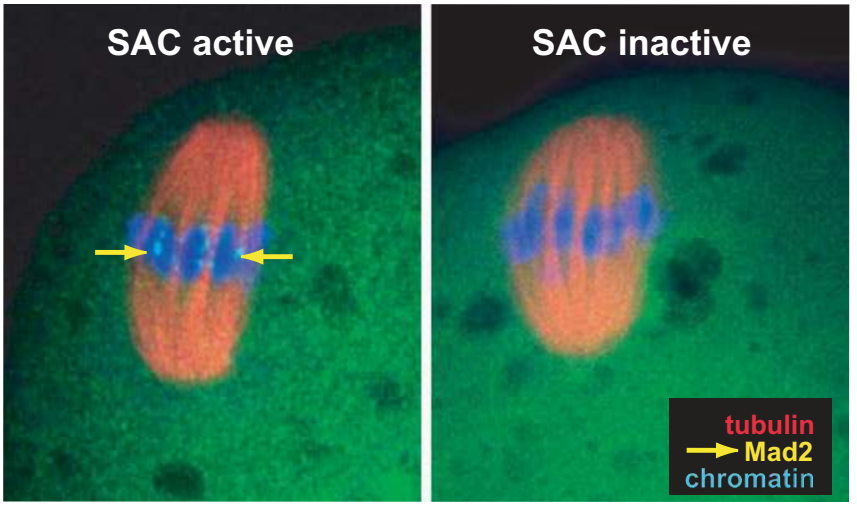

Fig. 6. Mad2 localization on kinetochores suggests that the spindle assembly checkpoint (SAC) is active in early metaphase II mouse oocytes. *SAC seems active one hour after the completion of the first meiotic division, as Mad2 localizes clearly on kinetochores. It becomes inactivated within the next 6-10 h, when Mad2 disappears from the kinetochores, despite the oocyte remaining arrested in metaphase II due to CSF activity.*

to establish SAC-mediated inhibition of cyclin B proteolysis in mouse oocytes showing that at least some elements of SAC control work effectively (Hoffmann, Kubiak and Polanski; in preparation).

It is not clear whether CSF, which could theoretically modulate the timing of the first meiotic anaphase, is already active at meiosis I (Ciemerych and Kubiak, 1998). However, in mice deprived of *mos* gene and protein, whose oocytes clearly lack CSF activity, the duration of the first meiotic M-phase remains unaffected (Verlhac *et al.*, 1996). This observation questions CSF as a possible candidate regulating the timing of anaphase I of meiosis.

MII-arrest and exit: Emi2, calcium and APC/C do the job, but what about ERK1/2 MAP kinases?

The completion of the first meiotic division results in the metaphase II-arrest of the oocyte (MII), in which chromosomes and the second meiotic spindle remain in a stable state for hours. However, the nuclear maturation of oocytes can be decoupled from the cytoplasmic one. In other words, MII oocyte needs some time to become fully "fertilizable" despite that it remains still in the same stage of meiosis. Different reactions of mouse oocytes, depending on their age after the first polar body extrusion, were described following fertilization or parthenogenetic activation (Kubiak, 1989). Right after the extrusion of the first polar body MII oocytes are not capable to inactivate MPF (Fulton and Whittingham, 1978; Polanski, 1995). Then, they develop this capacity. However, paradoxically, MPF inactivation is transitory. It is rapidly followed by the subsequent reactivation of MPF in maturing oocytes, and these oocytes enter the next stable M state, so called MIII (Kubiak, 1989). Such reaction clearly depends on the type of the activation stimulus. *In vitro* fertilization rarely results in MIII, but parthenogenetic stimuli induce an abortive meiotic MIII stage more frequently (Kubiak, 1989). Shortly after the second polar body extrusion, the MII oocytes are therefore programmed to reactivate the M-phase state after MPF inactivation. Such meiotic "phenotype" perpetuating M-phases without interphases is, therefore, not directly linked to the unique characteristic of the MII state, but rather to the age of the oocyte and the stage of its cytoplasmic maturation. The capacity to mobilize calcium stores seems to be the key event in the response of the oocyte to activation stimulus (Vincent *et al.*, 1992; Vitullo and Ozil, 1992; Dupont, 1998). In fertilized oocytes Ca^{2+} increase is caused by the sperm-brought PLCζ that catalyzes hydrolysis of PIP2 to inositole triphosphate (IP3) and diacylogrycerol (DAG) (Cox *et al.*, 2002; Saunders *et al.*, 2002; Halet, 2004; Swann *et al.*, 2004). Next, IP3 binds to its endocytoplasmic reticulum receptors (IP3R) and induces the release of Ca^{2+} oscillations (Markoulaki *et al.*, 2003; Markoulaki *et al.*, 2004). Moreover, in MII oocytes during early periods after the second polar body extrusion, Mad2 localizes on their kinetochores, as clear-cut fluorescent dots visible on immunofluorescence images. However, this location of Mad2 disappears gradually from the kinetochores during MII-arrest progression, showing that SAC is inactivated (Fig. 6, Sikora-Polaczek *et al.*, 2006). This behavior of Mad2 protein may correlate with the changes of the potential of oocytes to undergo normal activation. There are, however, no evidences that the changes in Mad2 localization (it seems that Mad2 disappearing from the kinetochores is translocated to the cytoplasm, where it could participate in the general inhibition of APC/C; Meraldi *et al.*, 2004) could play any role in cytoplasmic maturation.

Abortive activation of MII oocytes leading to MIII was described for the first time in the mouse. Since then it was reported in other mammals, including rat (Zernicka-Goetz, 1991), hamster (Tateno and Kamiguchi, 1997), rabbit (Collas *et al.*, 1995), cow (Li *et al.*, 2005, Liu *et al.*, 1998), pig (Ito *et al.*, 2003), and human (Balakier *et al.*, 2004). The phenomenon of reactivation of MPF following abortive activation of a MII oocyte suggests also that its triggering mechanism remains in a delicate equilibrium. In some cases even M III-arrested oocyte can react to an activating stimulus again by the decrease and the subsequent rise in MPF activity entering long-lasting MIV (Kubiak, 1989). This suggests the efficiency of the mechanism stabilizing MPF activity even under repeated abortive activations of oocytes.

The M-phase arrest is due to the action of CSF identified in MII oocytes of *Rana pipiens* (Masui and Markert, 1971) by experimental transfer of cytoplasm between mature oocytes and two-cell stage blastomeres (parallel to the discovery of MPF involving the cytoplasmic transfer between mature and non-mature oocytes; Masui and Markert, 1971) (Fig. 7). The formal proof for the CSF presence in mammalian oocyte came later from studies of mouse oocytes (Kubiak *et al.*, 1993), while the presence of MPF was confirmed earlier by Balakier and Czolowska (1977) (Fig. 8). Moreover, CSF seems to develop already during the first meiotic division in mouse oocytes (Ciemerych and Kubiak, 1998), however, it was shown unequivocally only in the oocytes of LT/Sv

strain (Ciemerych and Kubiak, 1998). LT/Sv oocytes have prolonged MI and therefore it was possible to demonstrate the CSF presence by cell fusion experiments, that revealed the capacity of metaphase I oocyte to arrest the mitotic cycle (Fig. 8). Similar experiments with wild type MI oocytes as fusion partners did not prove the presence of CSF during first meiotic M-phase simply because such oocytes spontaneously completed first meiotic division and reached the metaphase II stage during their maturation. However, these results do not contradict the possibility that CSF develops during MI. Thus, we believe that in maturing wild-type oocytes the CSF activity may develop during the first meiotic M-phase similarly as in the LT/Sv strain (how such oocytes could undergo MPF inactivation and complete the first meiotic division will be discussed below, after the description of the molecular identity of CSF). Alternatively, CSF development in LT/Sv maturing oocytes, as the cytoplasmic maturation itself, could be uncoupled from the nuclear one. If the CSF development were not affected in these oocytes, this activity would appear at the right time, i.e. when wild type oocytes reach the MII-arrest. In this case, the above comparison between LT/Sv MI oocytes and wild-type MI oocytes in terms of CSF activity could be erroneous. Future studies of molecular components of CSF should resolve this issue. However, as we mentioned previously, normal progression of the first meiotic division in *mos*-null oocytes questions the role of CSF in MI (Verlhac, *et al.*, 1996)

CSF induces the M-phase arrest by MPF stabilization. The molecular nature of CSF and its mode of action on MPF are not fully resolved. However, a great progress in understanding of CSF nature was marked recently when few key molecules and crucial events were discovered. The first molecule clearly involved in the CSF pathway was identified as Mos protooncogene in *Xenopus* oocytes (Watanabe *et al.*, 1989). Mos is MAP kinase kinase kinase activating MAP kinase kinase i.e. MEK1/2 and further ERK1 and ERK2 MAP kinases by series of phosphorylations (Kosako *et al.*, 1994) This pathway was shown to be of the key importance for the CSF activity both in *Xenopus* (Sagata *et al.,* 1988; Watanabe *et al.*, 1989) as well as in the mouse (Colledge *et al.*, 1994; Hashimoto *et al.*, 1994; Araki *et al.*, 1996; Verlhac *et al.*, 1996; Phillips *et al.*, 2002). However, the final interaction of this pathway with MPF resulting in stabilization of the latter was not so far clearly identified. In *Xenopus*, it seems that Mad2 and Bub1 checkpoint proteins down stream in the Mos/MAP kinase pathway) could play a direct role of APC/C inhibitors (Schwab *et al.*, 2001). However, in the mouse these proteins are clearly not involved (Tsurumi *et al.*, 2004). Similarly, the role of $p90^{rsk1}$, the direct substrate of ERK1 and ERK2 MAP kinases was shown to have the CSF activity in *Xenopus* (Gross *et al.*, 1999) but not in the mouse (Dumont *et al.*, 2005). These differences might be likely linked to species-dependent variants in the identity of CSF. It was also postulated by Dumont *et al.* (2005) that differences in functioning of MAPK mediated signal transduction in *Xenopus* and mouse oocytes might result from the oocyte size. In much bigger amphibian oocytes additional step of signal transduction might be necessary to effectively establish the metaphase arrest (Dumont *et al.*, 2005). Additional problem with Mos/MEK/ERK pathway lies in its permanent activity during all transitional periods in meiosis, when MPF is inactivated i.e. upon anaphase of the first and the second meiotic division. Apparently, the Mos/MEK/ERK pathway does not fulfill the postulate by Markert & Masui (1971) that CSF should stabilize MPF, and thus, should be inactivated before cyclin B degradation and MPF inactivation. Some review articles seemed to ignore this point (e.g. Dupont, 1998).

The real breakthrough in the "CSF science", and at least a partial explanation for the paradox described above, came from studies devoted to APC/C inhibition. Reiman *et al.* described a new protein acting as a potent APC/C inhibitor (Reimann *et al.*, 2001a; Reimann *et al.*, 2001b). They called it Emi1 - Early Mitotic Inhibitor. Soon, further studies have shown that Emi1 is a member of family of Early Mitotic Inhibitors when Emi2/Erp1 was discovered (Tung *et al.*, 2005). Emi2 was shown to have the CSF activity both in *Xenopus* (Liu and Maller, 2005; Rauh *et al.;* 2005; Liu *et al.,* 2006) and in the mouse (Shoji *et al.*, 2006).

