Mos is required for MAP kinase activation and is involved in microtubule organization during meiotic maturation in the mouse

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SUMMARY

Mos is normally expressed during oocyte meiotic maturation in vertebrates. However, apart from its cytostatic factor (CSF) activity, its precise role during mouse meiosis is still unknown. First, we analyzed its role as a MAP kinase kinase. Mos is synthesized concomitantly with the activation of MAP kinase in mouse oocytes. Moreover, MAP kinase is not activated during meiosis in oocytes from mos−/− mice. This result implies that Mos is necessary for MAP kinase activation in mouse oocytes. Raf-1, another MAP kinase kinase, is already present in immature oocytes, but does not seem to be active when MAP kinase is activated. Moreover, the absence of MAP kinase activation in mos−/− oocytes demonstrates that Raf-1 cannot compensate for the lack of Mos. These results suggest that Raf-1 is not involved in MAP kinase activation. Second, we analyzed the organization of the microtubules and chromosomes in oocytes from mos−/− mice. We observed that during the transition between two meiotic M-phases, the microtubules and chromosomes evolve towards an interphase-like state in mos−/− oocytes, while in the control mos+/− oocytes they remain in an M-phase configuration, as in the wild type. Moreover, after spontaneous activation, the majority of mos−/− oocytes are arrested for at least 10 hours in a third meiotic M-phase where they exhibit monopolar half-spindles. These observations present the first evidence, in intact oocytes, of a role for the Mos/.../MAP kinase cascade in the control of microtubule and chromatin organization during meiosis.

Key words: Mos, MAP kinase, microtubules, MTOCs, chromatin, mouse, meiosis

INTRODUCTION

The resumption of oocyte meiotic maturation is linked to the activation of a universal factor, the maturation promoting factor (MPF; Masui and Markert, 1971), a complex of cdc2 and Cyclin B (Lohka et al., 1988; Draetta et al., 1989; Labbé et al., 1989; Gautier et al., 1990). In Xenopus oocytes, the translation of mRNA encoding the Mos kinase is required for the activation of MPF kinase in mouse oocytes. Moreover, MAP kinase is not activated during meiosis in oocytes from mos−/− mice. This result implies that Mos is necessary for MAP kinase activation in mouse oocytes. Raf-1, another MAP kinase kinase, is already present in immature oocytes, but does not seem to be active when MAP kinase is activated. Moreover, the absence of MAP kinase activation in mos−/− oocytes demonstrates that Raf-1 cannot compensate for the lack of Mos. These results suggest that Raf-1 is not involved in MAP kinase activation. Second, we analyzed the organization of the microtubules and chromosomes in oocytes from mos−/− mice. We observed that during the transition between two meiotic M-phases, the microtubules and chromosomes evolve towards an interphase-like state in mos−/− oocytes, while in the control mos+/− oocytes they remain in an M-phase configuration, as in the wild type. Moreover, after spontaneous activation, the majority of mos−/− oocytes are arrested for at least 10 hours in a third meiotic M-phase where they exhibit monopolar half-spindles. These observations present the first evidence, in intact oocytes, of a role for the Mos/.../MAP kinase cascade in the control of microtubule and chromatin organization during meiosis.

Moreover, the analysis of mutant mos−/− mice has shown that, in this species, Mos is not required for GVBD although it is necessary for the arrest in metaphase II (Colledge et al., 1994; Hashimoto et al., 1994).

