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The metaphase II arrest in mouse oocytes is controlled through microtubule-dependent destruction of cyclin B in the presence of CSF

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In unfertilized eggs from vertebrates, the cell cycle is arrested in metaphase of the second meiotic division (metaphase II) until fertilization or activation. Maintenance of the long-term meiotic metaphase arrest requires mechanisms preventing the destruction of the maturation promoting factor (MPF) and the migration of the chromosomes. In frog oocytes, arrest in metaphase II (M II) is achieved by cytostatic factor (CSF) that stabilizes MPF, a heterodimer formed of cdc2 kinase and cyclin. At the metaphase/anaphase transition, a rapid proteolysis of cyclin is associated with MPF inactivation. In *Drosophila*, oocytes are arrested in metaphase I (M I); however, only mechanical forces generated by the chiasmata seem to prevent chromosome separation. Thus, entirely different mechanisms may be involved in the meiotic arrests in various species. We report here that in mouse oocytes a CSF-like activity is involved in the M II arrest (as observed in hybrids composed of fragments of metaphase II-arrested oocytes and activated mitotic mouse oocytes) and that the high activity of MPF is maintained through a continuous equilibrium between cyclin B synthesis and degradation. In addition, the presence of an intact metaphase spindle is required for cyclin B degradation. Finally, MPF activity is preferentially associated with the spindle after bisection of the oocyte. Taken together, these observations suggest that the mechanism maintaining the metaphase arrest in mouse oocytes involves an equilibrium between cyclin synthesis and degradation, probably controlled by CSF, and which is also dependent upon the three-dimensional organization of the spindle.

Key words: cyclin/cytostatic factor/maturation promoting factor/microtubule/mouse oocyte

Introduction

In unfertilized vertebrate eggs, the cell cycle is arrested in metaphase of the second meiotic division (metaphase II). An activity called cytostatic factor (CSF) has been demonstrated in amphibian oocytes (Masui and Markert, 1971; Masui, 1991). In metaphase II oocytes, CSF is active and maturation promoting factor (MPF) activity remains high. The nature of the interaction between MPF and CSF is not known. There is a growing body of information suggesting that either the c-*mos* kinase, or a complex including this kinase and cdk2 (cyclin-dependent kinase 2), might fulfil the role of CSF (Sagata *et al.*, 1989; Gabrielli *et al.*, 1993). MPF is destroyed at the exit from the metaphase arrest, but, unexpectedly, inactivation of CSF is not required for this transition (Lorca *et al.*, 1991; Watanabe *et al.*, 1991; Weber *et al.*, 1991). However, CSF activity does not regulate the metaphase arrest in all the organisms studied. In eggs from *Drosophila*, the metaphase I arrest appears to be controlled differently, through mechanical forces generated by the chiasmata that seem to prevent chromosome separation (McKim *et al.*, 1993). Mammalian oocytes, like amphibian oocytes, arrest at metaphase II, although it is not clear whether this arrest is maintained by CSF because this activity has not yet been demonstrated in mammalian oocytes. Since the synthesis of cyclin B, the regulatory component of MPF, is continued in metaphase-arrested mouse oocytes (Weber *et al.*, 1991) and the arrest can last for many hours, mechanisms must exist that maintain MPF activity at a steady level.

M-phase arrest can be induced in many eukaryotic cell types by microtubule depolymerizing drugs, leading to the idea that there is a checkpoint in the cell cycle at which proper spindle formation is monitored (reviewed in Hartwell and Weinert, 1989). Recently, genetic studies in the budding yeast have identified gene products required for this feedback control (Hoyt *et al.*, 1991; Li and Murray, 1991). We have examined the relationship between this checkpoint and the metaphase arrest in mouse oocytes. We have found that cyclin B turns over rapidly during the metaphase arrest, being both destroyed and synthesized, and that cyclin B degradation during the arrest requires the presence of microtubules. Furthermore, fusion of mitotic eggs with metaphase II-arrested oocytes illustrated the presence of a factor similar to CSF.