Identification of Emi2 as a principal molecule of the CSF pathway was possible when the mechanism of its inactivation was understood (Liu and Maller, 2005; Rauh *et al.*, 2005). It was revealed that Emi2 must be degraded to enable the full activation of APC/C which in turn allows cyclin B degradation and therefore MPF inactivation (Madgwick *et al.*, 2006). The mechanism of

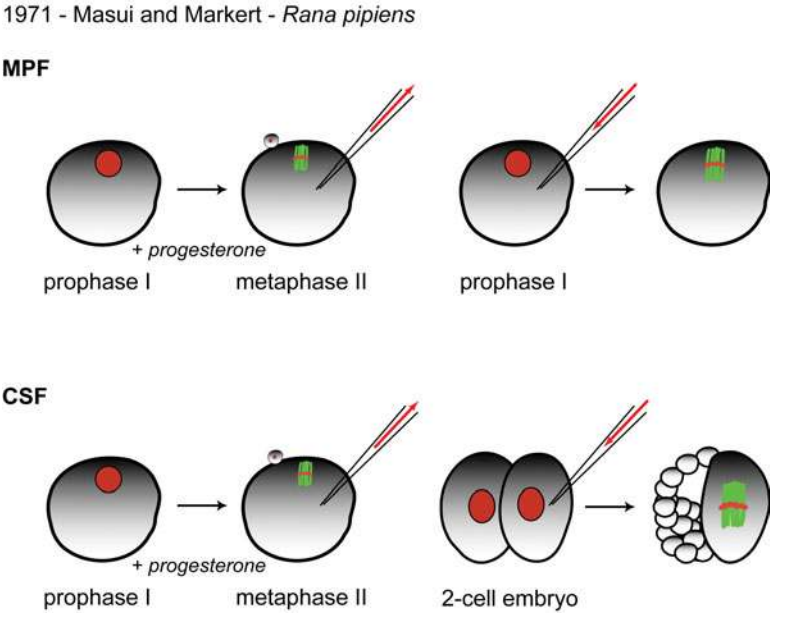

Fig. 7. Discovery of M-phase promoting factor (MPF) and cytostatic factor (CSF) in oocytes of *Rana pipiens*. (MPF) *In 1971, Masui and Markert induced frog prophase I oocytes to mature with progesterone (left), removed a portion of its cytoplasm (red arrow out) and transferred it to the cytoplasm of an uninduced prophase I oocyte (red arrow in; Masui and Markert, 1971). The injected oocyte resumed meiosis despite the lack of progesterone induction and reached the metaphase stage. These changes were attributed to the activity of the factor named as Maturation Promoting Factor.* **(CSF)** *In the same paper, they reported that the cytoplasm of metaphase II arrested frog oocytes injected into one of the two blastomeres of a dividing 2-cell embryo caused a permanent M-phase arrest. The control, uninjected blastomere followed cleavages and formed a multicellular morula.*

Emi2 degradation is rather complicated. It requires sequential phosphorylation of Emi2 by CaMKinase II and Plk1 (Schmidt *et al.*, 2005). Ca+2/calmodulin dependent kinase II (CaMKII) is activated as a result of IP3 to IP3R binding and the burst of ER-derived Ca^{2+} (Markoulaki *et al.*, 2003; Markoulaki *et al.*, 2004). Plk1 is active in MII oocyte, but cannot phosphorylate Emi2 before the CaMKII phosphorylation (Liu and Maller, 2005; Rauh *et al.*, 2005, Schmidt *et al.*, 2005).

Such a serial phosphorylation triggers polyubiquitination of Emi2 and its targeting for the proteasome-mediated degradation. In *Xenopus*, its proteolysis is necessary to trigger polyubiquitination of MPF complex on its cyclin B component (Nishiyama *et al.*, 2000), separation of both cyclin B and CDK1 resulting in MPF inactivation and further degradation of cyclin B by the proteasome (Nishiyama *et al.*, 2000; Chesnel *et al.*, 2006). Moreover, cyclin B dissociation from CDK1 appears to be a primary cause of MPF inactivation followed by CDK1 dephosphorylation on Thr-161 in *Xenopus laevis* cell-free extracts (Chesnel *et al.,* 2007). The latter was shown in cell-free extract during the first embryonic M-phase, but judging by similarities between the molecular mechanisms of MPF inactivation between meiosis (Nishiyama *et al.*, 2000; Chesnel *et al.*, 2005a) and mitosis (Chesnel *et al.*, 2006; Chesnel *et al.,* 2007) we believe that it is also the case for the activated MII oocytes both in *Xenopus* and in the mouse.

If Emi2 serves in MII oocytes as a major APC/C inhibitor what role could be played by Mos/MEK/MAP kinase pathway as the CSF components? Studies showing that CSF inactivation is a two-step event in mouse oocytes could suggest a connection between these two pathways. Indeed, cell fusion experiments between freshly activated mouse oocytes and mitotic one-cell embryos demonstrated that after rapid inactivation of the CSF its activity is restored and maintained for the additional 60 min, even in effectively activated oocytes (Ciemerych and Kubiak, 1999). This interval corresponds to the period of ERK1 and ERK2 inactivation in mouse oocytes (Verlhac *et al.*, 1994). The recent data concerning the mode of Emi2 degradation facilitate understanding of MPF inactivation in oocytes without Mos/MEK/MAP kinase pathway inactivation. The results concerning Emi2 suggest that the degradation of this protein corresponds to the first wave of CSF inactivation. Once Emi2 becomes degraded MPF inactivation occurs, however, still active Mos/MAK/MAP kinase pathway transiently restores the CSF activity, i.e. it remains active until Mos degradation and ERK1 and ERK2 inactivation (Weber *et al.*, 1991; Verlhac *et al.*, 1994). How this pathway could restore the CSF activity remains unclear and needs further studies (see below the hypothesis that Mos/MEK/MAP kinase pathway could interact with Emi2). Alternatively, Mos/MEK/MAP kinase pathway may stabilize Emi2.

The activities of ERK1 and ERK2 are necessary to slow down both the remodeling of the paternal and maternal chromatin via nuclear lamins phosphorylation (Peter *et al.*, 1990; Kubiak *et al.,* 1991; Prather *et al.*, 1991) and the reorganization of the interphase microtubule cytoskeleton (Szollosi *et al.*, 1993; Verlhac *et al.*, 1994) following activation of metaphase

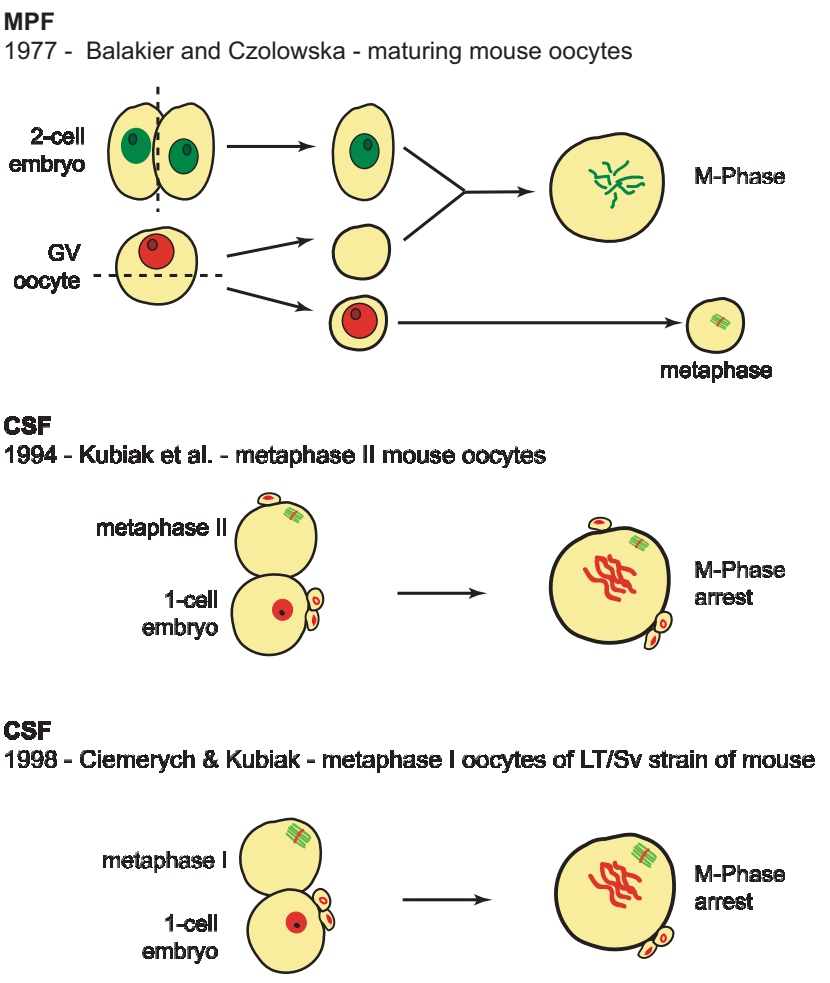

Fig. 8. Discovery of M-phase promoting factor (MPF) and cytostatic factor (CSF) in mouse oocytes. (MPF) *In 1977, Balakier and Czolowska bisected a GV stage oocyte of the mouse into nucleate and anucleate halves (Balakier and Czolowska, 1977). When nucleate halves resumed meiosis and reached metaphase, their anucleate counterparts (supposedly also entering into maturation) were fused with one of the blastomeres of a 2-cell mouse embryo. In the resulting hybrids, blastomere nuclei underwent nuclear envelope breakdown (NEBD) and chromatin condensation.* **(CSF)** *In 1994, Kubiak and collaborators (1994) induced fusion between a metaphase II-arrested mouse oocyte and a 1-cell parthenogenetic mouse embryo. In the resulting hybrids, the pronucleus underwent NEBD, condensed chromatin and remained permanently arrested in the M-phase. Luckily, Kubiak* et al. *used 1-cell embryos that resulted from parthenogenetical activation. In 1995 Zernicka-Goetz* et al. *and Kono* et al. *documented that hybrids of metaphase II-arrested oocytes with 1-cell parthenogenetic, but not with 1-cell zygotes, permanently arrest in M-phase. (Kono* et al., *1995, Zernicka-Goetz* et al., *1995). In hybrids of MII-arrest oocytes with zygotes, Ca2+ spikes occurring within zygote cytoplasm inactivate CSF present within the cytoplasm of the metaphase II-arrested component of the hybrid. In 1998, Ciemerych and Kubiak analyzing oocytes of the LT/Sv mouse strain, which are characterized by a prolonged first meiotic division, documented that CSF is also present at this stage of maturation by fusion of an MI oocyte with a partenogenetic one-cell embryo (Ciemerych and Kubiak, 1998).*

II-arrested oocyte. The restoration of CSF activity seems therefore to be a kind of a side effect of ERK1 and ERK2 activity. During physiological oocyte activation i.e. upon fertilization, the reappearance of the CSF activity has no role since MPF is definitely inactivated and becomes reactivated only at the end of the first embryonic cell cycle i.e. approximately 20 hours later. The embryo seems therefore to have a sufficient time to eliminate the meiotic activities present within the oocyte from which it originated. However, in the next chapter we will document that it is not necessary the whole truth of CSF inactivation.

Let's come back to the hypothesis that the first meiotic division (anaphase I) proceeds in the presence of CSF activity already developed in the oocyte to further elucidate the enigma of this factor. This apparent paradox is not more paradoxical than the onset of anaphase of the second meiotic division (anaphase II). Mos/ERK1/ERK2 MAP kinases pathway is not inactivated immediately upon fertilization, but some 60-90 min later (Weber *et al.*, 1991; Verlhac *et al.*; 1994). Thus, MPF inactivation and the anaphase II proceed in the presence of the activity of this key pathway for the CSF activity. The resolution of the mystery comes again from understanding Emi2 degradation which immediately, but transitionally removes the CSF activity. So, Emi2 is most probably also degraded upon anaphase I of meiosis in mouse oocytes (its degradation was recently shown to be essential for MI/MII transition in *Xenopus laevis*; Ohe *et al.,* 2007), and the presence of active MAP kinase pathway only helps to restore the CSF activity in MII oocyte following the first polar body extrusion. It seems therefore, that the CSF activity is a complex network of different pathways including direct APC/C inhibitors (like Emi2) and creating the appropriate meiotic environment (like Mos/MAP kinase one). It would be of great importance to understand the molecular relationship between the two major CSF pathways and specially to verify a speculation that Mos/MEK/MAP kinase pathway could activate or stabilize Emi2 (see below).

