Cell cycle modification during the transitions between meiotic M-phases in mouse oocytes

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Summary

When metaphase II-arrested mouse oocytes (M II) are activated very soon after ovulation, they respond abortively by second polar body extrusion followed by another metaphase arrest (metaphase III, M III; Kubiak, 1989). The M II/M III transition resembles the natural transition between the first and second meiotic metaphases (M I/M II). We observed that a similar sequence of events takes place during these two transitions: after anaphase, a polar body is extruded, the microtubules of the midbody disappear rapidly and a new metaphase spindle forms. The MPM-2 monoclonal antibody (which reacts with phosphorylated proteins associated with the centrosome during M-phase) stains discrete foci of peri-centriolar material only in metaphase arrested oocytes; during both transitional periods, a diffuse staining is observed, suggesting that these centrosomal proteins are dephosphorylated, as in a normal interphase. However, the chromosomes always remain condensed and an interphase network of microtubules is never observed during the transitional periods. Incorporation of 32P into proteins increases specifically during the transitional periods. Pulse-chase experiments, after labeling of the oocytes in M phase with 32P, showed that a 62 kDa phosphoprotein band disappears at the time of polar body extrusion. Histone H1 kinase activity (which reflects the activity of the maturation promoting factor) drops during both transitional periods to the level characteristic of interphase and then increases when the new spindle forms. Both the M I/M II and M II/M III transitions require protein synthesis as demonstrated by the effect of puromycin. These results suggest that the two M-phase/M-phase transitions are probably driven by the same molecular mechanism.

Key words: cell cycle, meiosis, microtubules, oocyte, MPF, parthenogenesis, phosphorylation, protein synthesis.

Introduction

Meiotic maturation is a complex process leading to the formation of female gametes ready for fertilization. During this process the cell cycle is modified. First, there are special events occurring during the first meiotic division: tetrad pairing and crossing-over in prophase followed by tetrad separation in anaphase. Second, instead of the usual succession of M phase, interphase (S phase with or without G1 and G2 phases), M phase, there is no evident interphase between the two meiotic M phases (metaphase I (M I) and metaphase II (M II)). The lack of DNA replication before the second meiotic division ensures that the M II oocytes contain two haploid sets of maternal chromosomes. Fertilization triggers the extrusion of the second polar body and the formation of the haploid female pronucleus. At the same time, the sperm nucleus decondenses and forms the haploid male pronucleus. Consequently, the diploid zygote has one set of haploid maternal and one set of haploid paternal chromosomes. The mechanism involved in the suppression of DNA replication between the two meiotic M phases is unknown. Moreover, there is normally a feedback that ensures that a new mitosis does not occur if the DNA is not correctly replicated. We are interested in those mechanisms that are involved in this peculiar transition between the M-phases during meiosis.

It was shown recently that the ability of mouse oocytes to respond to an activating stimulus (fertilization or artificial activation) develops gradually after the extrusion of the first polar body (Kubiak, 1989). Activation of very “young” metaphase II oocytes triggers the extrusion of the second polar body and entry into a subsequent metaphase, metaphase III (M III). It seems that this abortive activation leading to M III follows roughly the same pattern of events as the natural M I/M II meiotic transition. The M II/M III transition may therefore provide a useful experimental model for study of the cell cycle modification taking
place during meiotic maturation. The oocytes undergoing abortive activation have several advantages over those undergoing the natural M I/M II transition: (i) they are available in large numbers; (ii) they are easily recovered in the oviduct (whereas meiotic maturation normally takes place in the ovary); (iii) they are better synchronized. In this paper we present evidence that these two transitions are very similar and, thus, are most probably driven by the same mechanism. We show that the cdc2 histone H1 kinase activity drops during the transitory period and that MPM-2 staining is altered as during a normal interphase. However, the overall level of protein phosphorylation does not seem to decrease and most of the morphological events occurring during a normal interphase - nuclear envelope re-formation, chromatin decondensation and cytoskeletal rearrangements - are not seen.

