

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

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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, cytotostatic factor; GVBD, germinal vesicle breakdown; MPF, M-phase Promoting Factor; MI, metaphase I; MII, metaphase II; NEBD, nuclear envelopm breakdown; SAC, spindle assembly checkpoint.

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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 proteasome. Its neosynthesis 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

polyubiquitination 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 cyto-static 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

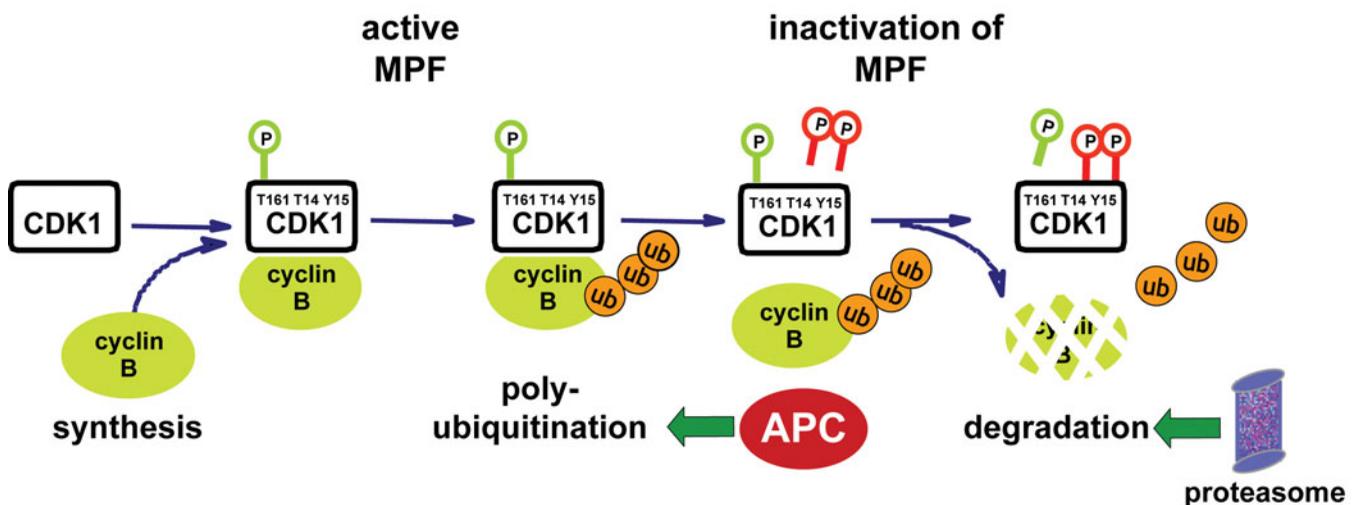
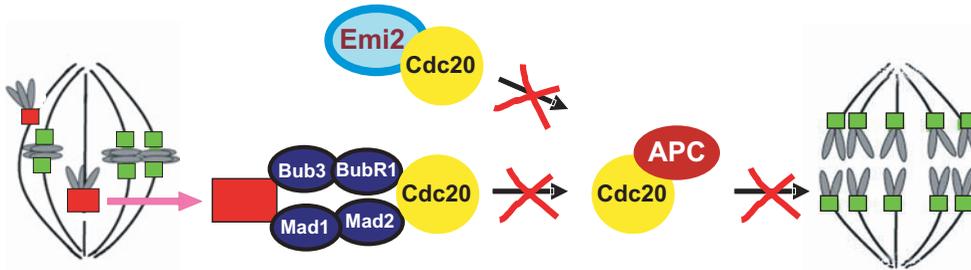
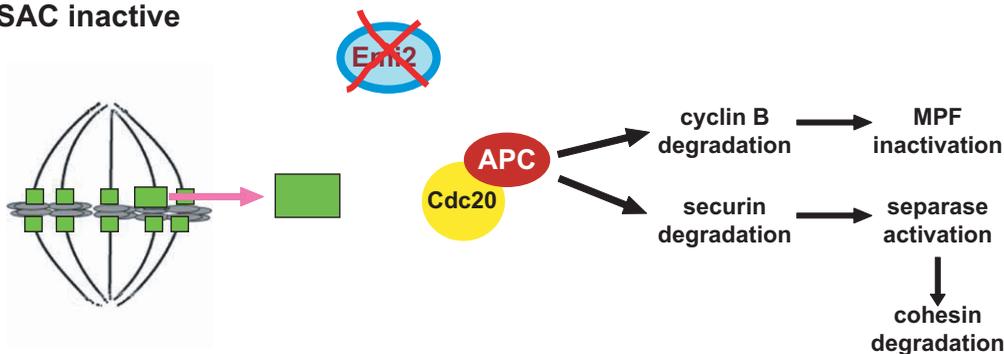