Another important characteristic of the CSF action is its capability to stabilize cyclin B and MPF activity in a dynamic manner. In MII oocytes of the mouse, a clear turnover of cyclin B was found (Kubiak *et al.*, 1993). Such a turnover was later confirmed in *Xenopus* oocytes as well (Thibier *et al.*, 1997). Cyclin B degradation is equilibrated by the continuous cyclin B synthesis. Upon oocyte activation this equilibrium is destroyed in favor to cyclin proteolysis, and the level of this protein dramatically falls down (Kubiak *et al.*, 1993). Moreover, during both the MII-arrest and upon oocyte activation cyclin B degradation requires a functional meiotic spindle (Kubiak *et al.*, 1993; Winston *et al.*, 1995). Spindle microtubules could be likely necessary to bring cyclin B and ubiquitine molecules together or the polyubiquitinated complex CDK1/cyclin B to the proteasome since both cyclin B and proteosomes are highly concentrated within the spindle. However, data concerning the role of SAC and Mad2 protein in mouse oocytes suggest another possibility (Tsurumi *et al.*, 2004; Sikora-Polaczek *et al.,* 2006). It seems that in MII oocytes two separate pathways stabilize cyclin B and MPF activity: namely SAC and CSF. We propose that the CSF (Emi2, Mos/MEK/MAP kinases pathway) efficiently slows down cyclin B degradation assuring the APC/C only partially active and this action is independent on microtubules. Disassembly of microtubules, as well as probably other spindle anomalies, switches the SAC pathway on, without affection the CSF activity, and results in a double inhibitory system with SAC as an emergency brake (Tsurumi *et al.,* 2004). SAC activation causes supplementary inhibition of APC/C via Mad2 (on kinetochores or in the cytoplasm) in a microtubule-dependent manner. Elucidation of the details of the interrelationship between CSF and SAC will certainly enable to verify this hypothesis.

It was shown recently in *Xenopus laevis* CSF extracts that CDK1 phosphorylates and inhibits Emi2 (Wu *et al.,* 2007). CDK1-phosphorylated sites are dephosphorylated by PP2A phosphatase, which antagonizes the effect of CDK1. According to Wu and colleagues, such equilibrium would be responsible for maintaining stable cyclin B levels and moderate APC/C activity (i.e. controlled cyclin B turnover) during the CSF arrest (Wu *et al.,* 2007). However, this mechanism might also be completed or reinforced by the Mos/MEK/MAP kinase pathway. This could explain a role of this pathway in the CSF arrest of oocytes. Mos/MEK/MAP kinase pathway could potentially be involved in phosphorylation of Emi2 and could affect it specifically in MII-arrested oocytes. Recently, such a hypothesis was successfully tested in *Xenopus laevis* extracts. Emi2 was shown to be a direct substrate of $p90^{rsk}$ kinase (Inoue *et al.*, 2007; Nishiyama *et al.*, 2007). Moreover, phosphorylation of Emi2 is necessary for the Emi2-dependent CSF activity. Therefore, this protein, at least in *Xenopus* oocytes, is the most downstream identified substrate in the Mos/MEK/MAP kinase ERK1 and ERK2 pathway. It is, however,

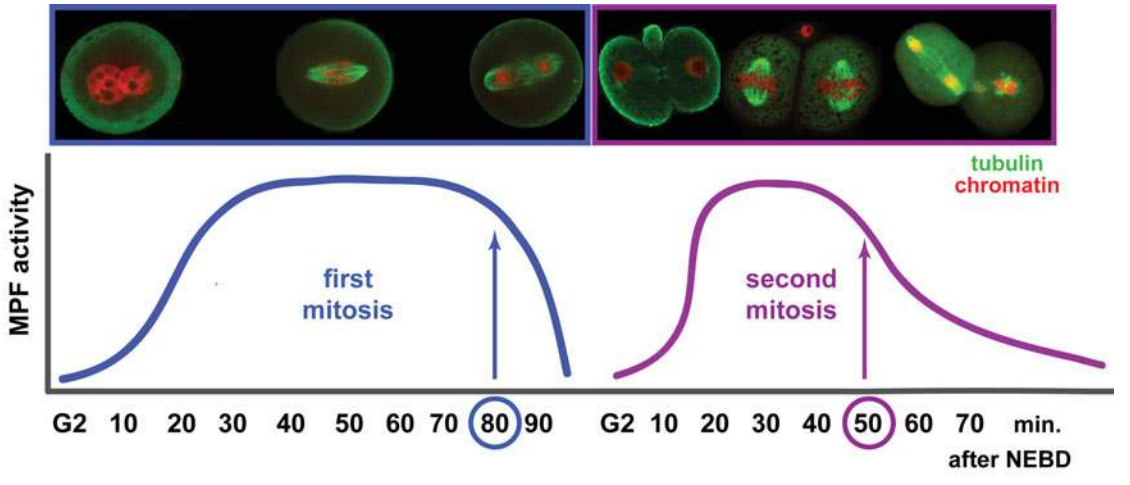

Fig. 9. M-phase promoting factor (MPF) dynamics during the first and second division of the mouse embryo. *The first mitotic division takes on average 120 min while the second one takes around 70 min. Time points when MPF activity (measured by histone H1 kinase activity) start to drop are depicted by circles around the specific time points. Upper lane: microtubules (green) and chromatin (red) during the first two embryonic M-phases.*

still unclear which kinase phosphorylates Emi2 in MII mouse oocytes, since, as explained above, $p90^{rsk}$ kinases were excluded as CSF component (Dumont *et al.,* 2005). Thus, identification of such kinase remains an open issue. On the other hand, it was also not investigated whether CDK1/PP2A balance operates also in mouse oocytes.

Finally, to complete our vision of CSF we have to mention a potential role od CDK2-cyclin E complex. It was shown to play a part of CSF activity in *Xenopus* oocytes via checkpoint protein Mps1 (Grimison, *et al.,* 2006) and is supposed to be a third, parallel component next to Mos/MEK/MAP kinase and Emi2 (Liu, *et al.,* 2007). However, there is no data suggesting such a role in the mouse oocyte. Moreover, there is no evidence that oocytes of mice lacking cyclin E1 and cyclin E2 (Geng, *et al.*, 2003; Parisi *et al.*, 2003) or CDK2 (Berthet, *et al.*, 2003, Ortega, *et al.*, 2003) exhibit any abnormalities in the regulation of MII arrest. Thus, we assume that the CDK2-cyclin E is not involved in the CSF activity in mouse oocytes.

First embryonic mitosis: meiosis-like mitosis, or how and why to prolong cell division of the zygote?

Several lines of evidence document that the first embryonic cell cycle differs from the subsequent ones. Progression of the first cell cycle relies on maternal factors i.e. mRNAs and proteins accumulated during growth phase of GV oocyte i.e. during the long arrest in the prophase of the first meiotic division (Bachvarova and De Leon, 1980; Pratt *et al.*, 1983; Bachvarova, 1985; Nothias *et al.*, 1995). The intense transcription ceases once oocyte reaches its maximal volume and becomes competent to resume meiotic division (Zuccotti *et al.*, 1995; Bouniol-Baly *et al.*, 1999; De La Fuente, 2006). Fertilization that triggers oocyte activation does not terminate transcriptional silencing. Despite that the first mRNAs transcribed from embryonic genome is detectable in G2 phase of the first cell cycle (Ram and Schultz, 1993; Bouniol *et al.*, 1995; Aoki *et al.*, 1997; Thompson *et al.*, 1998; Hamatani *et al.*, 2004) little is known about genes activated at that period of development. Currently, only single pieces of evidence document that proteins encoded by embryonic genome are both synthesized and active in one-cell embryo. Studies involving mice lacking functional RGS14 gene (a regulator of G protein signaling) showed that this mitotic spindle protein is necessary for the first mitotic division of the mouse embryo (Martin-McCaffrey *et al.*, 2004). Nevertheless, the burst of the major transcription, also described as zygotic genome activation (ZGA), occurs in the mouse during the second cell cycle when proteins originated from the embryonic genome become detectable (Flach *et al.*, 1982; Bensaude *et al.*, 1983; Bolton *et al.*, 1984; Schultz, 1993). Thus, the regulation of the first embryonic cell cycle depends largely on the mRNA and proteins accumulated already during meiosis. Consequently, during the early mitotic cycles of the embryo some meiotic pathways could also be present and eventually active.

Transcriptional silence is not the only characteristic that distinguishes the first cell cycle from the subsequent cycles. Among special features of the one-cell embryo one can list rapid deformation of its surface i.e. cortical activity that precedes the first, but not the next mitotic divisions (Waksmundzka *et al.*, 1984; Ciemerych, 1995; Ciemerych *et al.*, 1998; Liu *et al.*, 2002; Alikani *et al.*, 2005). Moreover, some phenomena such as cyclic activity of K+ ion channels (Day *et al.*, 1998) or cytoplasmic MPF activation are induced autonomously, i.e. independently from the nucleus, exclusively during the first cell cycle (Ciemerych *et al.*, 1998; Kubiak and Ciemerych, 2001). All these phenomena point to a high autonomy of the one cell embryo cytoplasm, comparable to the autonomy of the oocyte cytoplasm (Czolowska and Balakier, 1977; Hoffmann *et al.,* 2006).

Detailed analyses of cleaving preimplantation mouse embryos revealed yet another uniqueness of the one-cell embryo i.e. significant increase in the duration of the first mitosis in comparison to the subsequent ones. The first embryonic mitosis, assessed from the moment of nuclear envelope breakdown (NEBD) and till the beginning of cytokinesis, takes approximately 120 min., while the second one only 70 min. (Fig. 9; Ciemerych *et al.*, 1999). Importantly, similar differences are characteristic also for the first cleavage divisions of *X. laevis* (Chesnel *et al.*, 2005a; Chesnel *et al.*, 2005b), sea urchin *Sphaerechinus granularis* (J.Z. Kubiak and P. Cormier, unpublished observation), and *C. elegans* embryos (J.Z. Kubiak, F. Chesnel and P. Gonczy, unpublished observations). One of the possible explanations of the prolongation of the first mitosis observed in mouse embryos comes from the fact that female and male pronuclei are assembled via two different routes (Wright and Longo, 1988; Adenot *et al.*, 1991; Yanagimachi, 1994). In consequence these two sets of chromatin remain unconnected throughout the first embryonic cell cycle. Male and female pronuclei separately undergo DNA replication in S-phase. Importantly in mammalian zygotes two pronuclei do not fuse but independently and asynchronously undergo NEBD, and start chromatin condensation at G2/M transition (Ciemerych and Czolowska, 1993; Bomar *et al.*, 2002). Finally, they reunite during the formation of common metaphase plate and mitotic spindle (Donahue, 1972; Howlett and Bolton, 1985; Mayer *et al.*, 2000). It seems, therefore, possible that the prolongation of the first mitotic division facilitates and/or is caused by the necessity to combine two chromatin sets within single plate and spindle. However, the duration of the first mitosis does not differ between zygotes containing two pronuclei and haploid parthenogenotes with one haploid pronucleus and is cytoplasmatically controlled (Ciemerych *et al.*, 1999). Moreover, the development of zygotes that contain single diploid pronucleus is also not affected (Krukowska and Tarkowski, 2005). Therefore, the molecular machinery prolonging the first embryonic mitosis is again another manifestation of events that occur independently of the nucleus in the zygote. Nevertheless, it cannot be excluded that mechanisms operating within the cytoplasm of one-cell mouse embryo were evolutionary developed to assure the time necessary for the proper spatial arrangements of female and male chromosomes by extending the first mitosis in time.