Results

A CSF-like activity is present in metaphase II-arrested oocytes

When activated eggs at first mitosis were fused with metaphase II-arrested mouse oocytes, we found that the mitotic eggs remained arrested in M-phase for at least 24 h. Conversely, mitotic activated eggs fused with another mitotic egg continued development and divided during the first few hours after fusion (Table I). This 'CSF-like' activity was observed even when mitotic eggs were fused with cytoplasts or karyoplasts derived from bisected metaphase II oocytes.

Cyclin B turns over during the metaphase arrest

We then measured the H1 kinase activity during the metaphase II arrest to check whether the MPF activity changed during this period. When this experiment was performed between 16 and 45 h after human chorionic gonadotrophin (hCG) treatment, we observed a steady level

Table I. CSF activity in metaphase II-arrested mouse oocytes

Types of hybrid	Number of hybrids arrested in M-phase/total number	
	After 6 h of culture	After 24 h of culture
Meiosis II + meiosis II	32/32 (100%)	15/15 (100%)
Mitosis I + mitosis I	0/17 (0%)	0/10 (0%)
Meiosis II + mitosis I	36/36 (100%)	19/19 (100%)
Nucleated half + mitosis I	28/29 (97%)	27/29 (93%)
Enucleated half + mitosis I	15/15 (100%)	14/15 (93%)

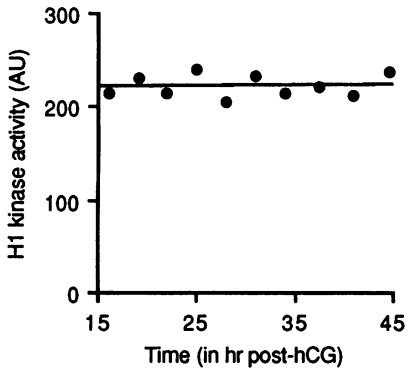

Fig. 1. Histone kinase activity during the metaphase II arrest in mouse oocytes. Groups of four oocytes were removed from culture between 15 and 45 h post-hCG, then lysed and assayed for histone H1 kinase activity. The line was traced using the linear least squares fit method (r^2 = 0.013).

of histone H1 kinase activity (Figure 1). Together, the relationships existing between cyclin concentration and cdc2 kinase activity (Clarke *et al.*, 1992), the steady level of H1 kinase activity in metaphase II-arrested oocytes and the fact that cyclin B can be labeled easily with [^{35}S]methionine during the same period (Weber *et al.*, 1991), suggested that cyclin B may turnover during the metaphase II arrest. By labeling newly synthesized proteins in metaphase II oocytes using [^{35}S]methionine and then immunoprecipitating cyclin B, we observed a single major band with a mol. wt of 62 kDa which was synthesized continuously between 15 and 19 h post-hCG (ovulation occurs at ~12–13 h post-hCG) (Figure 2a). This suggested that cyclin degradation occurs during the metaphase II arrest to maintain a constant protein level. To study cyclin B degradation, metaphase II-arrested oocytes were incubated for 1 h with [^{35}S]methionine and the newly synthesized proteins chased with cold methionine during the metaphase II arrest. A disappearance of cyclin B was observed during the 4 h chase period (Figure 2b). Treatment of the oocytes by nocodazole, a drug leading to the complete disappearance of microtubules in metaphase II-arrested oocytes (Maro *et al.*, 1986), inhibited cyclin B degradation (Figure 2b). Densitometry of the autoradiograms revealed that 58% of the protein was degraded after a 4 h chase period, whereas only 16% disappeared over the same period in the presence of nocodazole (Figure 2b′). Cyclin B degradation seems to be M-phase specific, since it was not observed in fertilized eggs (in late G_1/early S phase, 10–12 h post-fertilization), either in control medium or in the presence of nocodazole (Figure 2c). Thus, in mouse eggs (as in *Xenopus* oocytes; Xu *et al.*, 1992), cyclin B is much more stable during interphase than during M-phase. We also