Fig. 1. Activation and inactivation of M-phase promoting factor (MPF) during the M phase. Newly synthesized cyclin B associates with CDK1. The complex is phosphorylated on Thr-161, becomes active and provokes M-phase entry. At the end of M-phase, cyclin B becomes polyubiquitinated and targeted to the 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. Polyubiquitination of cyclin B is mediated by E3 ligase within the high molecular weight complex APC/C.

SAC active**SAC inactive**

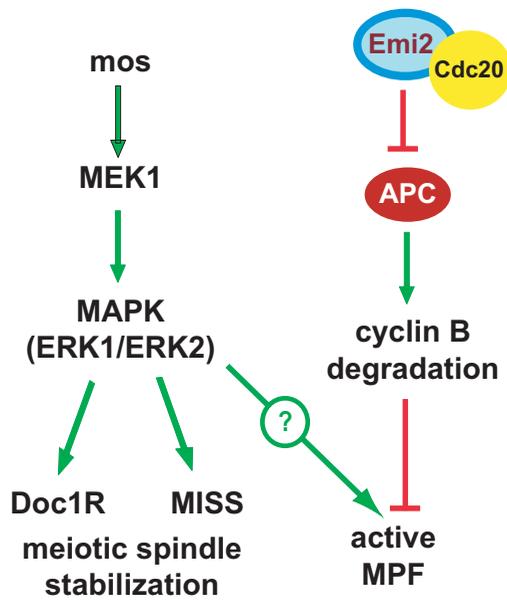
these proteins are also cohesins which act as a 'glue' holding together homologous chromosomes during the first meiotic division or two chromatids during second meiotic division or mitosis (Haering and Nasmyth, 2003; Uhlmann, 2004). Rec8 is a crucial cohesin involved in the binding of homologous chromosomes 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 (Siomou *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.

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.

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



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

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.