As we already mentioned, the formation of the metaphase plate and the spindle involves precise molecular mechanisms reassuring that kinetochores of all chromosomes form proper and stable connections with microtubules of the mitotic spindle (Fig. 2; Rieder *et al.*, 1994; Li and Nicklas, 1995; Rieder and Maiato, 2004). As it was documented in mitotically dividing somatic cells (Li and Benezra, 1996; Li *et al.*, 1997; Gorbsky *et al.*, 1998; Waters *et al.*, 1998; Fang *et al.*, 1999; Waters *et al.*, 1999; Chang *et al.*, 2004) and also in meiotically maturing oocytes (Kallio *et al.*, 2000; Wassmann *et al.*, 2003; Tsurumi *et al.*, 2004) active SAC prevents the anaphase onset. Inactivation of SAC accompanied by the loss

of Mad2 from kinetochores delineates the end of the prometaphase and the beginning of the metaphase and allows anaphase onset (Fig. 2; Li and Benezra, 1996; Waters *et al.*, 1998). Prometaphase can be distinguished from metaphase not only by the analysis of the localization of SAC proteins, but also by examination of chromosome movements. Oscillatory translocation of chromosomes pictures unbalanced pulling forces causing their displacement out of the forming plate (Howell *et al.*; 2000, Howell *et al.*, 2004; Meraldi *et al.*, 2004). Again this feature is characteristic not only for somatic cells but also for oocytes during metaphase I of meiosis (Brunet *et al.,* 1999). Thus, the presence of Mad2 at kinetochores and continuous chromosome movements delineates the prometaphase state.

In somatic cells, the prometaphase is relatively long. An average mitosis takes 50 min and the duration of metaphase is on average 10 min (Rieder *et al.*, 1994; Howell *et al.*, 2000; Jones *et al.*, 2004; Meraldi *et al.*, 2004; Meraldi and Sorger, 2005). These proportions could be changed or even inversed, e.g. during considerably prolonged first meiotic division of maturing oocytes, a prometaphase (characterized by the presence of Mad2 and Bub1on kinetochores and by chromosome movements) takes about 8 hrs, while metaphase only 1 hr or less (Brunet *et al.*, 1999; Brunet *et al.*, 2003; Wassmann *et al.*, 2003). Surprisingly, during the first and the second mitosis of the mouse embryo, Mad2 is localized at kinetochores for the similar period of time. Moreover, chromosome relocations within the metaphase plate were observed also for the comparable period in these two mitotic M-phases i.e. for 20-30 min, demonstrating that during both divisions the length of the prometaphase is similar (Sikora-Polaczek *et al.*, 2006). Thus, it is a metaphase itself that is prolonged in the first mitosis. The comparison of our previous results i.e. the dynamics of MPF activation and recently studied SAC inactivation showed that both events correlate during both mitotic divisions (Fig. 9, Ciemerych *et al.*, 1999; Sikora-Polaczek *et al.*, 2006). However, only during the first mitosis MPF becomes stabilized for over 60 min (Ciemerych *et al.*, 1999) (Fig. 10).

Noteworthy, the stabilization of MPF is also a landmark of oocytes arrested at the metaphase of the second mitotic division and is not achieved by SAC but by checkpoint-independent CSF activity (Tsurumi *et al.*, 2004; Sikora-Polaczek *et al.*, 2006). Mad2 becomes relocated from kinetochores to cytoplasm within 2h after the completion of the first meiotic division and the formation of the M II plate and the spindle. Importantly, such oocytes can be arrested for next several hours until being activated by penetrating spermatozooa. Thus, both M II oocytes and one cell embryos resemble each other in the dynamics of SAC inactivation (delineated by the disappearance of Mad2 from kinetochores and the cessation of chromosome movements). This similarity strongly suggests that also during the first mitotic division an initial role of SAC as a mechanism preventing anaphase onset is replaced by other MPF-stabilizing mechanism(s) (Sikora-Polaczek *et al.*, 2006).

The CSF activity drops after the M II oocyte activation i.e. at the very beginning of the first embryonic cell cycle. However, it cannot be excluded that transient stabilization of MPF during the first embryonic mitosis is a manifestation of a tuning mechanism possibly dependent on a residual activity of factor/factors previously (i.e. in M II) involved in the CSF activity. Since the switch from the meiotic to the mitotic type of control occurs during the first cell cycle of the embryo it is possible that some meiotic factors are present and remain active till the first mitosis. Similarly as during the meiotic arrest, these regulators could stabilize MPF activity via APC/C down-regulation, i.e. via prevention of cyclin B degradation. It would also be of importance to analyze whether the transitional metaphase arrest during the first embryonic mitotic division depends on a finely regulated turnover of cyclin B, as happens during the MII-arrest in oocytes (Kubiak *et al.,* 1993).

Mos/MEK/MAP kinase (ERK1/ERK2) pathway is necessary for CSF establishment in M II oocytes (Colledge *et al.*, 1994; Hashimoto, 1996; Phillips *et al.*, 2002). It influences positively the MPF activity and stabilizes the meiotic spindle via proteins like MISS (MAPK-interacting and spindle stabilizing) and DocR1 (Fig. 3; Lefebvre *et al.*, 2002; Terret *et al.*, 2003a). Several lines of evidence suggest that ERK1/ERK2 kinases play important role during the initiation and progression of mitosis in somatic cells (Peter *et al.*, 1992; Sanghera *et al.*, 1992; Chiri *et al.*, 1998; Shapiro *et al.*, 1998; Zecevic *et al.*, 1998; Harding *et al.*, 2003; Horne and Guadagno, 2003). This pathway does not seem, however, to be active during either the first or the second mitosis of the mouse embryo. During both divisions only inactive (i.e. non-phosphorylated) forms of ERK1/ERK2 were detectable in dividing embryos (Kalab *et al.*, 1996, Verlhac *et al.*, 1994). However, MAP kinase activity can be also assayed by its ability to phosphorylate MBP (myelin basic protein) in an *in vitro* assay. Importantly, during the first but not the second mitosis an MBP activity was detectable in such assay suggesting the possible activity of other MAP kinase(s) (Verlhac *et al.*, 1994; Haraguchi *et al.*, 1998). $p90^{rsk1}$

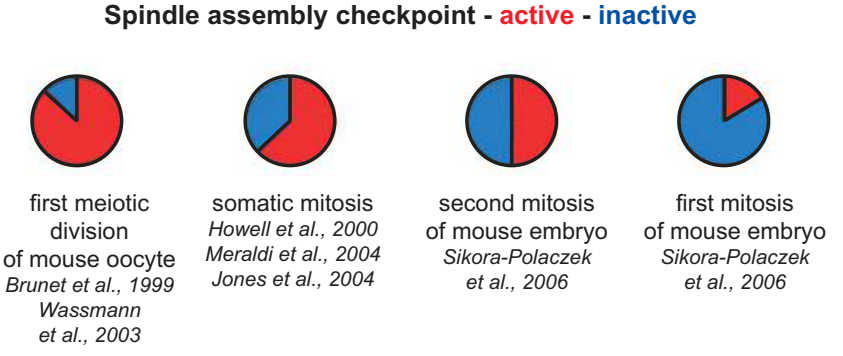

Fig. 10. Changes in the proportion of active and inactive spindle assembly checkpoint (SAC) during different M-phases in maturing oocytes, mitotically dividing mouse embryos and somatic cells. *Proportion of active (red) versus inactive (blue) SAC significantly differs during various M-phases. SAC activity is detectable throughout the almost whole first meiotic division (called metaphase I despite representing in fact prometaphase I) of mouse oocytes (Brunet* et al., *1999, Wassmann* et al., *2003). On the other hand, during the first mitotic division of the mouse embryo, SAC activity is detectable only for a proportionally short period of time (Sikora-Polaczek* et al., *2006). During subsequent mitotic divisions of the mouse embryo, these proportions resemble mitotically dividing somatic cells, suggesting transitional characteristic of the first embryonic M-phase (Howell* et al., *2000, Jones* et al., *2004, Meraldi* et al., *2004).*

figures among downstream targets of ERK1/ERK2 kinases. However, it is not involved in CSF activation in mouse oocytes (Dumont *et al.*, 2005) despite that its phosphorylation is an apparent manifestation of ERK1/ERK2 kinases activity (Kalab *et al.*, 1996; Verlhac *et al.*, 1996). Surprisingly, $p90^{rsk1}$ was shown to be partially phosphorylated during the first but not the second mitosis of the mouse embryo (Kalab *et al.*, 1996; our unpublished results). It cannot be also excluded that some modifications of MAP kinase pathway might be involved in MPF stabilization during the first mitosis of the mouse embryo and that a similar mode of APC/C inactivation operates during meiosis and during the first mitotic M-phase despite obvious differences between them. All these aspects await for elucidation.

It is well documented that the CSF activity involves factors preventing APC/C activation. As we mentioned previously these factors playing a role in SAC (such as Mad2, Mad1, Bub1) are involved neither in CSF in mouse oocytes (Tsurumi *et al.*, 2004) nor in M-phase prolongation in mitotically dividing one-cell embryos (Sikora-Polaczek *et al.*, 2006). Another potential factor involved in the prolongation of the first mitotic division might be one (or more) Emi protein(s). Emi1 was shown to bind Cdc20 and to inhibit APC/C during mitotic division (Reimann *et al.*, 2001a; Reimann *et al.*, 2001b; Margottin-Goguet *et al.*, 2003). It was proposed to be involved in CSF activation via $p90^{rsk1}$ dependent manner (Paronetto *et al.*, 2004). However, this hypothesis was questioned by the discovery that $p90^{rsk1}$ and other RSK family members are not involved in the metaphase II arrest (Dumont *et al.*, 2005). Nevertheless, studies on oocytes of *emi1*-null mice showed that Emi1 protein is crucial for the mitotic progression during preimplantation mouse development (Lee *et al.*, 2006a).

Emi2 has unquestionable role in CSF activation: its silencing causes parthenogenetic activation of oocytes and its levels drop in parthenogenetically activated oocytes (Shoji *et al.*, 2006). Interestingly, Emi2 degradation upon mouse oocyte activation proceeds relatively slow leaving high amount of this protein 6 hours after activation and easily detectable portion still present in the two-cell embryos (see Fig. 1H in Shoji *et al.,* 2006), which implies possible influence of Emi2 on the first mitosis. In *Xenopus*, during the first embryonic mitosis Emi2 seems not to play an essential role since it is present at too low levels to induce the M-phase arrest and it is not degraded as happens during the second meiotic division, but specifically phosphorylated (Liu, *et al.*, 2006). However, Emi2 depleted extracts accumulate less cyclin B during the first embryonic mitosis which result in a shortening of this M-phase duration (Liu, *et al.,* 2006) as shown before by Chesnel *et al.* (2005). The Emi1 and Emi2 localization and functionality was, however, not carefully tested in the first two embryonic mitoses during unperturbed mouse development. A paper describing briefly the effect of Emi2, as well as Emi1, knock-down using RNAi in mouse MII oocytes that were subsequently fertilized, suggests that these proteins have no essential role in mouse early embryonic development (Amanai, *et al.,* 2006) as in *Xenopus* (Liu, *et al.,* 2006). However, the duration of the first embryonic mitosis was not studied in such embryos. Thus, it cannot be excluded that Emi proteins also impinge on the machinery regulating the first mitotic division.

The fact that the first cell cycle bridges meiotic and mitotic type of cell cycle control directs a search of the factors involved in the regulation of the first mitosis towards the factors directly involved into the stabilization of the MPF activity. It is however, possible that other regulators or signal transduction pathways can also be engaged.

Concluding remarks

This overview points out similarities as well as striking differences between meiotic and mitotic M-phases of mouse oocytes and one-cell embryos. The M-phase regulatory machinery common for all eukaryotic cells is modified during this relatively short period of oogenesis and early development several times. These modifications result from different applications of either common molecular mechanisms (e.g. SAC) or stage-specific ones (e.g. CSF and the not yet specified mechanism prolonging the first embryonic mitosis). Each cycle and each M-phase differ substantially to assure harmonious preparation of oocytes for fertilization and early development.

Acknowledgements

The authors thank Franck Chesnel for the critical reading of this manuscript, Zuzanna Maciejewska for sharing the unpublished confocal image of one-cell mouse embryo at anaphase stage, and Ewa Borsuk for the phase-contrast image of fertilized oocyte. MAC is a recipient of L'Oreal for Women and Science Foundation, JZK acknowledges grants from ARC (4900), Ligue Contre le Cancer (Comité d'Ille-et-Vilaine et de la Vendée) and BQR (Université Rennes 1) and ZP from Deutsche Forschungsgemeinschaft (Schwerpunktprogramme 1109).