**Materials and methods**

**Recovery of oocytes**

Swiss female mice (5-6 weeks old; Animalerie Spécialisée de Villejuif, Centre National de la Recherche Scientifique, France) were superovulated by injection of 5 i.u. of pregnant mare's serum gonadotrophin (PMSG; Intervet) and human chorionic gonadotrophin (hCG; Intervet), 48 h apart. Freshly ovulated oocytes were obtained by puncturing the ampullae during a normal interphase; (iii) they are better synchronized. In this study Swiss mice were used instead of the two mouse strains. To visualize chromatin, propidium iodide (Molecular Probes; 5 μg/ml in PBS) or DAPI (5 μg/ml in PBS) was used.

**Oocyte fixation and immunocytochemical staining**

Histone H1 kinase assay

Histone H1 kinase activity was determined as described by Félix et al. (1989) in HK buffer (80 mM β-glycerophosphate, 20 mM EGTA, pH 7.3, 15 mM MgCl₂, 1 mM DTT, 1 mM PMSF, 10 μg/ml leupeptin, 10 μg/ml pepstatin, 10 μg/ml aprotinin) using exogenous histone H1 (H III-S from calf thymus, Sigma) as substrate. Samples each containing 50 oocytes in 5 μl of water were lysed by freezing and thawing three times, diluted twice in double concentrated HK buffer (2×HK) and incubated for 15 min at 20°C in the presence of 3.3 mg/ml histone H1, 1 mM ATP and 0.25 μCi/ml [γ²²P]ATP. The reaction was stopped by the addition of a similar volume of double concentrated sample buffer (Laemmli, 1970) and incubation for 2 min at 90°C. The samples were then electrophoresed on a 15% SDS-polyacrylamide gel (Laemmli, 1970). To test the specificity of the reaction, the p34cdc2 kinase
(histone H1 kinase) was removed by centrifugation from the control sample using p13Sul-coated Sepharose beads.

In the case of the M I/M II transition all oocytes entered M II. In the case of the M II/M III transition some oocytes remained in M II and some entered the first interphase. The first group was removed from the samples by using only the oocytes extruding the second polar body. However, it was not possible to distinguish during the transitional period between the M-phase/M-phase and the M-phase/interphase transitions. To calculate the relative percentages of M III and interphase oocytes, control groups were cultured for 5-6 h and scored for the presence of pronuclei. Alternatively, some samples were stained with Hoechst 33342 as described previously (Kubiak et al., 1991) and examined under the fluorescence microscope shortly after polar body extrusion. Only experiments giving more than 75% of M III oocytes were used. The values obtained in the histone H1 kinase assay were corrected to take into account the genuine percentage of M III within the extract.

Metabolic labeling
Oocytes were sampled in groups containing 40 oocytes when the first signs of polar body extrusion were visible and incubated in the labeling medium (M2+BSA containing 500 μCi/ml [35S]methionine or phosphate-free M2+BSA containing 500 μCi/ml [32P]orthophosphate) for 20 min starting at 0, 20, 40, 60 and 80 min after the sampling. Oocytes were then washed in three large drops of M2+BSA and collected in 5 μl double strength sample buffer and electrophoresed on a 10% SDS-PAGE (Laemmli, 1970). For pulse-chase experiments, approximately 500 M I oocytes or 1000 M II oocytes were labeled for 1 h as described above, washed in medium containing non-radioactive methionine (or phosphate), and then cultured in M2+BSA. For the M I/M II transition, oocytes were sampled in groups of 50 at the time of first polar body extrusion; for the M II/M III transition they were collected at various times after the sampling. Oocytes were then labeled for 1 h as described above, washed in medium containing non-radioactive methionine (or phosphate), and then cultured in M2+BSA. The values obtained in the histone H1 kinase assay were corrected to take into account the genuine percentage of M III within the extract.

Drugs
Puromycin (Sigma) was used to inhibit protein synthesis at a final concentration of 20 μM. Nocodazole (Aldrich) was used to block M I or M II oocytes in M phase at a final concentration of 10 μM.