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

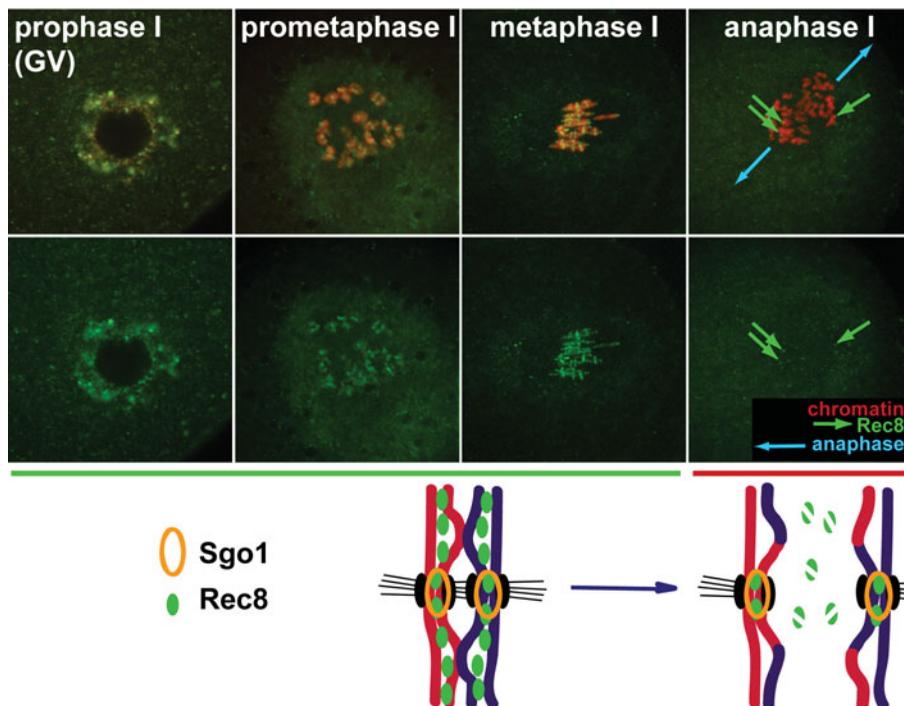
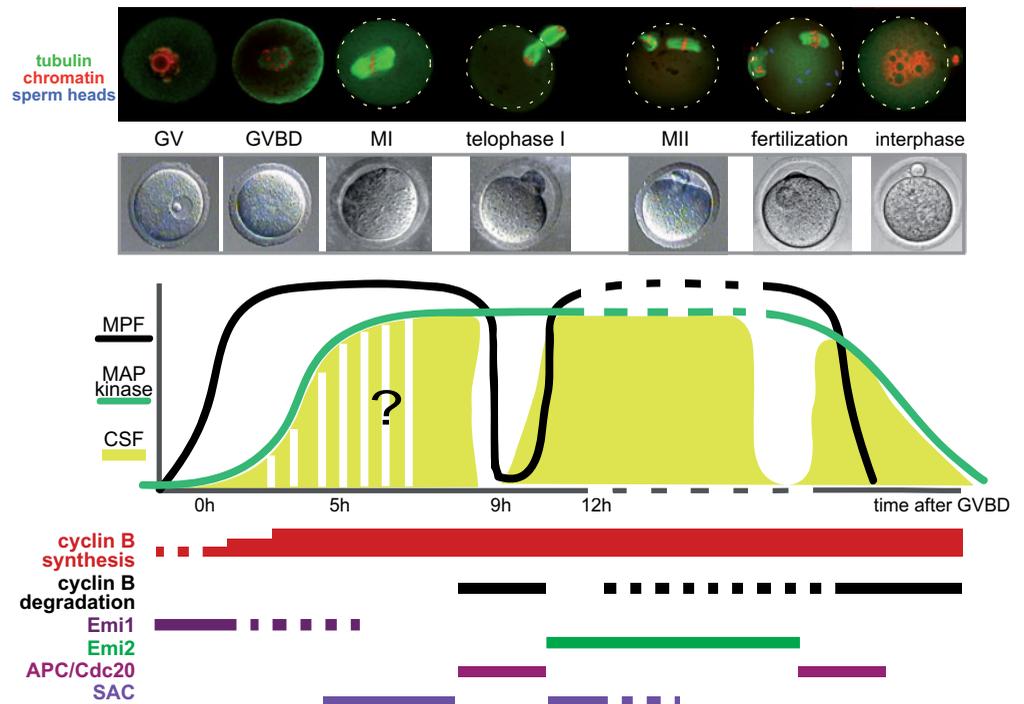


Fig. 4. Meiotic cohesin Rec8 in mouse oocytes. In the prophase I oocyte (GV stage), prometaphase I and metaphase of the first meiotic division Rec8 assures cohesion of homologous chromosomes. It colocalizes with shugoshin (Sgo1) in the centromere region. During anaphase of the first meiotic division, Rec8 localized so far along chromosome arms becomes degraded, allowing separation of homologous chromosomes. Sgo1 prevents degradation of Rec8 localized within the centromeres 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 MII-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

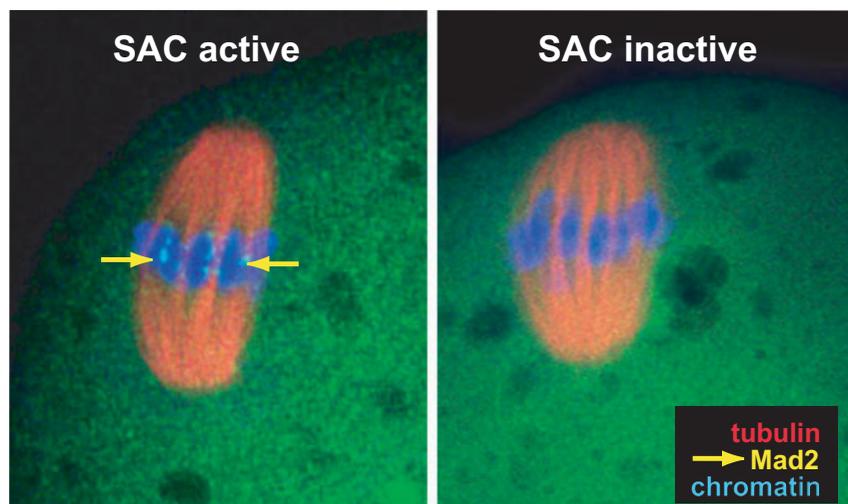


Fig. 6. Mad2 localization on kinetochores suggests that the spindle assembly checkpoint (SAC) is active in early metaphase II mouse oocytes. SAC seems active one hour after the completion of the first meiotic division, as Mad2 localizes clearly on kinetochores. It becomes inactivated within the next 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 PLC ζ that catalyzes hydrolysis of PIP2 to inositol triphosphate (IP3) and diacylglycerol (DAG) (Cox *et al.*, 2002; Saunders *et al.*, 2002; Halet, 2004; Swann *et al.*, 2004). Next, IP3 binds to its endocytosolic 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 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