In Xenopus oocytes at least three pathways can lead to p42mapk activation. One pathway involves Mos, and is activated mainly after progesterone stimulation (Nebreda and Hunt, 1993; Posada et al., 1993; Shibuya and Ruderman, 1993). The two other pathways depend upon receptor tyrosine kinases and act through the Ras GDP-exchange factor. However, whilst one pathway is Raf-1-dependent (Fabian et al., 1993; Muslin et al., 1993), the other one is Raf-1-independent (Itoh et al., 1993; Kuroda et al., 1995). All three pathways induce MAP kinase activation via MAP kinase kinase (MAPKK or MEK) in vitro and in vivo (Dent et al., 1992; Howe et al., 1992; Kyriakis et al., 1992; Muslin et al., 1993; Posada et al., 1993). The proteins involved in the Raf-1-dependent pathway are conserved from yeast to vertebrates (for review see Avruch et al., 1994). In contrast, Mos has been shown to be involved in MAP kinase activation only in oocytes.
from *Xenopus*. In addition, the relationships between the Raf-1 and Mos pathways in the triggering of MAP kinase activation in *Xenopus* oocytes are not clear and the data available are contradictory. Some results suggest that Raf-1 activation lies downstream of Mos (Muslin et al., 1993), while other results suggest that Raf-1 lies on the tyrosine receptor pathway at the same level as Mos (Fabian et al., 1993). In mouse oocytes little is known concerning MAP kinase activation. This activation is protein synthesis dependent (Verlhac et al., 1993), but no data are available concerning the roles of the Mos and Raf-1 kinases.

In addition to the roles of Mos and MAP kinase in the initiation of meiotic maturation and the metaphase II arrest, other roles have been suggested for these kinases during meiosis. It has been proposed that Mos is required for MPF reactivation in metaphase II (O’Keefe et al., 1991; Furuno et al., 1994; Gebauer et al., 1994) and that MAP kinase could be involved in microtubule and chromatin organization during the transition between the two meiotic M-phases (Verlhac et al., 1994). A role for Mos and MAP kinase in microtubule organization was also suggested from studies performed in tissue culture cells where Mos was found to be partly located in the mitotic spindle (Zhou et al., 1991) and in *Xenopus* extracts where the addition of MAP kinase to an interphase extract induced the shortening of the microtubules growing around exogenous centrosomes (Gotoh et al., 1991).

We show here that the Mos protein accumulates concomitantly with MAP kinase activation, suggesting that it might be involved in this event. This is demonstrated directly using oocytes from c-mos knock-out strains, where we observe no activation of MAP kinase. We also show that Raf-1 is already present in immature mouse oocytes, and thus is unable to compensate for the lack of Mos in MAP kinase activation. Moreover, in wild-type oocytes the electrophoretic mobility of Raf-1 does not change at the time of MAP kinase activation, reinforcing the idea that Raf-1 is not involved in ERK1 and ERK2 activation during the resumption of meiosis in mouse oocytes.

We show that the changes in MPF activity are similar in mos−/− compared to mos+/− oocytes demonstrating that Mos is not required for MPF reactivation during the M I to M II transition. The majority of oocytes from mos−/− mice are not arrested in metaphase II and extrude a second polar body as described by Colledge et al. (1994) and Hashimoto et al. (1994). However, we found that 76% of them do not stay in interphase, instead they undergo another transition from metaphase II to another M-phase (which we refer to as metaphase III, M III). During the transitions between M I/M II and M II/M III, we observe the formation of long microtubules resembling interphasic ones and partially decondensed chromosomes in the cytoplasm of mos−/− oocytes. These observations show that the Mos/MAP kinase pathway is normally required for the maintenance of microtubules and chromatin in a metaphasic state during the meiotic divisions. Moreover, the mos−/− metaphase III oocytes present abnormal monopolar spindles and remain arrested for at least 10 hours at this stage, suggesting that microtubule organizing centers (MTOCs) are altered in this mutant strain. All these data present the first evidence of a physiological role of the Mos/MAP kinase cascade in the control of microtubules as well as chromatin organization during meiosis in the mouse.