Results
The chromosomes, microtubules and MPM-2 antigens behave similarly during both transitions
When oocytes undergoing the M I/M II or the M II/M III transitions were stained with an anti-tubulin antibody YL1/2 and with propidium iodide, and observed under the confocal microscope, similar changes in microtubule organization and chromatin pattern were observed (Fig. 1A-L). In both cases, anaphase was followed by the extrusion of the polar body and formation of a broad microtubular structure joining the polar body and the oocyte through the midbody (Fig. 1B, H). These microtubules disappeared rapidly and formed a conical structure with the narrowest part located at the midbody proper, i.e. the region of contact between the polar body and the oocyte (Fig. 1C, I). In oocytes where midbody-associated microtubules had shrunk, new microtubules appeared next to the chromosomes on the side directed towards the center of the oocyte. The second polar body and the oocyte were still connected by a narrow remnant of the midbody microtubules (Fig. 1D, J). At that time, a new spindle developed in the oocyte as well as in the polar body. In the oocyte it formed perpendicular to the cell surface, while the midbodies had already disappeared (Fig. 1F, L). Chromosomes in both the oocytes and the polar bodies remained condensed during the whole transitional period. MPM-2, a monoclonal antibody that recognizes certain phosphorylated proteins associated with MTOCs in mitotic and meiotic cells, showed a diffuse staining during the transitions and was concentrated only in the area of the forming spindles (Fig. 2B). However, in all metaphase oocytes, it stained discrete foci at the spindle poles and within the cytoplasm (Fig. 2A, C).

We observed that the size of the spindles in the oocytes (M I, M II and M III) correlated with the number of chromatids present within them (Table 1; Fig. 3). The M II spindles were always well organized, while the M III spindles often had certain abnormalities. The oocytes that underwent normal activation formed a single pronucleus within the oocyte and an interphase nucleus within the second polar body (Fig. 4A, D). The midbody between the oocyte and the second polar body was broad and remained stable for hours (Fig. 4B), in contrast to what was observed in abortively activated oocytes, where it disappeared rapidly allowing the second polar body to separate easily from the oocyte. An extensive network of interphase microtubules was present within normally activated oocytes (Fig. 4B) and MPM-2 gave only a weak, diffuse signal in their cytoplasm with higher accumulation within the midbody (Fig. 4C).

Table 1. Sizes of metaphase I, metaphase II and metaphase III spindles

<table>
<thead>
<tr>
<th></th>
<th>Mean length±s.d. (μm)</th>
<th>Mean width±s.d. (μm)</th>
<th>Mean volume±s.d. (μm³)</th>
</tr>
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<tbody>
<tr>
<td>Metaphase I</td>
<td>13</td>
<td>25±3</td>
<td>17±4</td>
</tr>
<tr>
<td>Metaphase II</td>
<td>36</td>
<td>17±2</td>
<td>13±2</td>
</tr>
<tr>
<td>Metaphase III</td>
<td>52</td>
<td>15±2</td>
<td>8±1</td>
</tr>
</tbody>
</table>

*Calculated assuming that the spindles are cylinders.
Fig. 1
Fig. 1. Behavior of microtubules (left) stained with the anti-tubulin antibody YL1/2 (Kilmartin et al., 1982) and chromosomes (right) stained with propidium iodide, during M I/M II (A-F) and M II/M III (G-L) transitions viewed with confocal microscopy. Bar in G, 20 μm. (A-F) Metaphase I oocytes isolated from ovaries 7-8 h after hCG injection and fixed around the time of the first polar body extrusion (9-10.5 h after hCG). (G-L) Metaphase II oocytes treated with ethanol 14 h after hCG injection and fixed during the subsequent 1.5 h. Each pair of figures (A-G, B-H, etc.) represents similar stages in the two transitions. Open arrows indicate the direction of the polar body extrusion. Note that the chromosomes remain condensed during all transitional periods. (A,G) Metaphase I and II, respectively. (B,H) The beginning of polar body extrusion. Microtubules form a broad structure in the region of the midbody. (C,I) Microtubules of the midbody shrink and form conical structures. New microtubules appear next to the chromosomes in the oocyte. (D,E,J,K) While the shrinking of the midbody microtubules progresses, new spindles form both in the oocyte and in the polar body. The spindles in the oocytes form perpendicular to the oocyte surface (K) and then rotate to become parallel to the surface (D, E, J). The polar body is still connected to the oocyte by a very tiny microtubule bridge. (F,L) Approximately 1.5 h after polar body extrusion the M II (F) and the M III (L) spindles are not connected to the polar bodies.