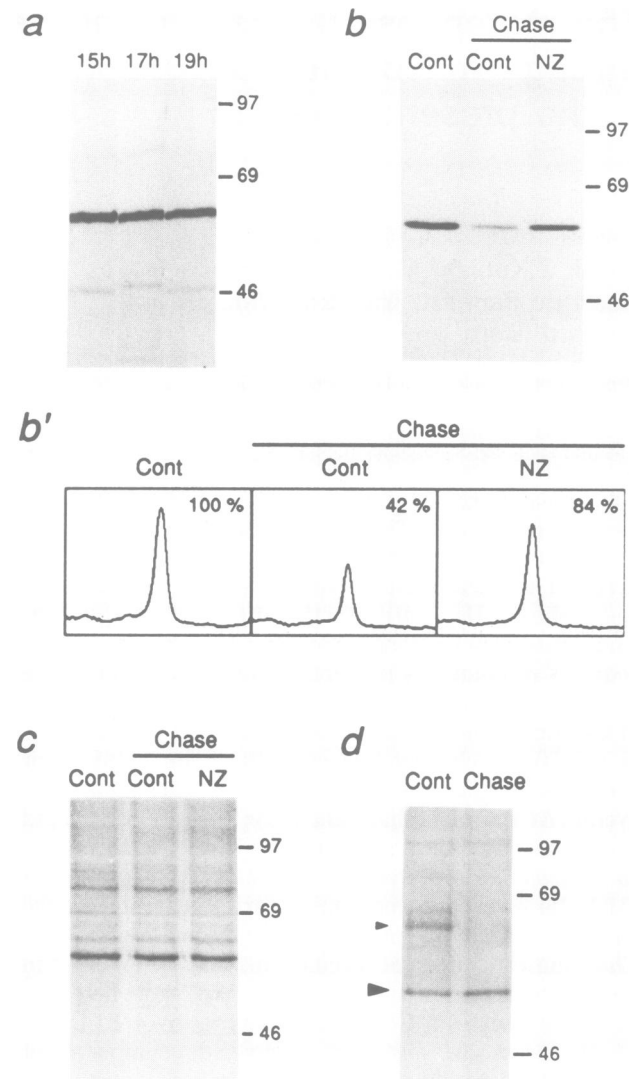

Fig. 2. Analysis of cyclin B turnover in mouse oocytes. **(a)** Continuous cyclin B synthesis during the meiotic metaphase II arrest. Groups of 675 oocytes were labeled for 1 h with [^{35}S]methionine and cyclin B was immunoprecipitated at the end of the labeling period at the times indicated (15, 17 and 19 h post-hCG). **(b)** Microtubule-dependent cyclin B degradation during the meiotic metaphase II arrest. Groups of 415 oocytes were labeled for 1 h with [^{35}S]methionine (Cont), washed in cold methionine and further incubated for 4 h in control medium (Chase, Cont) or in medium containing 10 μM nocodazole (Chase, NZ). **(b′)** Densitometry of the autoradiogram shown in b. **(c)** Cyclin B is not degraded in fertilized eggs. Groups of 210 fertilized eggs (10–12 h post-fertilization) were labeled for 1 h with [^{35}S]methionine (Cont), washed in cold methionine and further incubated for 4 h in control medium (Chase, Cont) or in medium containing 10 μM nocodazole (Chase, NZ). **(d)** β-Tubulin is not degraded during the meiotic metaphase II arrest. Groups of 206 oocytes were labeled for 1 h with [^{35}S]methionine (Cont), washed in cold methionine and further incubated for 4 h in control medium (Chase). Cyclin B and β-tubulin were immunoprecipitated together by using a mixture of the two specific antibodies. The positions on the gel of cyclin B (small arrowhead) and β-tubulin (large arrowhead) are indicated.

checked that this increase in protein turnover during M-phase was not a general feature of protein metabolism during the metaphase arrest by performing a chase experiment where both cyclin B and β-tubulin were immunoprecipitated. In contrast to cyclin B, β-tubulin was stable during the