**Fig. 1.** (A) Mos synthesis during maturation. Mos immunoblot of GV-arrested oocytes (lane 1), oocytes collected 1, 3 and 6 hours after GVBD (lanes 2 to 4 respectively), and M II-arrested oocytes (lane 5). Each sample contains 300 oocytes. (B) ERK1 and ERK2 activation during maturation. ERK1 immunoblot of GV-arrested oocytes (lane 1), oocytes collected 1, 3 and 6 hours after GVBD (lanes 2 to 4 respectively), and M II-arrested oocytes (lane 5). Each sample contains 10 oocytes. (C) Raf-1 electrophoretic mobility does not change during maturation. Raf-1 immunoblot of GV-arrested oocytes (lane 1), oocytes cultured in M2 medium until GVBD (lane 2), 1, 3 and 5 hours after GVBD (lanes 3 to 5 respectively), and M II-arrested oocytes, collected after superovulation (lane 6). Each sample contains 20 oocytes. Molecular mass markers, ×10−3, are on the right. BD, germinal vesicle breakdown.
The proteins were separated by electrophoresis in 10% polyacrylamide containing 0.1% SDS and electrically transferred onto nitrocellulose membranes (Schleicher and Schuell, pore size 0.45 μm). Following transfer and blocking for 2 hours in 3% skimmed milk in TBS (10 mM Tris, pH 7.5, 140 mM NaCl), containing 0.1% Tween-20 (TBS/Tween), the membrane was incubated overnight at 4°C with one of the primary antibodies diluted 1:300 in blocking solution. The three kinases Raf-1, Mos, and MAP kinases were detected using an anti-Raf-1 monoclonal antibody (Transduction Laboratories), an anti-Mos polyclonal antiserum (Gallick et al., 1985), and an anti-ERK polyclonal affinity purified antiserum (no. 691; Santa Cruz Biotechnology, Inc.). We used either an anti-rabbit immunoglobulin (when the polyclonal antibodies were used as first layers) or an anti-mouse immunoglobulin (for the monoclonal antibody) second layer antibodies conjugated to horseradish peroxidase (Amersham) diluted 1:1000 in 3% skimmed milk in TBS/Tween. The membranes were washed three times in TBS/Tween and then processed using the ECL detection system (Amersham). All experiments were repeated at least three times.

**Immunofluorescence**

The fixation and labeling of oocytes were performed as described by Kubiak et al. (1992). We used the rat monoclonal antibody YL1/2 specific for tyrosinated α-tubulin (Kilmartin et al., 1982) and the mouse monoclonal MPM-2 antibody, which stains MTOCs in M-phase (Rao et al., 1989). As second layers, we used either a fluorescein-conjugated anti-rat antibody (Miles) or a rhodamine-conjugated anti-mouse antibody (Miles). The chromatin was visualized using propidium iodide (Molecular Probes; 5 mg/ml in PBS). Samples were observed with a Bio-Rad MRC-600 confocal microscope.

**Kinase assays**

The MBP kinase and histone H1 kinase assays were performed as described by Félix et al. (1989) and Boulton and Cobb (1991). Three to five oocytes were transferred into 1 μl of M2 medium, frozen immediately in a mixture of dry ice and ethanol, and stored at -80°C. For the combined histone H1/MBP kinase assays, 2 μl of twice concentrated reaction buffer (180 mM EGTA, pH 7.3, 300 mM β-glycerophosphate, 40 mM MgCl2, 4 mM DTT, 4 mM benzamidine, 20 mM NaF, 0.4 mM sodium orthovanadate, 4 mM ATP) supplemented with protease inhibitors was added to the sample, which was then thawed and refrozen once to lyse the cells. The reaction was started by the addition of 2 μl of a solution containing either 1.5 mg/ml MBP or 66 μg/ml histone H1 and 0.5 mCi/ml [γ32P]ATP, and lasted 30 minutes at 30°C. The reaction was stopped by adding twice concentrated sample buffer (Laemmli, 1970) and boiling for 3 minutes. The samples were then analysed by electrophoresis in 15% SDS-PAGE, followed by autoradiography.