Fig. 2. Microtubules (left), MPM-2 (middle) and chromosomes (right) staining in M II (A), transitional (B) and M III (C) oocytes. Bar in C, 20 μm. A similar sequence of events was observed during the M I/M II transition. Microtubules are detected in the M II spindle (A, left), the midbody (B, left), the M III spindle and the second polar body (C, left). MPM-2 antibody decorates preferentially the foci of pericentriolar material at the spindle poles (arrowheads) and in the cytoplasm (small arrows) of the metaphase oocytes (A and C) as well as in the second polar body (open arrows). During the transitional period the foci of pericentriolar material cannot be detected by the MPM-2 antibody and only a diffuse staining is observed (B, middle). The middle part of C is composed of two slightly different focal planes of the same oocyte.
Protein phosphorylation follows the same pattern during both transitions

The diffuse staining obtained with the MPM-2 antibody during both transitional periods suggests interphase-like behavior of the phosphoproteins reacting with this antibody. We performed a pulse metabolic labeling of proteins with $^{32}$P phosphate for 20 min during the M I/M II and M II/M III transitions. Surprisingly, this labeling showed an increased incorporation of radioactive phosphate specifically during the transitional periods (Fig. 5). This increase in $^{32}$P incorporation was observed in all labeled bands, but it was particularly evident in the two most heavily labeled bands of phosphoproteins (approx. 71 kDa and the 35 kDa complex - a family of phosphoproteins typical of oocytes and early stages of murine development; Howlett, 1986).

We also performed pulse-chase experiments after metabolic labeling of proteins in M I and M II oocytes, which were then chased during the transitions to M II and M III, respectively, to see whether the pattern of phosphoproteins changed during these periods. These pulse-chase experiments showed that a band of 62 kDa disappeared during both M-phase/M-phase transitions (Fig. 6). This band is present in both M I and M II oocytes, as well as in the control oocytes arrested in M-phase by nocodazole and sampled every 30 min, when the experimental oocytes were undergoing the transitions. Its disappearance may be due to its dephosphorylation or to its proteolysis.
Fig. 5. Incorporation of phosphate into proteins increases during the M I/M II and the M II/M III transitions. Samples of 40 M I oocytes, M II oocytes, and oocytes undergoing M-phase/M-phase transitions, were collected every 20 min starting at the time of first or second polar body extrusion and labeled with \([^{32}P]\)orthophosphate for 20 min. Molecular mass is given on the right (in kDa).

Histone H1 kinase is transiently inactivated during both transitions

The behavior of chromosomes and microtubules during the two M-phase/M-phase transitions as well as the pattern of protein phosphorylation could suggest that there is no interphase separating the two metaphases. However, the disappearance of the punctate staining of MPM-2 and of the 62 kDa phosphoprotein band during these transitional periods suggests the opposite. To check whether a short interphase existed between the two metaphases, we measured the level of histone H1 kinase (which reflects the activity of the p34^{cd2} kinase) in oocytes during these two periods. We used extracts from groups of 50 oocytes collected every 30 min after polar body extrusion. We found that the histone H1 kinase activity dropped at the time of polar body extrusion and then increased progressively starting 60-75 min after polar body extrusion, i.e. the period when the M II or M III spindle formation starts (Fig. 7). Since during abortive activation of M II oocytes some oocytes undergo the normal transition to interphase and are not distinguishable at these early stages, some oocytes were collected 5 h later to estimate the percentage of oocytes undergoing normal activation in our samples. The graph presented in Fig. 8 shows corrected values. Despite this correction we observed that the level of histone H1 kinase in M III oocytes was not fully re-established in comparison to the level observed in M II oocytes. This activity was also slightly lower in M II oocytes when compared with M I oocytes.