References

ADENOT, P.G., SZOLLOSI, M.S., GEZE, M., RENARD, J.P. and DEBEY, P. (1991). Dynamics of paternal chromatin changes in live one-cell mouse embryo after natural fertilization. *Mol Reprod Dev* 28: 23-34.

ALIKANI, M., SCHIMMEL, T. and WILLADSEN, S.M. (2005). Cytoplasmic fragmentation in activated eggs occurs in the cytokinetic phase of the cell cycle, in lieu of normal cytokinesis, and in response to cytoskeletal disorder. *Mol Hum Reprod* 11: 335-44.

AMANAI, M., SHOJI, S., YOSHIDA, N., BRAHMAJOSYULA, M., PERRY, A.C. (2006). Injection of mammalian metaphase II oocytes with short interfering RNAs to dissect meiotic and early mitotic events. *Biol Reprod* 75:891-8.

ANGER, M., STEIN, P. and SCHULTZ, R.M. (2005). Cdc6 requirement for spindle formation during maturation of mouse oocytes. *Biol Reprod* 72: 188-94.

AOKI, F., WORRAD, D.M. and SCHULTZ, R.M. (1997). Regulation of transcriptional activity during the first and second cell cycles in the preimplantation mouse embryo. *Dev Biol* 181: 296-307.

ARAKI, K., NAITO, K., HARAGUCHI, S., SUZUKI, R., YOKOYAMA, M., INOUE, M., AIZAWA, S., TOYODA, Y. and SATO, E. (1996). Meiotic abnormalities of c-mos knockout mouse oocytes: Activation after first meiosis or entrance into third meiotic metaphase. *Biol Reprod* 55: 1315-24.

BACHVAROVA, R. (1985). Gene expression during oogenesis and oocyte development in mammals. *Dev Biol* 1: 453-524.

BACHVAROVA, R. and DE LEON, V. (1980). Polyadenylated RNA of mouse ova and loss of maternal RNA in early development. *Dev Biol* 74: 1-8.

BALAKIER, H. and CZOLOWSKA, R. (1977). Cytoplasmic control of nuclear maturation in mouse oocytes. *Exp Cell Res* 110: 466-9.

BALAKIER, H., SOJECKI, A., MOTAMEDI, G. and LIBRACH, C. (2004). Time-dependent capability of human oocytes for activation and pronuclear formation during metaphase ii arrest. *Hum Reprod* 19: 982-7.

BENSAUDE, O., BABINET, C., MORANGE, M. and JACOB, F. (1983). Heat shock proteins, first major products of zygotic gene activity in mouse embryo. *Nature* 305: 331-3.

BERTHET, C., ALEEM, E., COPPOLA, V., TESSAROLLO, L. and KALDIS, P. (2003). Cdk2 knockout mice are viable. *Curr Biol* 13: 1775-85.

BOLTON, V.N., OADES, P.J. and JOHNSON, M.H. (1984). The relationship between cleavage, DNA replication, and gene expression in the mouse 2-cell embryo. *J Embryol Exp Morphol* 79: 139-63.

BOMAR, J., MOREIRA, P., BALISE, J.J. and COLLAS, P. (2002). Differential regulation of maternal and paternal chromosome condensation in mitotic zygotes. *J Cell Sci* 115: 2931-40.

BOUNIOL-BALY, C., HAMRAOUI, L., GUIBERT, J., BEAUJEAN, N., SZOLLOSI, M.S. and DEBEY, P. (1999). Differential transcriptional activity associated with chromatin configuration in fully grown mouse germinal vesicle oocytes. *Biol Reprod* 60: 580-7.

BOUNIOL, C., NGUYEN, E. and DEBEY, P. (1995). Endogenous transcription occurs at the 1-cell stage in the mouse embryo. *Exp Cell Res* 218: 57-62.

BRADY, D.M. and HARDWICK, K.G. (2000). Complex formation between Mad1p, Bub1p and Bub3p is crucial for spindle checkpoint function. *Curr Biol* 10: 675-8.

BRUNET, S., MARIA, A.S., GUILLAUD, P., DUJARDIN, D., KUBIAK, J.Z. and MARO, B. (1999). Kinetochore fibers are not involved in the formation of the first meiotic spindle in mouse oocytes, but control the exit from the first meiotic m phase. *J Cell Biol* 146: 1-12.

BRUNET, S. and MARO, B. (2005). Cytoskeleton and cell cycle control during meiotic maturation of the mouse oocyte: Integrating time and space. *Reproduction* 130: 801-11.

BRUNET, S., PAHLAVAN, G., TAYLOR, S. and MARO, B. (2003). Functionality of the spindle checkpoint during the first meiotic division of mammalian oocytes. *Reproduction* 126: 443-50.

BRUNET, S. and VERNOS, I. (2001). Chromosome motors on the move. From motion to spindle checkpoint activity. *EMBO Rep* 2: 669-73.

CHANG, H.Y., LEVASSEUR, M. and JONES, K.T. (2004). Degradation of APC^{cdc20} and APC^{cdh1} substrates during the second meiotic division in mouse eggs. *J Cell Sci* 117: 6289-96.

CHESNEL, F., BAZILE, F., PASCAL, A. and KUBIAK, J.Z. (2006). Cyclin B dissociation from cdk1 precedes its degradation upon MPF inactivation in mitotic extracts of Xenopus laevis embryos. *Cell Cycle* 5: 1687-98.

CHESNEL, F., BAZILE, F., PASCAL, A. and KUBIAK, J.Z. (2007). Cyclin B2/CDK1 dissociation precedes cyclin B2 degradation and CDK1 Thr-161 dephosphorylation during histone H1 kinase inactivation in mitotic *Xenopus laevis* embryo cell-free extract. *Int J Dev Biol* 51: 297-305.

CHESNEL, F., GAUTIER, I., RICHARD-PARPAILLON, L. and KUBIAK, J.Z. (2005a). Each mitosis can be different: How the cell cycle machinery modulates early embryonic M-phases. In *New impact on protein modifications in the regulation of reproductive system.*, (ed. TOKUMOTO, T.). Research Signpost, 155-167.

CHESNEL, F., VIGNAUX, F., RICHARD-PARPAILLON, L., HUGUET, A. and KUBIAK, J.Z. (2005b). Differences in regulation of the first two M-phases in Xenopus laevis embryo cell-free extracts. *Dev Biol.* 285: 358-375.

CHIRI, S., DE NADAI, C. and CIAPA, B. (1998). Evidence for MAP kinase activation during mitotic division. *J Cell Sci* 111 (Pt 17): 2519-27.

CIEMERYCH, M.A. (1995). Chromatin condensation activity and cortical activity during the first three cell cycles of a mouse embryo. *Mol Reprod Dev* 41: 416-24.

CIEMERYCH, M.A. and CZOLOWSKA, R. (1993). Differential chromatin condensation of female and male pronuclei in mouse zygotes. *Mol Reprod Dev* 34: 73-80.

CIEMERYCH, M.A. and KUBIAK, J.Z. (1998). Cytostatic activity develops during meiosis i in oocytes of lt/sv mice. *Dev Biol* 200: 198-211.

CIEMERYCH, M.A. and KUBIAK, J.Z. (1999). Transient reactivation of CSF in parthenogenetic one-cell mouse embryos. *Biol Cell* 91: 641-7.

CIEMERYCH, M.A., MARO, B. and KUBIAK, J.Z. (1999). Control of duration of the first two mitoses in a mouse embryo. *Zygote* 7: 293-300.

CIEMERYCH, M.A., TARKOWSKI, A.K. and KUBIAK, J.Z. (1998). Autonomous activation of histone H1 kinase, cortical activity and microtubule organization in one- and two-cell mouse embryos. *Biol Cell* 90: 557-64.

COHEN-FIX, O. and KOSHLAND, D. (1997). The metaphase-to-anaphase transition: avoiding a mid-life crisis. *Curr Opin Cell Biol* 9: 800-6.

COLLAS, P., CHANG, T., LONG, C. and ROBL, J.M. (1995). Inactivation of histone H1 kinase by Ca2+ in rabbit oocytes. *Mol Reprod Dev* 40: 253-8.

COLLEDGE, W.H., CARLTON, M.B., UDY, G.B. and EVANS, M.J. (1994). Disruption of c-mos causes parthenogenetic development of unfertilized mouse eggs. *Nature* 370: 65-8.

COX, L.J., LARMAN, M.G., SAUNDERS, C.M., HASHIMOTO, K., SWANN, K. and LAI, F.A. (2002). Sperm phospholipase Czeta from humans and cynomolgus monkeys triggers Ca2+ oscillations, activation and development of mouse oocytes. *Reproduction* 124: 611-23.

DAY, M.L., JOHNSON, M.H. and COOK, D.I. (1998). A cytoplasmic cell cycle controls the activity of a K+ channel in pre-implantation mouse embryos. *EMBO J* 17: 1952-60.

DE LA FUENTE, R. (2006). Chromatin modifications in the germinal vesicle (GV) of mammalian oocytes. *Dev Biol* 292: 1-12.

DONAHUE, R.P. (1972). Fertilization of the mouse oocyte: Sequence and timing of nuclear progression to the two-cell stage. *J Exp Zool* 180: 305-18.

DUMONT, J., UMBHAUER, M., RASSINIER, P., HANAUER, A. and VERLHAC, M.H. (2005). p90rsk is not involved in cytostatic factor arrest in mouse oocytes. *J Cell Biol* 169: 227-31.

DUPONT, G. (1998). Link between fertilization-induced Ca2+ oscillations and relief from metaphase II arrest in mammalian eggs: A model based on calmodulin-dependent kinase II activation. *Biophys Chem* 72: 153-67.

FANG, G., YU, H. and KIRSCHNER, M.W. (1998). The checkpoint protein Mad2 and the mitotic regulator cdc20 form a ternary complex with the anaphase-promoting complex to control anaphase initiation. *Genes Dev* 12: 1871-83.

FANG, G., YU, H. and KIRSCHNER, M.W. (1999). Control of mitotic transitions by the anaphase-promoting complex. *Philos Trans R Soc Lond B Biol Sci* 354: 1583-90.

FLACH, G., JOHNSON, M.H., BRAUDE, P.R., TAYLOR, R.A. and BOLTON, V.N. (1982). The transition from maternal to embryonic control in the 2-cell mouse embryo. *EMBO J* 1: 681-6.

FRY, A.M. and YAMANO, H. (2006). Apc/c-mediated degradation in early mitosis: How to avoid spindle assembly checkpoint inhibition. *Cell Cycle* 5: 1487-91.

FULTON, B.P. and WHITTINGHAM, D.G. (1978). Activation of mammalian oocytes by intracellular injection of calcium. *Nature* 273: 149-51.

GAUTIER, J., MINSHULL, J., LOHKA, M., GLOTZER, M., HUNT, T. and MALLER, J.L. (1990). Cyclin is a component of maturation-promoting factor from Xenopus. *Cell* 60: 487-94.

GENG, Y., YU, Q., SICINSKA, E., DAS, M., SCHNEIDER, J.E., BHATTACHARYA, S., RIDEOUT, W.M., BRONSON, R.T., GARDNER, H. and SICINSKI, P. (2003). Cyclin E ablation in the mouse. *Cell* 114: 431-43.

GONG, D., POMERENING, J.R., MYERS, J.W., GUSTAVSSON, C., JONES, J.T., HAHN, A.T., MEYER, T. and FERRELL, J.E., JR. (2007). Cyclin A2 regulates nuclear-envelope breakdown and the nuclear accumulation of cyclin B1. *Curr Biol* 17: 85-91.

GORBSKY, G.J., CHEN, R.H. and MURRAY, A.W. (1998). Microinjection of antibody to Mad2 protein into mammalian cells in mitosis induces premature anaphase. *J Cell Biol* 141: 1193-205.