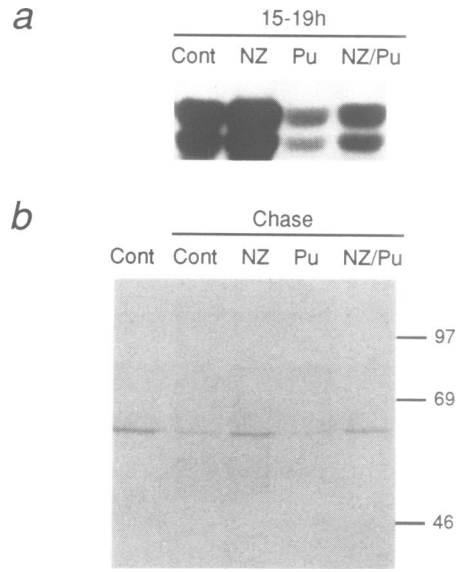

Fig. 3. Nocodazole increases histone H1 kinase activity and prevents cyclin B degradation in metaphase II-arrested mouse oocytes. (**a**) Groups of 40 oocytes, 15 h post-hCG, were cultured for 4 h in control medium (Cont), in medium containing 10 μM nocodazole (NZ), 20 μM puromycin (Pu), or both nocodazole and puromycin (NZ/Pu), then lysed and assayed for histone H1 kinase activity. Similar results were obtained using 2 and 6 h culture periods (not shown). (**b**) Groups of 275 oocytes were labeled for 1 h with [^{35}S]methionine from 13–14 h post-hCG (Cont), washed in cold methionine and further incubated for 4 h either in control medium (Chase, Cont), in medium containing 10 μM nocodazole (Chase, NZ), 20 μM puromycin (Chase, Pu), or both nocodazole and puromycin (Chase, NZ/Pu).

metaphase arrest, even in the absence of nocodazole (Figure 2d).

Cyclin concentration controls H1 kinase activity

To correlate changes in cyclin metabolism with changes in MPF activity, histone H1 kinase activity and cyclin B degradation were measured in parallel. Nocodazole induced an increase in histone H1 kinase activity that was abolished when the protein synthesis inhibitor puromycin was added to the culture medium (Figure 3a). This suggested that the nocodazole-induced rise was related to newly synthesized cyclin molecules. Inhibition of protein synthesis by puromycin alone induced a drop in histone kinase activity, probably corresponding to an absence of cyclin synthesis with a maintained destruction. These ideas were confirmed directly by immunoprecipitation of cyclin B after a pulse–chase experiment in the presence of nocodazole and/or puromycin. Cyclin B degradation was inhibited by nocodazole, either in the absence or presence of puromycin (Figure 3b). To eliminate the possibility that the effect of nocodazole on MPF activity was not related to the depolymerization of microtubules, we tested the impact of various microtubule inhibitors on H1 kinase activity. Treatment of the oocytes for 4 h with colcemid, griseofulvin or vinblastine also led to a rise in H1 kinase activity, identical to the one observed with nocodazole (Figure 4), thus demonstrating that the increase in H1 kinase activity was due to the absence of the spindle and not to a side effect of nocodazole or its solvent (see Materials and methods).

Mouse oocytes can be activated parthenogenetically by protein synthesis inhibitors (Siracusa *et al.*, 1978). To determine whether this activation is due to the microtubule-dependent degradation of cyclin B in the absence of cyclin B synthesis, oocytes were treated with both puromycin and nocodazole. Activation rates were measured by counting the number of oocytes containing a pronucleus. We observed that the puromycin-induced parthenogenetic activation could be inhibited by nocodazole (Figure 5), suggesting that oocyte activation by puromycin is related to the microtubule-dependent cyclin destruction.

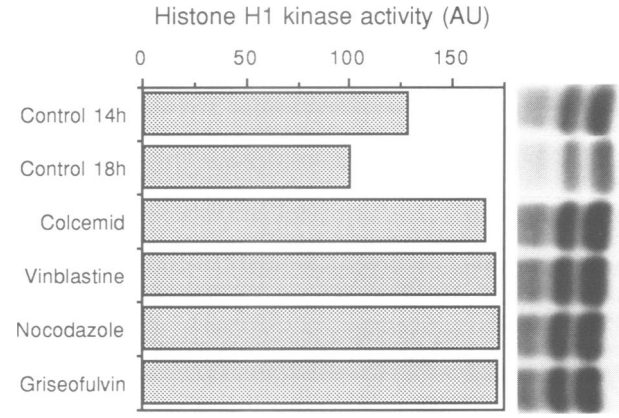

Fig. 4. Microtubule inhibitors increase histone H1 kinase activity. Groups of 40 oocytes were treated for 4 h (14–18 h post-hCG) with either 2×10^{-5} M colcemid, 5×10^{-6} M vinblastine, 10^{-5} M nocodazole or 2×10^{-4} M griseofulvin, lysed and assayed for histone H1 kinase activity. Immunofluorescence analysis showed that the solvents had no effect on the microtubule network and that spindle microtubules were depolymerized by all the drugs. Tubulin paracrystals were observed after vinblastine treatment.