**RESULTS**

**Mos accumulates concomitantly with MAP kinase activation during maturation of mouse oocytes**

We examined mouse oocytes at different stages of meiotic maturation using anti-Mos polyclonal antibodies previously characterized for immunoblotting in mouse oocytes (Weber et al.,
We observed that Mos was synthesized after GVBD. The Mos kinase could be detected by immunoblotting 3 hours after GVBD and reached a maximal level in metaphase II-arrested oocytes (Fig. 1A). At the same time the electrophoretic mobility of ERK1 and ERK2 was reduced, demonstrating that their activation had occurred through phosphorylation (Fig. 1B). Thus, in mouse oocytes, Mos is synthesized concomitantly with ERK1 and ERK2 activation, well after GVBD.

The electrophoretic mobility of Raf-1 does not change during maturation

A previous study has shown that Raf-1 mRNAs are present in mouse oocytes (Pal et al., 1993). To determine if the Raf-1 kinase is present in mouse oocytes during maturation and whether it is phosphorylated, oocytes were collected at different stages of maturation and examined by immunoblotting using a monoclonal antibody directed against a sequence of human Raf-1. In GV oocytes, a unique protein of relative molecular mass 72-74×10³ (the molecular mass of vertebrate Raf-1) was detected by the antibody as shown in Fig. 1C. The electrophoretic mobility of this protein did not change during in vitro maturation (lanes 1-5). However, in M II-arrested oocytes, bands of higher molecular mass were recognized by the anti-Raf-1 antibody (lane 6). Thus, the electrophoretic mobility of Raf-1 remains constant and high during the process of meiosis in mouse oocytes, suggesting that it is not phosphorylated and thus not active (Morrison et al., 1989), at least until the metaphase II stage.

Mos is not involved in the timing of meiotic maturation and the control of MPF activity but is required for MAP kinase activation

To check whether Mos was involved in the activation of MAP kinase in mouse oocytes, we used the mutant strain of mos⁻/⁻ mice (Colledge et al., 1994). Maturing oocytes, either from mos⁻/⁻ mice or from mos⁺/⁺ mice, were collected and synchronized by scoring for GVBD, which allowed us to follow precisely their progression into the meiotic cell cycles. We analysed the kinetics of meiotic maturation and samples were collected at various times after GVBD to measure histone H1 kinase activity. We assessed the phosphorylation state of ERK1 and ERK2 and the activity of myelin basic protein (MBP) kinase. Fig. 2A shows that the timing of GVBD (occurring between 0.5 and 1 hour after the beginning of
Oocytes from mos−/− mice, collected in metaphase I at 3 and 5 hours after GVBD (Fig. 4A lanes 1 and 2), or after extrusion of the first polar body (Fig. 3A lane 3) contained the fast migrating forms of ERK1 and ERK2, which correspond to the inactive forms of MAP kinases normally present in immature oocytes (Fig. 3A lane 6). In contrast, control oocytes from mos+/− mice showed a normal pattern of ERK1 and ERK2 activation (Fig. 3A lanes 4 and 5). We never detected a significant rise in the activity of MBP kinase in oocytes from mos−/− mice, while MBP kinase activity increased normally in mos+/− oocytes (Fig. 3B). These results show that ERK1 and ERK2 are not activated during meiosis in oocytes from mos−/− mice. Thus, Raf-1 cannot compensate for the lack of Mos in these oocytes. However, the lack of MAP kinase activity in mos−/− oocytes does not interfere with the progression of meiotic maturation and the changes in MPF activity until the metaphase II arrest.