1971 - Masui and Markert - *Rana pipiens*

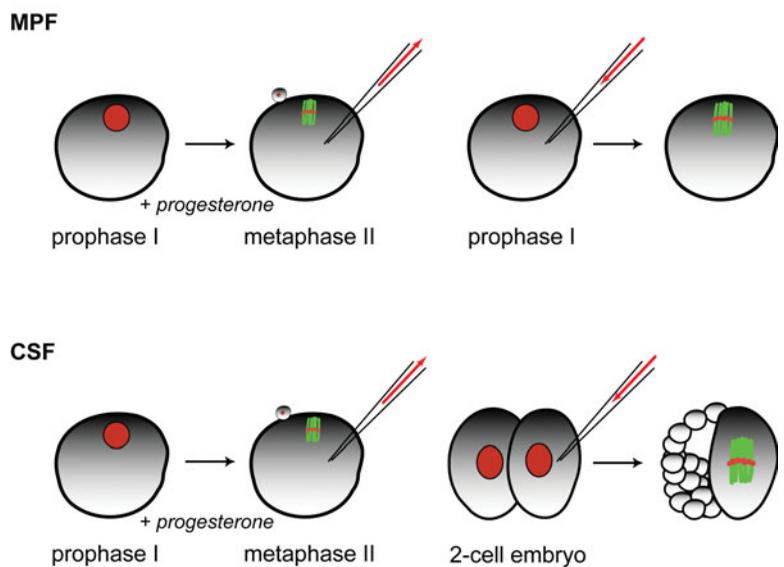
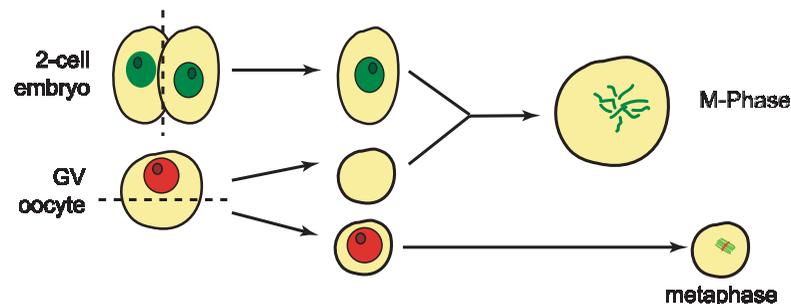


Fig. 7. Discovery of M-phase promoting factor (MPF) and cytostatic factor (CSF) in oocytes of *Rana pipiens*. (MPF) In 1971, Masui and Markert induced frog prophase I oocytes to mature with progesterone (left), removed a portion of its cytoplasm (red arrow out) and transferred it to the cytoplasm of 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). Ca²⁺/calmodulin dependent kinase II (CaMKII) is activated as a result of IP₃ to IP₃R binding and the burst of ER-derived Ca²⁺ (Markoulaki *et al.*, 2003; Markoulaki *et al.*, 2004).