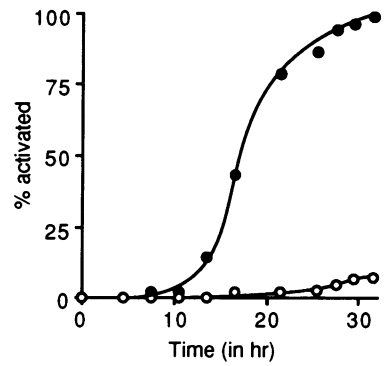

Fig. 5. Nocodazole inhibits activation of mouse oocytes by puromycin. Freshly ovulated mouse ocytes, 14 h post-hCG, were incubated continuously in 20 μM puromycin (●; n = 128) or in the presence of both 20 μM puromycin and 10 μM nocodazole (○; n = 124) for various times. They were scored for the presence of pronuclei (indicating that activation had taken place 5 h earlier).

H1 kinase activity is associated with the spindle

Our results show that cyclin destruction is somehow associated with the spindle in metaphase II-arrested oocytes. In other systems, an association between cyclin B and the spindle has been reported using immunofluorescence with anti-cyclin antibodies (Pines and Hunter, 1991; Bailly *et al.*, 1992; Ookata *et al.*, 1992). To test directly whether MPF itself is predominantly associated with the spindle, we bisected metaphase II-arrested mouse oocytes and assayed the two halves for histone H1 activity (Figure 6). There was a

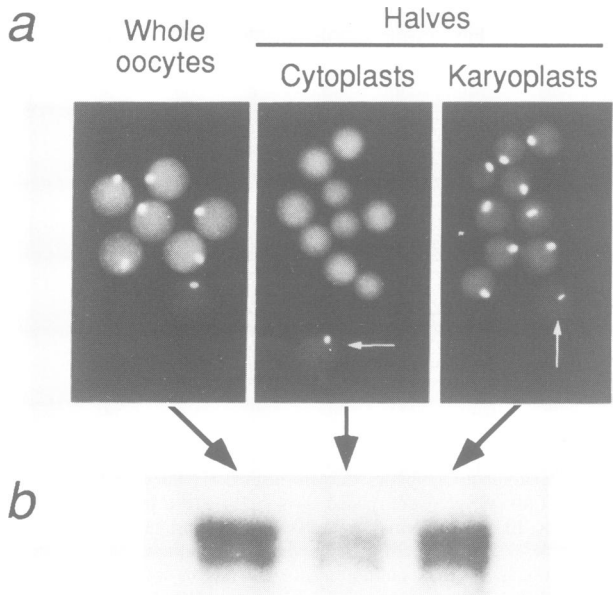

Fig. 6. Histone H1 kinase activity in whole oocytes, cytoplasts and karyoplasts. (**a**) Live whole and halved oocytes stained with Hoechst 33342 and observed under the fluorescence microscope. In the karyoplast and cytoplast groups, a whole oocyte has been included for comparison (white arrows). (**b**) Histone kinase assay performed using groups of 33 oocytes or 65 cytoplasts or karyoplasts. Photographs taken after the separation were used to estimate the volume of the cytoplasts and karyoplasts. In the experiment shown, the difference between the total volume of the two groups was ~1%. Bar = 100 μm.

marked difference in H1 kinase activity between the two halves, with most of the activity (Figure 6b) being found in the half containing the metaphase spindle (karyoplast; Figure 6a).

Discussion

Cyclin turnover in metaphase II-arrested mouse oocytes

The modulation of cyclin concentration by synthesis and degradation is of central importance in the control of MPF activity (Evans *et al.*, 1983; Draetta *et al.*, 1989; Murray and Kirschner, 1989; Murray *et al.*, 1989). Cyclins are degraded by the ubiquitin pathway (Glotzer *et al.*, 1991; Hershko *et al.*, 1991) and this degradation is triggered by MPF itself (Félix *et al.*, 1990).