Fig. 6. Pulse-chase experiment showing the disappearance of a 62 kDa phosphoprotein during the M-phase/M-phase transitions. M I and M II oocytes were incubated in the presence of \([^{32}P]\)orthophosphate (500 \(\mu\)Ci/ml) during 1 h, then \([^{32}P]\)orthophosphate was washed out. For the M II/M III transition, the oocytes were activated as described in Materials and methods. Samples of 80 (M I/M II; lane PB1) or 150 (M II/M III; lane PB2) oocytes were collected at the time of polar body extrusion. Identical numbers of control oocytes were placed after labeling in a medium containing 10 \(\mu\)M nocodazole to prevent the metaphase/anaphase transition, and they were sampled at the same time as the oocytes undergoing the transition (lanes M I and M II). The 62 kDa band is marked with an arrow.

Fig. 7. Histone H1 kinase activity during the two M-phase/M-phase transitions in samples of 25 oocytes (data gathered from two separate experiments for each transition). Note that the level of histone H1 kinase activity drops during the transitional periods and is not fully re-established in M II (and M III) oocytes when compared with M I (and M II) oocytes.
Protein synthesis is required for both transitions

The inactivation of the cdc2 histone H1 kinase at the end of a normal mitosis is correlated with the proteolytic degradation of cyclins (Murray et al., 1989). Thus, its rapid activation on entry into M II or M III might require de novo synthesis of these proteins. We tested whether protein synthesis is required for the M I/M II and the M II/M III transitions using puromycin as a protein synthesis inhibitor. For the M I/M II transition, the oocytes were placed in a medium containing 20 μM puromycin just before the estimated time of the first polar body extrusion. For the M II/M III transition, they were placed in the drug 15 min after ethanol treatment, i.e. at the moment of anaphase II. Oocytes undergoing the M I/M II or M II/M III transitions in puromycin-free medium were used as controls. M II oocytes placed in puromycin-supplemented and puromycin-free media without the activating treatment were used as additional controls for the M II/M III transition. We scored the oocytes for the presence of an interphase nucleus 3 h after polar body extrusion. First, we confirmed that the presence of puromycin does not interfere with the timing and dynamics of polar body extrusion in both transitions (data not shown). As shown in Table 2, the presence of a protein synthesis inhibitor during the two M-phase/M-phase transitions induced the formation of interphase nuclei 3 h after polar body extrusion. None of the oocytes that extruded the polar body in the presence of puromycin returned to a metaphase state. The oocytes that remained in M I or M II during this period, had condensed chromosomes. Control M II oocytes incubated in the medium with puromycin for 3 h did not form interphase nuclei, with the exception of some (7%) that had extruded the second polar body (spontaneous activation) during the 3 h culture (3% in puromycin-free medium). These data show that protein synthesis is necessary for both M-phase/M-phase transitions. In the absence of protein synthesis anaphase is not inhibited; however, interphase nuclei form instead of metaphase plates.

To determine the critical period during which protein

Table 2. Effects of puromycin on the types of oocytes obtained during the M I/M II and the M II/M III transitions