GRIMISON, B., LIU, J., LEWELLYN, A.L., MALLER, J.L. (2006). Metaphase arrest by cyclin E-Cdk2 requires the spindle-checkpoint kinase Mps1. *Curr Biol* 16:1968-73.

GROSS, S.D., SCHWAB, M.S., LEWELLYN, A.L. and MALLER, J.L. (1999). Induction of metaphase arrest in cleaving Xenopus embryos by the protein kinase p90rsk. *Science* 286: 1365-7.

HAERING, C.H. and NASMYTH, K. (2003). Building and breaking bridges between sister chromatids. *Bioessays* 25: 1178-91.

HAGTING, A., DEN ELZEN, N., VODERMAIER, H.C., WAIZENEGGER, I.C., PETERS, J.M. and PINES, J. (2002). Human securin proteolysis is controlled by the spindle checkpoint and reveals when the APC/C switches from activation by cdc20 to cdh1. *J Cell Biol* 157: 1125-37.

HALET, G. (2004). PKC signaling at fertilization in mammalian eggs. *Biochim Biophys Acta* 1742: 185-9.

HAMATANI, T., CARTER, M.G., SHAROV, A.A. and KO, M.S. (2004). Dynamics of global gene expression changes during mouse preimplantation development. *Dev Cell* 6: 117-31.

HAMPL, A. and EPPIG, J.J. (1995). Translational regulation of the gradual increase in histone H1 kinase activity in maturing mouse oocytes. *Mol Reprod Dev* 40: 9-15.

HARAGUCHI, S., NAITO, K. and SATO, E. (1998). MAP kinase cascade, but not ERKs, activated during early cleavage of mouse embryos. *Mol Reprod Dev* 51: 148-55.

HARDING, A., GILES, N., BURGESS, A., HANCOCK, J.F. and GABRIELLI, B.G. (2003). Mechanism of mitosis-specific activation of MEK1. *J Biol Chem* 278: 16747-54.

HASHIMOTO, N. (1996). Role of c-mos proto-oncogene product in the regulation of mouse oocyte maturation. *Horm Res* 46 Suppl 1: 11-4.

HASHIMOTO, N., WATANABE, N., FURUTA, Y., TAMEMOTO, H., SAGATA, N., YOKOYAMA, M., OKAZAKI, K., NAGAYOSHI, M., TAKEDA, N., IKAWA, Y. *et al.* (1994). Parthenogenetic activation of oocytes in c-mos-deficient mice. *Nature* 370: 68-71.

HASSOLD, T., HUNT, P.A. and SHERMAN, S. (1993). Trisomy in humans: Incidence, origin and etiology. *Curr Opin Genet Dev* 3: 398-403.

HERBERT, M., LEVASSEUR, M., HOMER, H., YALLOP, K., MURDOCH, A. and MCDOUGALL, A. (2003). Homologue disjunction in mouse oocytes requires proteolysis of securin and cyclin b1. *Nat Cell Biol* 5: 1023-5.

HOFFMANN, S., TSURUMI, C., KUBIAK, J. Z. and POLANSKI, Z. (2006). Germinal vesicle material drives meiotic cell cycle of mouse oocyte through the 3'UTR-dependent control of cyclin B1 synthesis. *Dev Biol* 292: 46-54.

HOFFMANN, I., CLARKE, P.R., MARCOTE, M.J., KARSENTI, E. and DRAETTA, G. (1993). Phosphorylation and activation of human cdc25-c by cdc2—cyclin B and its involvement in the self-amplification of MPF at mitosis. *EMBO J* 12: 53-63.

HOMER, H.A., MCDOUGALL, A., LEVASSEUR, M. and HERBERT, M. (2005a). Restaging the spindle assembly checkpoint in female mammalian meiosis i. *Cell Cycle* 4.

HOMER, H.A., MCDOUGALL, A., LEVASSEUR, M., MURDOCH, A.P. and HERBERT, M. (2005b). Mad2 is required for inhibiting securin and cyclin B degradation following spindle depolymerisation in meiosis I mouse oocytes. *Reproduction* 130: 829-43.

HOMER, H.A., MCDOUGALL, A., LEVASSEUR, M., YALLOP, K., MURDOCH, A.P. and HERBERT, M. (2005c). Mad2 prevents aneuploidy and premature proteolysis of cyclin B and securin during meiosis I in mouse oocytes. *Genes Dev* 19: 202-7.

HORNE, M.M. and GUADAGNO, T.M. (2003). A requirement for MAP kinase in the assembly and maintenance of the mitotic spindle. *J Cell Biol* 161: 1021-8.

HOWELL, B.J., HOFFMAN, D.B., FANG, G., MURRAY, A.W. and SALMON, E.D. (2000). Visualization of Mad2 dynamics at kinetochores, along spindle fibers, and at spindle poles in living cells. *J Cell Biol* 150: 1233-50.

HOWELL, B.J., MOREE, B., FARRAR, E.M., STEWART, S., FANG, G. and SALMON, E.D. (2004). Spindle checkpoint protein dynamics at kinetochores in living cells. *Curr Biol* 14: 953-64.

HOWLETT, S.K. and BOLTON, V.N. (1985). Sequence and regulation of morphological and molecular events during the first cell cycle of mouse embryogenesis. *J Embryol Exp Morphol* 87: 175-206.

HYSLOP, L. A., NIXON, V. L., LEVASSEUR, M., CHAPMANN, F., CHIBA, K., MCDOUGALL, A., VENABLES, J. P., ELLIOTT, D. J. and JONES, K. T. (2004). Ca(2+)-promoted cyclin B1 degradation in mouse oocytes requires the establishment of a metaphase arrest. *Dev Biol* 269: 206-19.

INOUE, D., OHE, M., KANEMORI, Y., NOBUI, T. and SAGATA, N. (2007). A direct link of the Mos-MAPK pathway to Erp1/Emi2 in meiotic arrest of Xenopus laevis eggs. *Nature* 446: 1100-4

ITO, J., SHIMADA, M. and TERADA, T. (2003). Effect of protein kinase c activator on mitogen-activated protein kinase and p34(cdc2) kinase activity during parthenogenetic activation of porcine oocytes by calcium ionophore. *Biol Reprod* 69: 1675-82.

JACKMAN, M., LINDON, C., NIGG, E.A. and PINES, J. (2003). Active cyclin B1-cdk1 first appears on centrosomes in prophase. *Nat Cell Biol* 5: 143-8.

JONES, J.T., MYERS, J.W., FERRELL, J.E. and MEYER, T. (2004). Probing the precision of the mitotic clock with a live-cell fluorescent biosensor. *Nat Biotechnol* 22: 306-12.

KALAB, P., KUBIAK, J.Z., VERLHAC, M.H., COLLEDGE, W.H. and MARO, B. (1996). Activation of p90rsk during meiotic maturation and first mitosis in mouse oocytes and eggs: MAP kinase-independent and -dependent activation. *Development* 122: 1957-64.

KALLIO, M., ERIKSSON, J.E. and GORBSKY, G.J. (2000). Differences in spindle association of the mitotic checkpoint protein Mad2 in mammalian spermatogenesis and oogenesis. *Dev Biol* 225: 112-23.

KONO, T., CARROLL, J., SWANN, K. and WHITTINGHAM, D.G. (1995). Nuclei from fertilized mouse embryos have calcium-releasing activity. *Development* 121: 1123-8.

KOSAKO, H., GOTOH, Y. and NISHIDA, E. (1994). Regulation and function of the MAP kinase cascade in Xenopus oocytes. *J Cell Sci Suppl* 18: 115-9.

KRUKOWSKA, A. and TARKOWSKI, A.K. (2005). Mouse zygotes with one diploid pronucleus formed as a result of ICSI can develop normally beyond birth. *Mol Reprod Dev.* 72:346-53.

KUBIAK, J.Z. (1989). Mouse oocytes gradually develop the capacity for activation during the metaphase II arrest. *Dev Biol* 136: 537-45.

KUBIAK, J.Z. and CIEMERYCH, M.A. (2001). Cell cycle regulation in early mouse embryos. *Novartis Found Symp* 237: 79-89; discussion 89-99.

KUBIAK, J.Z., PRATHER, R.S., MAUL, G.G. and SCHATTEN, G. (1991). Cytoplasmic modification of the nuclear lamina during pronuclear-like transformation of mouse blastomere nuclei. *Mech Dev* 35: 103-11.

KUBIAK, J.Z., WEBER, M., DE PENNART, H., WINSTON, N.J. and MARO, B. (1993). The metaphase II arrest in mouse oocytes is controlled through microtubule-dependent destruction of cyclin B in the presence of CSF. *EMBO J* 12: 3773-8.

LEDAN, E., POLANSKI, Z., TERRET, M.E. and MARO, B. (2001). Meiotic maturation of the mouse oocyte requires an equilibrium between cyclin B synthesis and degradation. *Dev Biol* 232: 400-13.

LEE, H., LEE, D.J., OH, S.P., PARK, H.D., NAM, H.H., KIM, J.M. and LIM, D.S. (2006a). Mouse Emi1 has an essential function in mitotic progression during early embryogenesis. *Mol Cell Biol* 26: 5373-81.

LEE, J., IWAI, T., YOKOTA, T. and YAMASHITA, M. (2003). Temporally and spatially selective loss of rec8 protein from meiotic chromosomes during mammalian meiosis. *J Cell Sci* 116: 2781-90.

LEE, J., OKADA, K., OGUSHI, S., MIYANO, T., MIYAKE, M. and YAMASHITA, M. (2006b). Loss of rec8 from chromosome arm and centromere region is required for homologous chromosome separation and sister chromatid separation, respectively, in mammalian meiosis. *Cell Cycle* 5: 1448-55.

LEFEBVRE, C., TERRET, M.E., DJIANE, A., RASSINIER, P., MARO, B. and VERLHAC, M.H. (2002). Meiotic spindle stability depends on MAPK-interacting and spindle-stabilizing protein (Miss), a new MAPK substrate. *J Cell Biol* 157: 603-13.

LEMAIRE-ADKINS, R., RADKE, K. and HUNT, P.A. (1997). Lack of checkpoint control at the metaphase/anaphase transition: A mechanism of meiotic nondisjunction in mammalian females. *J Cell Biol* 139: 1611-9.

LEW, D.J. and BURKE, D.J. (2003). The spindle assembly and spindle position checkpoints. *Annu Rev Genet* 37: 251-82.

LI, G.P., LIU, Y., BUNCH, T.D., WHITE, K.L. and ASTON, K.I. (2005). Asymmetric division of spindle microtubules and microfilaments during bovine meiosis from metaphase I to metaphase III. *Mol Reprod Dev* 71: 220-6.

LI, X. and NICKLAS, R.B. (1995). Mitotic forces control a cell-cycle checkpoint. *Nature* 373: 630-2.

LI, Y. and BENEZRA, R. (1996). Identification of a human mitotic checkpoint gene: Hsmad2. *Science* 274: 246-8.

LI, Y., GORBEA, C., MAHAFFEY, D., RECHSTEINER, M. and BENEZRA, R. (1997). Mad2 associates with the cyclosome/anaphase-promoting complex and inhibits its activity. *Proc Natl Acad Sci USA* 94: 12431-6.

LIU, L., JU, J.C. and YANG, X. (1998). Parthenogenetic development and protein patterns of newly matured bovine oocytes after chemical activation. *Mol Reprod Dev* 49: 298-307.

LIU, L., TRIMARCHI, J.R., SMITH, P.J. and KEEFE, D.L. (2002). Checkpoint for DNA integrity at the first mitosis after oocyte activation. *Mol Reprod Dev* 62: 277-88.

LIU, J., GRIMISON, B., LEWELLYN, A.L., MALLER, J.L. (2006). The anaphase-promoting complex/cyclosome inhibitor Emi2 is essential for meiotic but not mitotic cell cycles. *J Biol Chem* 281:34736-41.

LIU, J., GRIMISON, B., MALLER, J.L. (2007). New insight into metaphase arrest by

cytostatic factor: from establishment to release. *Oncogene* 26:1286-9.