Our data demonstrate that cyclin B turns over during the metaphase II arrest in mouse oocytes, being both destroyed and synthesized, and that cyclin B degradation during the arrest requires the presence of intact microtubules. In eggs from the marine invertebrate *Patella*, it has been suggested that the metaphase I arrest is also controlled through cyclin turnover, but in the absence of CSF (van Loon *et al.*, 1991). However, in these experiments cyclin degradation during the arrest was induced by the protein synthesis inhibitor used for the chase (P.Colas, personal communication). In colchicine-arrested and KCl-activated clam embryos, cyclin was shown to accumulate in M-phase. Moreover, the rapid proteolysis of cyclin and exit from M-phase required the presence of a mitotic spindle (Minshull *et al.*, 1989; Hunt *et al.*, 1992) showing that an intact spindle is required to induce the rapid cyclin degradation that takes place at the metaphase–anaphase transition. However, these obervations do not give any indication about the role of the spindle in the degradation mechanism, i.e. whether it is involved in the transmission of the degradation signal or in the actual degradation machinery. Our observations suggest that microtubule-depolymerizing drugs are able to arrest the cell cycle once the cyclin degradation machinery has been turned on by inhibiting the cyclin degradation mechanism itself. However, this spindle checkpoint is not present in all vertebrate eggs since, for example in *Xenopus* eggs, microtubule depolymerizing drugs are not able to arrest the cell cycle (Gerhart *et al.*, 1984; Clute and Masui, 1992).

CSF-like activity in metaphase II-arrested oocytes

Until now, the presence of CSF activity has not been demonstrated in mouse oocytes arrested at metaphase of the second meiotic division. We have performed fusion experiments which demonstrate the presence of an activity similar to CSF. We did not perform injection experiments, as used with *Xenopus* oocytes, because mouse oocytes activate very easily under these conditions and it is impossible to inject a large amount of cytoplasm into these very fragile eggs. Our data show that the small amount of cytoplasm in oocyte fragments contain enough of the CSF-like activity to arrest the cell cycle in M-phase (Table I). In addition, the c-*mos* protein, the catalytic component of CSF (Sagata *et al.*, 1989), is present in mouse oocytes and is not destroyed until after the exit from the metaphase II arrest (Weber *et al.*, 1991), as in *Xenopus* eggs (Lorca *et al.*, 1991; Watanabe *et al.*, 1991). In *Xenopus*, the product of the c-*mos* proto-oncogene is stable and has CSF activity when phosphorylated on Ser3 (Nishizawa *et al.*, 1992). This is the case during the metaphase II arrest, but not during the preceding steps of maturation (Nishizawa *et al.*, 1992; Xu *et al.*, 1992). In mouse oocytes, the c-*mos* gene product is also required for cyclin B accumulation (O'Keefe *et al.*, 1991).

Mechanisms involved in the metaphase II arrest in mouse oocytes

We have shown that the metaphase II arrest is maintained for several hours throughout the continuous balanced synthesis and degradation of cyclin (Figure 7a). The equilibrium between these two processes is probably dependent to some extent upon CSF. Roy *et al.* (1990) have demonstrated that the c-*mos* kinase is able to phosphorylate cyclin B *in vitro*, and this may make cyclin degradation more difficult. Alternatively, c-*mos* could inhibit directly the degradation machinery.

Mouse oocytes fertilized in the presence of nocodazole remain in M-phase as long as they are kept in the drug (Maro *et al.*, 1986), indicating that cyclin degradation induced by oocyte activation is also dependent upon the presence of microtubules. This suggests that the same degradation machinery may be used both during the metaphase arrest and after oocyte activation (Figure 7b). However, we cannot rule out that two different mechanisms are used. In *Xenopus* eggs, a Ca^{2+}–calmodulin-dependent event releases the cyclin degradation pathway from its 'CSF-inhibited state' at fertilization (Lorca *et al.*, 1991). Moreover, in the budding yeast, a mutant that is insensitive to microtubule disruption is altered in a gene coding for a calcium-binding protein (Li and Murray, 1991). It has been proposed that this MAD gene product is involved in the coupling between calcium