<table>
<thead>
<tr>
<th></th>
<th>Activation</th>
<th>n</th>
<th>% M I</th>
<th>% M II</th>
<th>% M III</th>
<th>% Pronucleus</th>
</tr>
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<tbody>
<tr>
<td><strong>M I/M II transition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>68</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Puromycin</td>
<td></td>
<td>75</td>
<td>3</td>
<td>0</td>
<td>97</td>
<td></td>
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<tr>
<td><strong>M II/M III transition</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td></td>
<td>205</td>
<td>26</td>
<td>44</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Puromycin</td>
<td></td>
<td>201</td>
<td>7</td>
<td>0</td>
<td>93</td>
<td></td>
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<tr>
<td>Control</td>
<td></td>
<td>70</td>
<td>97</td>
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<td>3</td>
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</tr>
<tr>
<td>Puromycin</td>
<td></td>
<td>69</td>
<td>93</td>
<td>0</td>
<td>7</td>
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*20 μM puromycin added during M I.
†120 μM puromycin added 15 min after activation with 8% ethanol for 6.5 min.
synthesis is required to enter into a new metaphase, we treated oocytes undergoing the M I/M II transition with short pulses of puromycin (30, 45, 60, 75 min) starting at the time of first polar body extrusion (Fig. 8A). Even short pulses of puromycin during the transitional period were sufficient to induce the formation of interphase nuclei. However, these nuclei underwent condensation a few hours after removal of the drug, indicating that once protein synthesis was re-established the protein(s) necessary for entry into M-phase appeared in the oocytes. Moreover, delayed treatment with puromycin after first polar body extrusion did not induce the formation of interphase nuclei, indicating that the protein(s) necessary for entry into M-phase were already synthesized (Fig. 8B). Taken together, these data suggest that the protein(s) involved in the reactivation of MPF during the M I/M II transition are synthesized during a very short period of 30-60 min after the extrusion of the first polar body.

We tried to identify the protein(s) synthesized specifically during this period by metabolic labeling with 35S-methionine. However, we observed the same pattern of protein synthesis in M I, M II and M III oocytes and during both transitional periods (data not shown). This suggests that this(these) protein(s) is(are) synthesized constitutively, or is(are) synthesized during the transition in a very low quantity, making its(there) detection impossible in such experiments.

Discussion

The cell cycle of oocytes undergoing meiosis is modified when compared with the mitotic cell cycle. During the transition between the first and the second meiotic division there is no nuclear formation and no DNA synthesis. After extrusion of the first polar body the oocyte enters a new phase M, metaphase II (M II) (Donahue, 1968). The mouse oocyte remains arrested in M II until fertilization. Premature activation (parthenogenetic activation or fertilization in vitro) results in abortive activation of M II oocytes leading to a new M phase, M III (Kubiak, 1989). This experimentally induced M II/M III transition seems to be similar to natural M I/M II transition. In this paper we compared these two transitions at the morphological (chromosomes, microtubules and MPM-2 antigen) and biochemical levels (histone H1 kinase activity, protein phosphorylation, requirement for protein synthesis).

M I/M II and M II/M III transitions are similar

We observed that the microtubules, chromosomes and antigens detected by the MPM-2 monoclonal antibody behave similarly during both transitions: the chromosomes remain condensed, no interphase network of microtubules is ever formed and a diffuse staining with MPM-2 is observed during both transitional periods. The relatively fast disassembly of the midbody microtubules (within 1.5 h after activation) may be responsible for the fragility of the link between the polar body and the metaphase arrested oocyte. Moreover, histone H1 kinase activity drops to the interphase level, phosphate incorporation into proteins increases and a 62 kDa phosphoprotein band disappears during both transitional periods. Finally, the re-entry into M phase is clearly dependent upon new protein(s) synthesis during the transitional period. On the basis of these observations we can conclude that the M II/M III transition faithfully mimics the M I/M II transition.

Peculiarities of the M II/M III transition

The chromosomes present in M III oocytes are not always well arranged within the spindle. This could be linked to the fact that the chromosomes consist of a single chromatid with a single kinetochore. Despite this, a bipolar spindle can still form (see Figs 1-3), without half-spindle fusion as observed in Xenopus egg extracts (Sawin and Mitchison, 1991). This observation demonstrates that paired kinetochores are not essential for spindle assembly but facilitate the correct positioning of the chromosomes on the metaphase plate. After activation of M III oocytes, a variable number of chromosomes (11-13) are extruded in the polar body, showing that it is not possible to control chromosome segregation in these conditions (Kubiak, 1989). We are currently studying the ultrastructure of such M III spindles using both the confocal and the electron microscope.