LIU, J., MALLER, J.L. (2005). Calcium elevation at fertilization coordinates phosphorylation of XErp1/Emi2 by Plx1 and CaMK II to release metaphase arrest by cytostatic factor. *Curr Biol* 15:1458-68.

MADGWICK, S., HANSEN, D.V., LEVASSEUR, M., JACKSON, P.K. and JONES, K.T. (2006). Mouse Emi2 is required to enter meiosis ii by reestablishing cyclin B1 during interkinesis. *J Cell Biol* 174: 791-801.

MALMANCHE, N., MAIA, A. and SUNKEL, C.E. (2006). The spindle assembly checkpoint: Preventing chromosome mis-segregation during mitosis and meiosis. *FEBS Lett* 580: 2888-95.

MARANGOS, P. and CARROLL, J. (2004). Fertilization and Insp3-induced Ca2+ release stimulate a persistent increase in the rate of degradation of cyclin B1 specifically in mature mouse oocytes. *Dev Biol* 272: 26-38.

MARANGOS, P., VERSCHUREN, E.W., CHEN, R., JACKSON, P.K. and CARROLL, J. (2007). Prophase i arrest and progression to metaphase i in mouse oocytes are controlled by Emi1-dependent regulation of apccdh1. *J Cell Biol* 176: 65-75.

MARGOTTIN-GOGUET, F., HSU, J.Y., LOKTEV, A., HSIEH, H.M., REIMANN, J.D. and JACKSON, P.K. (2003). Prophase destruction of Emi1 by the scf(betatrcp/slimb) ubiquitin ligase activates the anaphase promoting complex to allow progression beyond prometaphase. *Dev Cell* 4: 813-26.

MARKOULAKI, S., MATSON, S., ABBOTT, A.L. and DUCIBELLA, T. (2003). Oscillatory CaMKII activity in mouse egg activation. *Dev Biol* 258: 464-74.

MARKOULAKI, S., MATSON, S. and DUCIBELLA, T. (2004). Fertilization stimulates long-lasting oscillations of CaMKII activity in mouse eggs. *Dev Biol* 272: 15-25.

MARTIN-MCCAFFREY, L., WILLARD, F.S., OLIVEIRA-DOS-SANTOS, A.J., NATALE, D.R., SNOW, B.E., KIMPLE, R.J., PAJAK, A., WATSON, A.J., DAGNINO, L., PENNINGER, J.M. *et al.* (2004). Rgs14 is a mitotic spindle protein essential from the first division of the mammalian zygote. *Dev Cell* 7: 763-9.

MASUI, Y. (2001). From oocyte maturation to the in vitro cell cycle: The history of discoveries of maturation-promoting factor (MPF) and cytostatic factor (CSF). *Differentiation* 69: 1-17.

MASUI, Y. and MARKERT, C.L. (1971). Cytoplasmic control of nuclear behavior during meiotic maturation of frog oocytes. *J Exp Zool* 177: 129-45.

MAYER, W., SMITH, A., FUNDELE, R. and HAAF, T. (2000). Spatial separation of parental genomes in preimplantation mouse embryos. *J Cell Biol* 148: 629-34.

MERALDI, P., DRAVIAM, V.M. and SORGER, P.K. (2004). Timing and checkpoints in the regulation of mitotic progression. *Dev Cell* 7: 45-60.

MERALDI, P. and SORGER, P.K. (2005). A dual role for bub1 in the spindle checkpoint and chromosome congression. *EMBO J* 24: 1621-33.

MURRAY, A.W., SOLOMON, M.J. and KIRSCHNER, M.W. (1989). The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature* 339: 280-6.

NASMYTH, K. (2005). How do so few control so many? *Cell* 120: 739-46.

NISHIYAMA, A., TACHIBANA, K., IGARASHI, Y., YASUDA, H., TANAHASHI, N., TANAKA, K., OHSUMI, K. and KISHIMOTO, T. (2000). A nonproteolytic function of the proteasome is required for the dissociation of cdc2 and cyclin B at the end of m phase. *Genes Dev* 14: 2344-57.

NISHIYAMA, T., OHSUMI, K. and KISHIMOTO, T. (2007). Phosphorylation of Erp1 by p90rsk is required for cytostatic factor arrest in Xenopus laevis eggs. *Nature* 446: 1096-9

NOTHIAS, J.Y., MAJUMDER, S., KANEKO, K.J. and DEPAMPHILIS, M.L. (1995). Regulation of gene expression at the beginning of mammalian development. *J Biol Chem* 270: 22077-80.

NURSE, P. (1990). Universal control mechanism regulating onset of M-phase. *Nature* 344: 503-8.

OHE, M., INOUE, D., KANEMORI, Y. and SAGATA, N. (2007). Erp1/Emi2 is essential for the meiosis I to meiosis II transition in Xenopus oocytes. *Dev Biol* 303: 157-64.

ORTEGA, S., PRIETO, I., ODAJIMA, J., MARTIN, A., DUBUS, P., SOTILLO, R., BARBERO, J.L., MALUMBRES, M. and BARBACID, M. (2003). Cyclin-dependent kinase 2 is essential for meiosis but not for mitotic cell division in mice. *Nat Genet* 35: 25-31.

PARISI, T., BECK, A.R., ROUGIER, N., MCNEIL, T., LUCIAN, L., WERB, Z. and AMATI, B. (2003). Cyclins E1 and E2 are required for endoreplication in placental trophoblast giant cells. *EMBO J* 22: 4794-803.

PARONETTO, M.P., GIORDA, E., CARSETTI, R., ROSSI, P., GEREMIA, R. and SETTE, C. (2004). Functional interaction between p90(rsk2) and Emi1 contributes to the metaphase arrest of mouse oocytes. *EMBO J* 23: 4649-4659.

PASSMORE, L.A. (2004). The anaphase-promoting complex (APC): The sum of its parts? *Biochem Soc Trans* 32: 724-7.

PETER, M., NAKAGAWA, J., DOREE, M., LABBE, J.C. and NIGG, E.A. (1990). In vitro disassembly of the nuclear lamina and m phase-specific phosphorylation of lamins by cdc2 kinase. *Cell* 61: 591-602.

PETER, M., SANGHERA, J.S., PELECH, S.L. and NIGG, E.A. (1992). Mitogen-activated protein kinases phosphorylate nuclear lamins and display sequence specificity overlapping that of mitotic protein kinase p34cdc2. *Eur J Biochem* 205: 287-94.

PETERS, J.M. (2002). The anaphase-promoting complex: Proteolysis in mitosis and beyond. *Mol Cell* 9: 931-43.

PHILLIPS, K.P., PETRUNEWICH, M.A., COLLINS, J.L., BOOTH, R.A., LIU, X.J. and BALTZ, J.M. (2002). Inhibition of MEK or cdc2 kinase parthenogenetically activates mouse eggs and yields the same phenotypes as mos(-/-) parthenogenotes. *Dev Biol* 247: 210-23.

PINES, J. (2006). Mitosis: A matter of getting rid of the right protein at the right time. *Trends Cell Biol* 16: 55-63.

PINES, J. and HUNTER, T. (1991). Human cyclins A and B1 are differentially located in the cell and undergo cell cycle-dependent nuclear transport. *J Cell Biol* 115: 1-17.

POLANSKI, Z. (1995) Activation of in vitro matured mouse oocytes arrested at first or second meiotic metaphase. *Int J Dev Biol* 39: 1015-1020.

POLANSKI, Z. (1997a). Genetic background of the differences in timing of meiotic maturation in mouse oocytes: A study using recombinant inbred strains. *J Reprod Fertil* 109: 109-14.

POLANSKI, Z. (1997b). Strain difference in the timing of meiosis resumption in mouse oocytes: Involvement of a cytoplasmic factor(s) acting presumably upstream of the dephosphorylation of p34cdc2 kinase. *Zygote* 5: 105-9.

POLANSKI, Z., HOFFMANN, S. and TSURUMI, C. (2005). Oocyte nucleus controls progression through meiotic maturation. *Dev Biol* 281: 184-95.

POLANSKI, Z., LEDAN, E., BRUNET, S., LOUVET, S., VERLHAC, M.H., KUBIAK, J.Z. and MARO, B. (1998). Cyclin synthesis controls the progression of meiotic maturation in mouse oocytes. *Development* 125: 4989-97.

PRATHER, R.S., KUBIAK, J., MAUL, G.G., FIRST, N.L. and SCHATTEN, G. (1991). The expression of nuclear lamin A and C epitopes is regulated by the developmental stage of the cytoplasm in mouse oocytes or embryos. *J Exp Zool* 257: 110-4.

PRATT, H. P., BOLTON, V. N. and GUDGEON, K. A. (1983). The legacy from the oocyte and its role in controlling early development of the mouse embryo. *Ciba Found Symp* 98: 197-227.

PRIETO, I., TEASE, C., PEZZI, N., BUESA, J.M., ORTEGA, S., KREMER, L., MARTINEZ, A., MARTINEZ, A.C., HULTEN, M.A. and BARBERO, J.L. (2004). Cohesin component dynamics during meiotic prophase I in mammalian oocytes. *Chromosome Res* 12: 197-213.

RAM, P.T. and SCHULTZ, R.M. (1993). Reporter gene expression in G2 of the 1-cell mouse embryo. *Dev Biol* 156: 552-6.

RAUH, N.R., SCHMIDT, A., BORMANN, J., NIGG, E.A. and MAYER, T.U. (2005). Calcium triggers exit from meiosis II by targeting the APC/C inhibitor xerp1 for degradation. *Nature* 437: 1048-52.

REDDY, S.K., RAPE, M., MARGANSKY, W.A. and KIRSCHNER, M.W. (2007). Ubiquitination by the anaphase-promoting complex drives spindle checkpoint inactivation. *Nature* 446: 921-5.

REIMANN, J.D., FREED, E., HSU, J.Y., KRAMER, E.R., PETERS, J.M. and JACKSON, P.K. (2001a). Emi1 is a mitotic regulator that interacts with cdc20 and inhibits the anaphase promoting complex. *Cell* 105: 645-55.

REIMANN, J.D., GARDNER, B.E., MARGOTTIN-GOGUET, F. and JACKSON, P.K. (2001b). Emi1 regulates the anaphase-promoting complex by a different mechanism than Mad2 proteins. *Genes Dev* 15: 3278-85.

REIS, A., CHANG, H. Y., LEVASSEUR, M. and JONES, K. T. (2006). APCcdh1 activity in mouse oocytes prevents entry into the first meiotic division. *Nature*

Cell Biol 8: 539-40.

RIEDER, C.L. and MAIATO, H. (2004). Stuck in division or passing through: What happens when cells cannot satisfy the spindle assembly checkpoint. *Dev Cell* 7: 637-51.

RIEDER, C.L., SCHULTZ, A., COLE, R. and SLUDER, G. (1994). Anaphase onset in vertebrate somatic cells is controlled by a checkpoint that monitors sister kinetochore attachment to the spindle. *J Cell Biol* 127: 1301-10.

SAGATA, N., OSKARSSON, M., COPELAND, T., BRUMBAUGH, J. and VANDE WOUDE, G. F. (1988). Function of c-mos proto-oncogene product in meiotic maturation in Xenopus oocytes. *Nature* 335: 519-25.

SANGHERA, J.S., PETER, M., NIGG, E.A. and PELECH, S.L. (1992). Immunological characterization of avian map kinases: Evidence for nuclear localization. *Mol Biol Cell* 3: 775-87.

SAUNDERS, C.M., LARMAN, M.G., PARRINGTON, J., COX, L.J., ROYSE, J., BLAYNEY, L.M., SWANN, K. and LAI, F.A. (2002). Plc zeta: A sperm-specific trigger of Ca(2+) oscillations in eggs and embryo development. *Development* 129: 3533-44.

