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

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...
and targeted to the proteasome. The lid of the proteasome induces dissociation of polyubiquitinated cyclin B from CDK1 and its deubiquitination. CDK1, once dissociated from cyclin, becomes inactivated by dephosphorylation of Thr-161 and phosphorylation of Thr 14/Tyr-15. 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 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

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**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.
in consequence Rec8 proteolysis (Herbert et al., 2003; Homer 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 catalyzed by separase is a prerequisite step for the proper chromosome segregation (Siomos et al., 2001; Terret et al., 2003b; Uhlmann, 2003). 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).

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 (M). 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

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

The first meiotic M-phase in oocytes is governed by a unique machinery based on the first meiotic division checkpoint, which is responsible for prolongation of metaphase I, and activation of the second meiotic division (Fig. 2). It is important to note that this mechanism is different from the mitotic cycle. In mitotic cells, the metaphase arrest is caused by the interaction of checkpoint proteins Mad1, Mad2, Bub3, BubR1, that bind and inactivate the APC/C activator Cdc20. This mechanism is responsible for prolongation of metaphase I and anaphase II, which is required for proper chromosome segregation (Siomos et al., 2001). In contrast, in meiotic cells, the metaphase arrest is caused by activation of the SAC, which prevents the activation of the APC/C. The SAC is activated by the binding of checkpoint proteins Mad1, Mad2, Bub3, BubR1, that bind and inactivate the APC/C activator Cdc20. This mechanism is responsible for prolongation of metaphase I 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. 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
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

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).

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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.

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 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.
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 with high level of cyclin B synthesis (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
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 (Ciemieszch 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., 1998). 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 MII (Kubiak, 1989). Such reaction clearly depends on the type of the activation stimulus. *In vitro* fertilization rarely results in MII, but parthenogenetic stimuli induce an abortive meiotic MII 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 cytoplasmatic 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 Ca²⁺ increase is caused by the sperm-brought PLCζ 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 endocyttoplasmic reticulum receptors (IP3R) and induces the release of Ca²⁺ 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 cytoplasmatic maturation.

Abortive activation of MII oocytes leading to MII 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 cytoplasmatic 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 (Ciemieszch and Kubiak, 1998), however, it was shown unequivocally only in the oocytes of LT/Sv

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**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.
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 MI-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 downstream 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 p90rsk1, 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 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*

**MPF**

+ progesterone

prophase I → metaphase II → prophase I

**CSF**

+ progesterone

prophase I → metaphase II → 2-cell embryo

![Fig. 7. Discovery of M-phase promoting factor (MPF) and cytostatic factor (CSF) in oocytes of *Rana pipiens*.](image)
Emi2 degradation is rather complicated. It requires sequential phosphorylation of Emi2 by CaMKKinase 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). 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 (Nishiya et al., 2000), separation of both cyclin B and CDK1 resulting in MPF inactivation and further degradation of cyclin B by the proteasome (Nishiya 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 (Nishiya 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 (Ciernyerek 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 CSF 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 paradoxic 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 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 MI- 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<sup>th</sup> 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,
still unclear which kinase phosphorylates Emi2 in MII mouse oocytes, since, as explained above, p90RK kinases were excluded as CSF components (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 mitotic division of the mouse embryo (Martin-McCaffrey et al., 1995) 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 originating 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 mitotic 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 separate 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 reversed, 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 2 hr 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 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 Quagano, 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).

**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\textsuperscript{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\textsuperscript{rsk1} dependent manner (Paronetto et al., 2004). However, this hypothesis was questioned by the discovery that p90\textsuperscript{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 parthenogenetically activated 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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