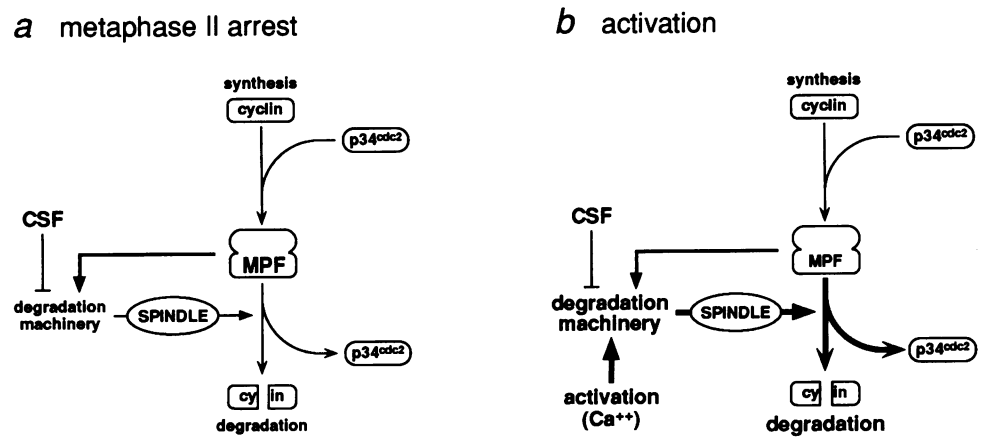

Fig. 7. A model of the proposed control of the metaphase arrest in mouse oocytes. (**a**) CSF-induced metaphase arrest; (**b**) release of the metaphase arrest by activation. Arrows = activation/stimulation (degree represented by the thickness of the line); bars = inhibition.

transients and the exit from mitosis by inactivation of inhibitors of cell cycle progression (Li and Murray, 1991).

Components of MPF (Bailly *et al.*, 1989, 1992; Pines and Hunter, 1991; Ookata *et al.*, 1992), the kinase activity itself (this paper) and c-*mos* (Zhou *et al.*, 1991) are all associated with the metaphase spindle. Our results suggest that the cyclin degradation machinery is somehow also linked to the spindle (microtubules and/or chromosomes). The observation that the formation of pronuclei takes place only in enucleated fragments of metaphase II mouse oocytes fertilized in the presence of colcemid, but not in the nucleated fragments (Borsuk and Manka, 1988), suggests that the chromosomes are also involved in this machinery. Both cyclin stabilization and degradation may thus be controlled by the three-dimensional organization of the spindle, a very dynamic structure in metaphase II-arrested mouse oocytes (de Pennart *et al.*, 1988). Cyclin degradation may normally be triggered in the oocyte once spindle formation has been completed correctly. In the budding yeast, it has been proposed that the function of the BUB gene product may be to prevent cyclin degadation when microtubule function is compromised (Hoyt *et al.*, 1991). However, in the mouse oocyte, because of the presence of CSF, cyclin destruction is slowed down and the disappearing cyclin can be replaced by newly synthesized protein. This triggering of cyclin destruction is similar to that during mitotic metaphase, where the destruction of cyclin is complete and the transition to anaphase takes place. As yet, the cyclin degradation machinery has not been identified. Biochemical characterization of the proteolytic activities associated with the spindle may provide a useful complement to genetic studies for the identification of this machinery.

Materials and methods

Recovery of oocytes

Swiss female mice [8–11 weeks old; Animalerie Spécialisée de Villejuif, Centre National de la Recherche Scientifique (CNRS), France] were superovulated by injection of 5 IU of pregnant mare's serum gonadotrophin and hCG 48 h apart. Ovulated oocytes were obtained by puncturing the ampullae of oviducts between 12 and 18 h post-hCG in 150 IU/ml hyaluronidase dissolved in phosphate-buffered saline. Fertilized eggs were obtained after mating of the females with Swiss males and were recovered from the oviducts 25–27 h post-hCG. We chose to use activated eggs for the early events (polar body extrusion and formation of the pronuclei) because this technique allows a better synchronization of the samples.