**Mos is necessary for normal organization of the microtubules and chromatin during the transitions between two meiotic M-phases**

To check for an eventual role of the Mos/.../MAP kinase cascade in microtubules and chromatin organization during the M I to M II transition (Verlhac et al., 1994), we processed oocytes for immunofluorescent staining of the microtubules and the chromosomes. We observed the classical M I to M II transition in mos+/− mice: the M I spindle appeared 6 hours after GVBD (Fig. 4B), the M II spindle was visible 10 hours after GVBD (Fig. 4C) and was still present 4 hours later, due to the CSF-induced arrest (Fig. 4D). In mos−/− mice, after the formation of the M I spindle (Fig. 5A), the oocytes extruded the first polar body and then progressed to metaphase II (Fig. 5D). However, during the M I to M II transition, instead of short microtubules localized in the vicinity of the condensed chromosomes, we could observe long microtubules radiating from a mass of slightly decondensed chromatin (Fig. 5B and compare C and C¢ with C¢¢). Thus, in mos−/− oocytes, the organization of microtubules and chromatin evolves towards an interphase-like state during the M I to M II transition.

We found that 76% of the mos−/− oocytes that had extruded a first polar body, expelled subsequently a second polar body and entered a transition from M II (Fig. 5D) to a third M-phase stage (Fig. 5F), that we will call metaphase III (M III). Here again, during the transition from M II to M III, we observed the presence of long microtubules and slightly decondensed chromosomes (Fig. 5E). The remaining 24% either did not extrude the second polar body, or its extrusion was greatly delayed (4 to 6 hours later). Of the remaining 24% mos−/− oocytes which did not extrude their second polar body in time, we observed that 2/3 were arrested at a very peculiar state 24 hours after GVBD. Asters of microtubules had gathered in the oocyte center, near to the condensed chromatin and no spindle-like structures were evident (Fig. 6C). Only 1/3 of the 24% were in interphase at this time and possessed a well formed network of cortical microtubules and nuclei with decondensed chromatin (Fig. 6D). These oocytes always possessed two pronuclei, indicating abnormal chromosomal segregation.

Together these results suggest that in the absence of Mos, the microtubules and chromatin evolve towards an interphase-like state during the meiotic transitions. They also show that 24% of the mos−/− oocytes present severe defects in M II spindle formation and/or function.

**The metaphase III mos−/− oocytes are arrested with monopolar half-spindles**

A second meiotic transition leading to a third meiotic M-phase, the so-called metaphase III (M III), can be induced in wild-type oocytes by ethanol activation of freshly ovulated oocytes (13-15 hours post-hCG; Kubiak, 1989). The organization of the microtubule network in M III-arrested mos−/− oocytes was very unusual compared to those from control mos+/− M III oocytes and extruded its second polar body between 11 and 14 hours after GVBD (Fig. 6). They remained in metaphase II for 2-4 hours and then arrested in metaphase II (Fig. 2B). The MPF activity in both types of oocyte decreased during the M I to M II transition and increased again in M II (Fig. 2B), as previously observed during maturation (Kubiak et al., 1992). However, in mos−/− oocytes, MPF activity decreased again at the time of second polar body extrusion (Fig. 2B).

**Fig. 6.** Organization of the microtubules and chromatin in mos−/− mice 24 hours after GVBD. Microtubules are shown in green and chromosomes in red. (A) Higher magnification of a mos−/− oocyte which expelled its second polar body between 11 and 14 hours after GVBD and was arrested for 10 hours in metaphase III, arborizing a monopolar spindle. This oocyte was also stained with the MPM-2 monoclonal antibody (in blue). (B) Control mos+/− oocyte, which has been activated with ethanol 14 hours after GVBD, and is arrested in M III. (C,D) mos−/− oocytes, which either did not extrude the second polar body or extruded it after a 4- to 6-hour delay.
obtained after ethanol activation 14 hours post GVBD (Kubiak, 1989). Monopolar half-spindles were observed in 90% of mos−/− oocytes and we could only detect a single pole after staining with the MPM-2 antibody (Fig. 6A). This third M-phase is not a real metaphase since the spindles are not bipolar, however, to simplify the terminology we will refer to it as M III. All the M III spindles present in control ethanol-activated mos−/− oocytes were found to be bipolar (Fig. 6B). Thus the monopolar spindles present in the M III mos−/− oocytes are not an artefact due to in vitro culture, but their presence is related either directly or indirectly to the loss of Mos function. Moreover, the M III oocytes from mos−/− mice possessed unphosphorylated and inactive forms of ERK1 and ERK2, which was not the case for M III-arrested mos+/− oocytes obtained after the parthenogenetic activation of in vitro matured oocytes (Fig. 7). Finally, we observed that the M III mos−/− oocytes were arrested in M-phase for at least 10 hours, probably as a consequence of the spindle checkpoint, since no CSF is present in these oocytes.