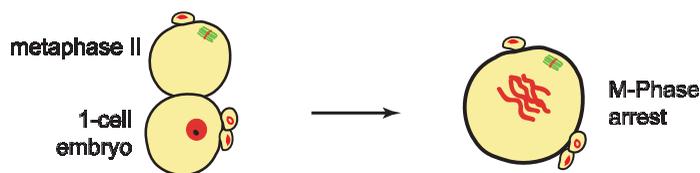
MPF

1977 - Balakier and Czolowska - maturing mouse oocytes



CSF

1994 - Kubiak et al. - metaphase II mouse oocytes



CSF

1998 - Ciemerych & Kubiak - metaphase I oocytes of LT/Sv strain of mouse

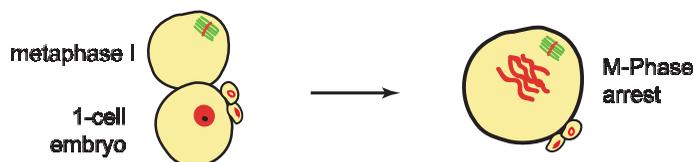


Fig. 8. Discovery of M-phase promoting factor (MPF) and cytotostatic factor (CSF) in mouse oocytes. (MPF) In 1977, Balakier and Czolowska bisected a GV stage oocyte of the mouse into nucleate and anucleate halves (Balakier and Czolowska, 1977). When nucleate halves resumed meiosis and reached metaphase II, 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 parthenogenetic one-cell embryo (Ciemerych and Kubiak, 1998).

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

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 ubiquitin molecules together or the polyubiquitinated complex CDK1/cyclin B to the proteasome since both cyclin B and proteasomes 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,

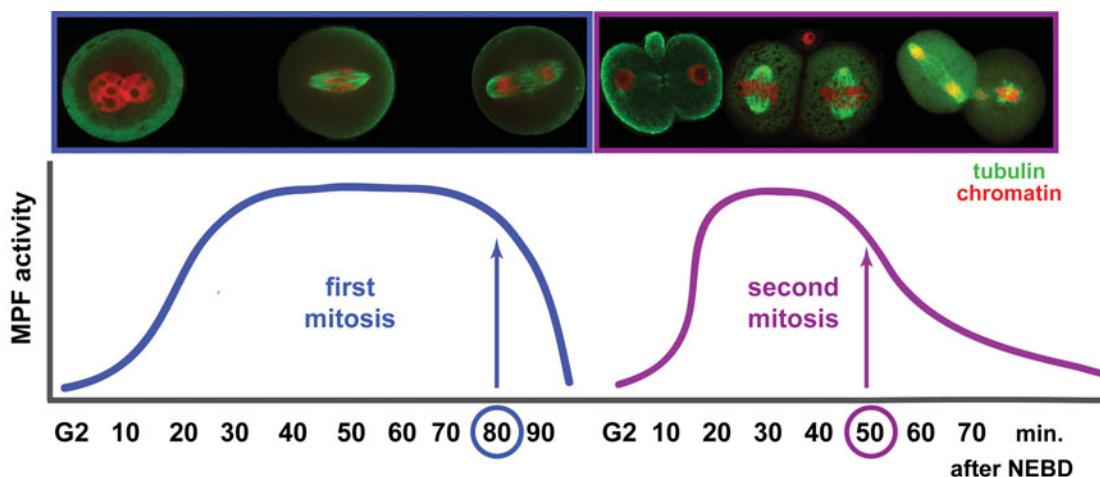


Fig. 9. M-phase promoting factor (MPF) dynamics during the first and second division of the mouse embryo. The first mitotic division takes on average 120 min while the second one takes around 70 min. Time points when MPF activity (measured by histone H1 kinase activity) start to drop are depicted by 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 of 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 cytoplasmically 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 inverted, e.g. during considerably prolonged first meiotic division of maturing oocytes, a prometaphase (characterized by the presence of Mad2 and Bub1 on 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 spermatozoa. 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 embry-

onic 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}

Spindle assembly checkpoint - active - inactive

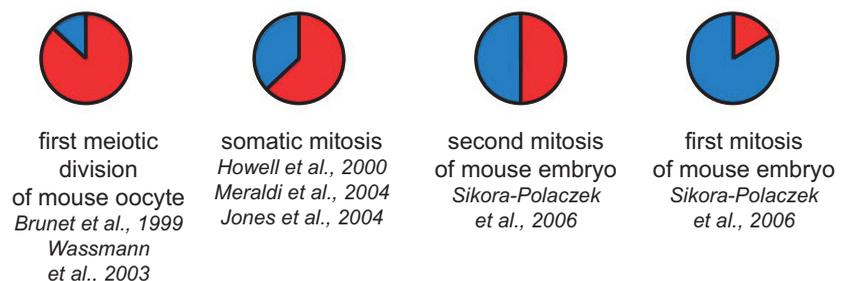


Fig. 10. Changes in the proportion of active and inactive spindle assembly checkpoint (SAC) during different M-phases in maturing oocytes, mitotically dividing mouse embryos and somatic cells. Proportion of active (red) versus inactive (blue) SAC significantly differs during various M-phases. SAC activity 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.

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