Oocyte activation

Oocytes were activated 18 h post-hCG according to the method of Cuthberson (1983) by a 6.5 min exposure to freshly prepared 8% ethanol (Merck) in M2 medium containing 4 mg/ml bovine serum albumin (BSA) (Whittingham, 1971; Goodall and Maro, 1986). The alcohol was removed by washing the oocytes carefully in three large drops of M2. Cells were cultured in T6 medium containing 4 mg/ml BSA (T6 + BSA) (Howlett *et al.*, 1987), under paraffin oil at 37°C with 5% CO_2 in air.

Oocyte bisection

Small groups of oocytes were bisected manually (Czolowska *et al.*, 1986). They were then stained with Hoechst to check under the fluorescence microscope for the presence of a metaphase plate of chromosomes (Kubiak *et al.*, 1991), photographed and either frozen in water for histone H1 kinase assay or used for fusion experiments. The halves were collected <30 min after bisection.

Electrofusion

Activated oocytes at the first mitotic stage (12–14 h post-activation) were agglutinated with either whole oocytes or oocyte halves (12–14 h post-hCG) in phytohemagglutinin (PHA; Sigma) diluted in M2 without BSA at a final concentration of 400 μg/ml for 5 min on 1% agarose at 37°C. Aggregated pairs were washed in 0.25 M glucose made in bidistilled water supplemented with 100 μg/ml BSA and electrofused in this solution according to the technique described previously (Kubiak and Tarkowski, 1985). Two electrofusion protocols were used, consisting of either two direct current pulses of 120 V, 100 μs duration with a 100 μs interval and a distance of 300 μm between the electrodes, or two direct current pulses of 50 V, 25 μs duration with a 25 μs interval and a distance of 120 μm from a Pulse Generator II (Brodowicz co., Warsaw, Poland). The pairs were transferred to drops of T6 + BSA under liquid paraffin at 37°C. They were examined 6 and 24 h post-fusion, and then fixed for immunocytochemical staining.

Histone H1 kinase activity

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 $MgCl_2$, 1 mM dithiothreitol (DTT), 1 mM phenylmethylsulfonyl fluoride (PMSF), 10 μg/ml leupeptin, 10 μg/ml pepstatin, 10 μg/ml aprotinin] using exogenous histone H1 (HIII-S from calf thymus, Sigma) as substrate. Samples containing the oocytes in M2 + BSA were lysed by freezing and thawing three times, diluted twice in two times concentrated HK buffer and incubated for 15 min at 20°C in the presence of 3.3 mg/ml histone H1, 1 mM ATP and 0.25 mCi/ml [^{32}P]ATP (Kubiak *et al.*, 1991). The reaction was stopped by the addition of a similar volume of two times 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). In order to test the specificity of the reaction, the p34^{cdc2} kinase was removed by centrifugation from a metaphase II control sample using p13^{suc1}-coated Sepharose beads (not shown).

Immunoprecipitation

Oocytes were labeled for 1 h with 500 μCi/ml of [^{35}S]methionine (sp. act. 1000 Ci/mM; SJ 1015 Amersham) in M2 + BSA, then washed three times in M2 containing cold methionine. Oocytes were collected in M2 + PVP

and frozen at −70°C. Samples were resuspended in RIPA buffer and cyclin B or β-tubulin was immunoprecipitated using either an antibody directed against cyclin B (Pines and Hunter, 1989) or a monoclonal antibody directed against β-tubulin (Amersham). Immunoprecipitates were lysed in SDS sample buffer and analyzed using a 10% SDS−polyacrylamide gel (Laemmli, 1970).

Drugs

Nocodazole and griseofulvin were purchased from Aldrich, and colcemid and vinblastine from Sigma. Stock solutions were at a concentration of 10^{-2} M in dimethylsulfoxide (DMSO) for nocodazole, 2×10^{-1} M in dimethylformamide (DMF) for griseofulvin, 2×10^{-2} M in methanol for colcemid and 5×10^{-3} M in methanol for vinblastine. All these stocks were diluted 1000 times in M2 + BSA for the experiments. We checked that DMSO, DMF and methanol had no effect on the microtubule network at the doses used, and that spindle microtubules were depolymerized by all drugs (in addition, tubulin paracrystals were observed after vinblastine treatment).

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