In conclusion, we must point out that all mos−/− oocytes showed defects in spindle microtubule organization during the late stages of meiotic maturation that may be due to alterations in MTOCs: 76% of them had monopolar M III spindles, while 24% had severe defects in M II spindle formation and/or function.

DISCUSSION

This work was performed to analyse the role of Mos and Raf-1 in the control of MAP kinase activation, and also to determine some physiological roles of Mos during mouse meiotic maturation.

Mos, but not Raf-1, is required for MAP kinase activation

Two pathways can trigger the resumption of meiosis and activation of p42mapk in Xenopus oocytes: the physiological progesterone pathway and the receptor tyrosine kinase pathway. Mos would appear to play a role mainly in the progesterone pathway, and Raf-1 in the receptor tyrosine kinase pathway, with a possible interaction of these two pathways (Fabian et al., 1993). We observed a good correlation between the accumulation of Mos and MAP kinase activation in mouse oocytes, suggesting that there is a link between Mos synthesis and MAP kinase activation. This correlation has also been described in Xenopus oocytes (Nebreda and Hunt, 1993). We then showed that MAP kinase activation does not occur in mos−/− mice, implying that Mos is normally required for MAP kinase activation. We also demonstrate that, similar to Xenopus oocytes, Raf-1 is present in immature mouse oocytes (Fabian et al., 1993; Muslin et al., 1993), but does not compensate for Mos in the MAP kinase activation pathway in oocytes from mos−/− mice, thus suggesting strongly that Raf-1 is not involved in this process in the mouse oocyte.

In Xenopus oocytes, the electrophoretic mobility of Raf-1 shifts to the slower migrating form at the time of germinal vesicle breakdown, when MAP kinase is activated (Fabian et al., 1993; Muslin et al., 1993). However, in mouse oocytes the electrophoretic mobility of Raf-1 does not change during the early stages of maturation and corresponds to the fast migrating form of the kinase. It is not clear whether the slow migrating form of Raf-1 corresponds to the active kinase, however the fast migrating species corresponds to the inactive form of the enzyme (at least in vertebrate cells; Morrison et al., 1989; Samuels et al., 1993). This likely lack of Raf-1 activation in mouse oocytes when MAP kinase activation occurs normally strengthens the idea that Raf-1 is not required for MAP kinase activation during the resumption of meiosis. This result is consistent with the fact that the synthesis of Ras, which lies upstream of Raf-1, is not required for meiosis in mouse oocytes, but is required for progression to the 2-cell stage (Yamauchi et al., 1994). However, we do not exclude the possibility that Raf-1 plays a role later during fertilization, since M II-arrested mouse oocytes were found to contain the slower migrating form that corresponds to the phosphorylated form of Raf-1.

The fact that in mouse oocytes, Mos but not Raf-1, is required for MAP kinase activation is an important and new result, which suggests that Mos might be involved specifically in the physiological control of MAP kinase activation during vertebrate meiotic maturation.