A short puromycin treatment (60-75 min) during the M I/M II and the M II/M III transitions resulted in the formation of interphase nuclei. However, in the first case, the nuclei were unstable and underwent condensation upon drug removal, while in the second (M II/M III) this treatment was sufficient to induce the formation of stable nuclei. This behavior supports the preceding observations that the capacity for activation of mouse oocytes develops during M II arrest (Kubiak, 1989). Clarke and Masui (1983) have shown that the treatment of M I and M II oocytes with puromycin resulted in the formation of interphase nuclei. In the first case, these nuclei were unstable and did not replicate DNA, while, in the second case, DNA replication took place and the oocytes entered the first cell cycle. However, activation of M II oocytes by inhibitors of protein synthesis requires, at least, a 6 h incubation in the presence of the drug (Clarke and Masui, 1983; Siracusa et al., 1978; and our unpublished observations), indicating that some long-lived proteins are involved in the maintenance of metaphase arrest. Our results show that, in young oocytes, a short inhibition of protein synthesis is sufficient to induce the transition to a normal interphase, when metaphase arrest is released by ethanol activation.

Differences between these transitions and the transition to interphase

The rapid disassembly of the midbody microtubules in M II and M III oocytes contrasts with the stability
of these structures in normally activated oocytes. de Pennart et al. (1988) and Schatten et al. (1988) demonstrated that the midbody microtubules in activated mouse oocytes are acetylated. Using the same antibodies (6-11B-1 (Piperno and Fuller, 1985) for acetylated microtubules and YL1/2 (Kilmartin et al., 1982) for tyrosinated microtubules) we have observed that the microtubules of the shrinking M III midbody are also acetylated (data not shown). This indicates that the midbodies formed after abortive activation are also composed of more-stable microtubules, but that their behavior is modified by the cytoplasmic conditions in the oocyte: entry into M-phase favors their disassembly, whereas they are very stable in interphase.

During the M-phase/M-phase transitions a short interphase takes place, as demonstrated by the drop in H1 kinase activity, but a characteristic interphase network of microtubules (composed of longer and more stable microtubules) is never observed. Verde et al. (1990) have shown that histone H1 kinase (or cdc2 kinase) activity induces a decreased stability of microtubules during M phase and that the lengthening of microtubules in interphase correlates with the inactivation of the cdc2 kinase. However, if the behavior of microtubules depends entirely on the activity of this major cell cycle kinase, we should observe the transient formation of an interphase network during the transitional period between two M phases. Since this was not the case, it is tempting to propose that the behavior of microtubules during the M-phase/M-phase transition is influenced by the activity(ies) of other intermediate kinase(s). The existence of such intermediate kinase(s) is also suggested by the increased incorporation of phosphate into proteins during the period of histone H1 kinase inactivation. At the same time, we observed the disappearance of a 62 kDa phosphoprotein. This may be linked with either the activation of some specific phosphorylates or, more probably, the degradation of this protein, since the molecular mass of 62 kDa corresponds to that of cyclin B in mouse oocytes (Weber et al., 1991). This is reinforced by our observations that protein synthesis is necessary for re-entry into the subsequent M phase during both the M I/M II and the M II/M III transitions, which suggests that the protein(s) whose synthesis is required are cyclins. It was recently shown by O'Keefe et al. (1991) that the synthesis of cyclin B takes place in oocytes undergoing the M I/M II transition and that the c-mos protooncogene product is necessary to stabilize the cyclin B synthesized during this transition. We are now studying in more detail the behavior of the components of MPF (p34^cd^ and cyclins) and of CSF (c-mos) to obtain information concerning the mechanism responsible for re-entry into M-phase. The M II/M III transition should be of great help in these biochemical studies, since it enables us to collect large numbers of synchronous oocytes.

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