SCHMIDT, A., DUNCAN, P.I., RAUH, N.R., SAUER, G., FRY, A.M., NIGG, E.A. and MAYER, T.U. (2005). Xenopus polo-like kinase Plx1 regulates xerp1, a novel inhibitor of APC/C activity. *Genes Dev* 19: 502-13.

SCHMIDT, A., RAUH, N.R., NIGG, E.A. and MAYER, T.U. (2006). Cytostatic factor: An activity that puts the cell cycle on hold. *J Cell Sci* 119: 1213-8.

SCHULTZ, R.M. (1993). Regulation of zygotic gene activation in the mouse. *Bioessays* 15: 531-8.

SCHWAB, M.S., ROBERTS, B.T., GROSS, S.D., TUNQUIST, B.J., TAIEB, F.E., LEWELLYN, A.L. and MALLER, J.L. (2001). Bub1 is activated by the protein kinase p90(rsk) during Xenopus oocyte maturation. *Curr Biol* 11: 141-50.

SHAH, J.V. and CLEVELAND, D.W. (2000). Waiting for anaphase: Mad2 and the spindle assembly checkpoint. *Cell* 103: 997-1000.

SHAPIRO, P.S., VAISBERG, E., HUNT, A.J., TOLWINSKI, N.S., WHALEN, A.M., MCINTOSH, J.R. and AHN, N.G. (1998). Activation of the MKK/ERK pathway during somatic cell mitosis: Direct interactions of active ERK with kinetochores and regulation of the mitotic 3F3/2 phosphoantigen. *J Cell Biol* 142: 1533-45.

SHOJI, S., YOSHIDA, N., AMANAI, M., OHGISHI, M., FUKUI, T., FUJIMOTO, S., NAKANO, Y., KAJIKAWA, E. and PERRY, A.C. (2006). Mammalian Emi2 mediates cytostatic arrest and transduces the signal for meiotic exit via cdc20. *EMBO J* 25: 834-45.

SIKORA-POLACZEK, M., HUPALOWSKA, A., POLANSKI, Z., KUBIAK, J.Z. and CIEMERYCH, M.A. (2006). The first mitosis of the mouse embryo is prolonged by transitional metaphase arrest. *Biol Reprod* 74: 734-43.

SIOMOS, M.F., BADRINATH, A., PASIERBEK, P., LIVINGSTONE, D., WHITE, J., GLOTZER, M. and NASMYTH, K. (2001). Separase is required for chromosome segregation during meiosis I in Caenorhabditis elegans. *Curr Biol* 11: 1825-35.

STEGMEIER, F., RAPE, M., DRAVIAM, V.M., NALEPA, G., SOWA, M.E., ANG, X.L., MCDONALD, E.R., 3RD, LI, M.Z., HANNON, G.J., SORGER, P.K. *et al.* (2007). Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. *Nature* 446: 876-81.

SWANN, K., LARMAN, M.G., SAUNDERS, C.M. and LAI, F.A. (2004). The cytosolic sperm factor that triggers Ca2+ oscillations and egg activation in mammals is a novel phospholipase c: Plc zeta. *Reproduction* 127: 431-9.

SZOLLOSI, M.S., KUBIAK, J.Z., DEBEY, P., DE PENNART, H., SZOLLOSI, D. and MARO, B. (1993). Inhibition of protein kinases by 6-dimethylaminopurine accelerates the transition to interphase in activated mouse oocytes. *J Cell Sci* 104 (Pt 3): 861-72.

TATENO, H. and KAMIGUCHI, Y. (1997). Parthenogenetic activation of chinese hamster oocytes by chemical stimuli and its cytogenetic evaluation. *Mol Reprod Dev* 47: 72-8.

TERRET, M.E., LEFEBVRE, C., DJIANE, A., RASSINIER, P., MOREAU, J., MARO, B. and VERLHAC, M.H. (2003a). Doc1r: A map kinase substrate that control microtubule organization of metaphase ii mouse oocytes. *Development* 130: 5169-77.

TERRET, M.E., WASSMANN, K., WAIZENEGGER, I., MARO, B., PETERS, J.M. and VERLHAC, M.H. (2003b). The meiosis I-to-meiosis II transition in mouse oocytes requires separase activity. *Curr Biol* 13: 1797-802.

THIBIER, C., DE SMEDT, V., POULHE, R., HUCHON, D., JESSUS, C. and OZON, R. (1997). In vivo regulation of cytostatic activity in Xenopus metaphase II-arrested oocytes. *Dev Biol* 185: 55-66.

THOMPSON, E.M., LEGOUY, E. and RENARD, J.P. (1998). Mouse embryos do not wait for the MBT: Chromatin and RNA polymerase remodeling in genome activation at the onset of development. *Dev Genet* 22: 31-42.

TSURUMI, C., HOFFMANN, S., GELEY, S., GRAESER, R. and POLANSKI, Z. (2004). The spindle assembly checkpoint is not essential for CSF arrest of mouse oocytes. *J Cell Biol* 167: 1037-50.

TUNG, J.J., HANSEN, D.V., BAN, K.H., LOKTEV, A.V., SUMMERS, M.K., ADLER, J.R., 3RD and JACKSON, P.K. (2005). A role for the anaphase-promoting complex inhibitor Emi2/xerp1, a homolog of early mitotic inhibitor 1, in cytostatic factor arrest of Xenopus eggs. *Proc Natl Acad Sci USA* 102: 4318-23.

TUNQUIST, B.J. and MALLER, J.L. (2003). Under arrest: Cytostatic factor (CSF)-mediated metaphase arrest in vertebrate eggs. *Genes Dev* 17: 683-710.

UHLMANN, F. (2003). Chromosome cohesion and separation: From men and molecules. *Curr Biol* 13: R104-14.

UHLMANN, F. (2004). The mechanism of sister chromatid cohesion. *Exp Cell Res* 296: 80-5.

VERLHAC, M.H., KUBIAK, J.Z., CLARKE, H.J. and MARO, B. (1994). Microtubule and chromatin behavior follow MAP kinase activity but not MPF activity during meiosis in mouse oocytes. *Development* 120: 1017-25.

VERLHAC, M.H., KUBIAK, J.Z., WEBER, M., GERAUD, G., COLLEDGE, W.H., EVANS, M.J. and MARO, B. (1996). Mos is required for MAP kinase activation and is involved in microtubule organization during meiotic maturation in the mouse. *Development* 122: 815-22.

VINCENT, C., CHEEK, T.R. and JOHNSON, M.H. (1992). Cell cycle progression of parthenogenetically activated mouse oocytes to interphase is dependent on the level of internal calcium. *J Cell Sci* 103 (Pt 2): 389-96.

VITULLO, A.D. and OZIL, J.P. (1992). Repetitive calcium stimuli drive meiotic resumption and pronuclear development during mouse oocyte activation. *Dev Biol* 151: 128-36.

WAKSMUNDZKA, M., KRYSIAK, E., KARASIEWICZ, J., CZOLOWSKA, R. and TARKOWSKI, A.K. (1984). Autonomous cortical activity in mouse eggs controlled by a cytoplasmic clock. *J Embryol Exp Morphol* 79: 77-96.

WANG, J.Y., LEI, Z.L., NAN, C.L., YIN, S., LIU, J., HOU, Y., LI, Y.L., CHEN, D.Y. and SUN, Q.Y. (2007). RNA interference as a tool to study the function of Mad2 in mouse oocyte meiotic maturation. *Mol Reprod Dev* 74: 116-24.

WASSMANN, K., NIAULT, T. and MARO, B. (2003). Metaphase I arrest upon activation of the Mad2-dependent spindle checkpoint in mouse oocytes. *Curr Biol* 13: 1596-608.

WATANABE, N., VANDE WOUDE, G.F., IKAWA, Y. and SAGATA, N. (1989). Specific proteolysis of the c-mos proto-oncogene product by calpain on fertilization of Xenopus eggs. *Nature* 342: 505-11.

WATANABE, Y. and KITAJIMA, T.S. (2005). Shugoshin protects cohesin complexes at centromeres. *Philos Trans R Soc Lond B Biol Sci* 360: 515-21, discussion 521.

WATERS, J.C., CHEN, R.H., MURRAY, A.W., GORBSKY, G.J., SALMON, E.D. and NICKLAS, R.B. (1999). Mad2 binding by phosphorylated kinetochores links error detection and checkpoint action in mitosis. *Curr Biol* 9: 649-52.

WATERS, J.C., CHEN, R.H., MURRAY, A.W. and SALMON, E.D. (1998). Localization of mad2 to kinetochores depends on microtubule attachment, not tension. *J Cell Biol* 141: 1181-91.

WEBER, M., KUBIAK, J.Z., ARLINGHAUS, R.B., PINES, J. and MARO, B. (1991). C-mos proto-oncogene product is partly degraded after release from meiotic arrest and persists during interphase in mouse zygotes. *Dev Biol* 148: 393-7.

WINSTON, N., BOURGAIN-GUGLIELMETTI, F., CIEMERYCH, M.A., KUBIAK, J.Z., SENAMAUD-BEAUFORT, C., CARRINGTON, M., BRECHOT, C. and SOBCZAK-THEPOT, J. (2000). Early development of mouse embryos null mutant for the cyclin A2 gene occurs in the absence of maternally derived cyclin A2 gene products. *Dev Biol* 223: 139-53.

WINSTON, N.J. (1997). Stability of cyclin B protein during meiotic maturation and the first mitotic cell division in mouse oocytes. *Biol Cell* 89: 211-9.

WINSTON, N.J., MCGUINNESS, O., JOHNSON, M.H. and MARO, B. (1995). The exit of mouse oocytes from meiotic M-phase requires an intact spindle during intracellular calcium release. *J Cell Sci* 108 (Pt 1): 143-51.

WRIGHT, S.J. and LONGO, F.J. (1988). Sperm nuclear enlargement in fertilized hamster eggs is related to meiotic maturation of the maternal chromatin. *J Exp Zool* 247: 155-65.

WU, Q., GUO Y., YAMADA, A., PERRY J.A., WANG M.Z., ARAKI M., FREEL C.D., TUNG J.J., TANG W., MARGOLIS, S.S,, JACKSON, P.K,, YAMANO, H., ASANO, M. and KORNBLUTH S. (2007). A Role for Cdc2- and PP2A-Mediated Regulation of Emi2 in the Maintenance of CSF Arrest. *Curr Biol* 17: 213-24.

YANAGIMACHI, R. (1994). Mammalian fertilization. In *The physiology of reproduction*, vol. 1 (ed. KNOBIL, E., NEILL J.D.). Raven Press, New York, pp.189-317.

YIN, S., WANG, Q., LIU, J.H., AI, J.S., LIANG, C.G., HOU, Y., CHEN, D.Y., SCHATTEN, H. and SUN, Q.Y. (2006). Bub1 prevents chromosome misalignment and precocious anaphase during mouse oocyte meiosis. *Cell Cycle* 5: 2130-7.

YU, H. (2002). Regulation of apc-cdc20 by the spindle checkpoint. *Curr Opin Cell Biol* 14: 706-14.

ZECEVIC, M., CATLING, A.D., EBLEN, S.T., RENZI, L., HITTLE, J.C., YEN, T.J., GORBSKY, G.J. and WEBER, M.J. (1998). Active MAP kinase in mitosis: Localization at kinetochores and association with the motor protein CENP-E *J Cell Biol* 142: 1547-58.

ZERNICKA-GOETZ, M. (1991). Spontaneous and induced activation of rat oocytes. *Mol Reprod Dev* 28: 169-76.

ZERNICKA-GOETZ, M., CIEMERYCH, M.A., KUBIAK, J.Z., TARKOWSKI, A.K. and MARO, B. (1995). Cytostatic factor inactivation is induced by a calcium-dependent mechanism present until the second cell cycle in fertilized but not in parthenogenetically activated mouse eggs. *J Cell Sci* 108 (Pt 2): 469-74.

ZUCCOTTI, M., PICCINELLI, A., GIORGI ROSSI, P., GARAGNA, S. and REDI, C.A. (1995). Chromatin organization during mouse oocyte growth. *Mol Reprod Dev* 41: 479-85.