Mos and MAP kinase are not involved in MPF reactivation during the M I to M II transition

The timing of germinal vesicle breakdown and the extrusion of the first polar body, as well as changes in MPF activity, were similar in mos−/− and mos+/− oocytes. These results show that MPF reactivation occurs normally during the M I to M II transition. Moreover, the fact that most (76%) mos−/− oocytes undergo a second meiotic transition leading to M III reinforces the idea that Mos is not required for MPF reactivation. Together, our results prove that in mouse oocytes, in contradiction to previous studies performed both in Xenopus and mouse oocytes (O’Keefe et al., 1991; Furuno et al., 1994; Gebauer et al., 1994), Mos is not required for the reactivation of MPF during meiosis.
Mos is required for normal microtubule and chromatin organization during meiosis in mouse oocytes

We did not observe any major differences in the formation of the first meiotic spindles in oocytes from mos−/− compared to mos+/+. However, the organization of the microtubules and chromatin during the M I to M II (and M II to M III) transition was very different in mos−/− oocytes compared to the classical organization observed in mos+/+ oocytes. The lack of Mos and/or active MAP kinase in mos−/− oocytes induces the reorganization of the microtubules and chromatin towards an interphase-like state during these transitions. These results reinforce the hypothesis of a role for MAP kinase in the maintenance of a metaphasic organization of the microtubules and chromatin in meiosis, when MPF is inactive (Verlhac et al., 1994).

We have also presented here evidence that Mos is required for proper meiotic spindle formation. Surprisingly, mos−/− oocytes activate spontaneously in M II but do not directly enter interphase. Most of them (76%) undergo another meiotic transition (M II to M III) and remain arrested with monopolar spindles in M III. The occurrence of an M II to M III transition can be explained by the work of (Kubiak, 1989), which showed that ethanol activation of very young ovulated oocytes induces an M II to M III transition, whereas the same treatment on older ovulated oocytes induces an M II to interphase transition. The mos−/− oocytes also activate early in M II (since they do not arrest in M II), and consistently most of them then progress to metaphase III. The mos−/− oocytes which show a delay in extrusion of the second polar body have a tendency to enter interphase. However, M III spindles obtained after activation of in vitro or in vivo matured oocytes are normally bipolar (Kubiak et al., 1992; this work), and not monopolar as observed in mos+/+ oocytes. This result suggests strongly that MTOCs function is altered in mos−/− oocytes and thus impairs the proper formation of the M III spindle. We also presented evidence that mos−/− oocytes show defects in M II spindle function, since 24% of them either cannot extrude the second polar body or extrude it after a 4- to 6-hour delay. The abnormal formation and/or function of the M II spindle in this population of oocytes is reinforced by the observation of abnormal chromosomal segregation (oocytes with two pronuclei of different sizes). The possibility that MTOC function is altered in mos−/− mice, which lack MAP kinase activity, is consistent with previous observations showing that a fraction of MAP kinase localizes to the MTOCs in mouse oocytes (Verlhac et al., 1993). Based on these microtubules abnormalities observed in vivo, our results strengthen the idea of an important role for Mos and activated MAP kinase in controlling microtubule organization during meiotic maturation.

Oocytes from mos−/− mice do not arrest in metaphase II but arrest in a third M-phase

We observe that 76% of mos−/− oocytes are arrested for at least 10 hours at a stage that we call metaphase III. In these M III-arrested mos−/− oocytes, the chromosomes remained condensed, the microtubules remained short as in M-phase, and we could observe a positive staining with the MPM-2 monoclonal antibody, indicating that they are indeed arrested in metaphase. This arrest explains why it takes so long for mos−/− oocytes to reform pronuclei (nuclei appear 24 hours after GVBD) and why they do so rarely (in 8% of the cases after in vitro culture, and in 40% of the cases after in vivo culture; Colledge et al., 1994). It has been shown previously that mouse oocytes possess a spindle checkpoint which prevents them exiting from metaphase II after activation if the spindle is not properly formed (Kubiak et al., 1993; Winston et al., 1995). The majority of mos−/− oocytes are arrested in metaphase III not because of a CSF activity (Mos is absent and we show here that MAP kinase is not active), but probably because their M III spindles are monopolar and so are not functional. The lack of CSF activity in these oocytes is somehow compensated for by the absence of a functional M III spindle. Whether this arrest in M III can lead to fertilized embryos possessing the normal ploidy requires further investigation.

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