Kinetics of MPF and histone H1 kinase activity differ during the G2- to M-phase transition in mouse oocytes

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ABSTRACT   Maturation promoting factor (MPF) is universally recognized as the biological entity responsible for driving the cell cycle from G_2- to M-phase. Histone H1 kinase activity is widely accepted as a biochemical indicator of $p34^{cdc2}$ protein kinase complex activity and therefore MPF activity. In this paper we present results which indicate that during the G_2- to M-phase transition in mouse oocytes the dynamic of $p34^{cdc2}$ related histone H1 kinase activity differs markedly from the biological activity of MPF as measured by classical cell fusion procedures. MPF is activated just before germinal vesicle breakdown (GVBD) whereas histone H1 kinase is activated 5-7 h later coincident with the formation of the definitive first metaphase plate. The biological activity of MPF is merely reduced to about 50% of control levels by a short period of protein synthesis inhibition (1-2 h) and completely suppressed after a prolonged period of inhibition (4-5 h). By contrast, inhibition of protein synthesis in mouse oocytes results in a rapid and complete suppression of histone H1 kinase activity. Therefore, biological MPF and histone H1 kinase activity should not be used in an interchangeable manner during the G_2- to M-phase transition in mouse oocytes.

KEY WORDS: *mouse, oocyte, maturation promoting factor, histone H1 kinase*

Introduction

MPF is universally recognized as the biological entity responsible for driving the cell cycle from the G_2- to M-phase (Dorée, 1990; Maller, 1990; Nurse, 1990; Meikrantz and Schlegel, 1992) as measured by cytoplasmic injection or cell fusion (biological MPF). During meiosis, MPF activity appears shortly before GVBD and fluctuates thereafter in accord with the cell cycle; activity is high at metaphase I (MI) and II (MII) and low during anaphase I (AI) and telophase I (TI) (Masui and Markert, 1971; Kishimoto and Kanatani, 1976; Dorée et al., 1983; Gerhart et al., 1984; Fulka et al., 1992). In yeast, marine invertebrates and amphibia histone H1 kinase activity parallels that of biological MPF (Cicirelli et al., 1988) and has become the accepted biochemical indicator of MPF activity (Arion et al., 1988; Dunphy et al., 1988; Gautier et al., 1988; Meijer et al., 1989).

In vivo fully grown mouse oocytes are arrested at the G_2- to M-phase border and normally resume meiosis in response to a gonadotrophic signal. Resumption of meiosis also occurs when oocytes are removed from follicles and cultured in vitro. GVBD occurs within 1 to 2 h after release from the follicle and the first metaphase plate is formed 6-7 h later. G_2-phase arrest can, however, be maintained in vitro by the addition of dibutyryl cyclic AMP (dbcAMP) to the medium (Cho et al., 1974).

To test the hypothesis that histone H1 kinase activity is a biochemical indicator of biological MPF activity in mammalian oocytes, as it is in marine invertebrate and amphibian oocytes, we determined both activities in mouse oocytes in parallel experiments. We have utilized the dbcAMP maintained germinal vesicle (GV) stage arrest of mouse oocytes in the measurement of the biological activity of MPF by assessing the capacity of maturing oocytes to induce membrane breakdown and chromatin condensation in GV-arrested reporter oocytes (Balakier, 1978). To ascertain whether the dynamics of histone H1 kinase activity correspond with that of biologically active MPF, single mouse oocytes were analyzed using histone H1 as the exogenous substrate and protein kinase inhibitor peptide to block cyclic AMP-dependent protein kinase (Arion et al., 1988; Fulka et al., 1992). The specificity of histone H1 kinase activity as a measure for the activity of the $p34^{cdc2}$ protein kinase complex was shown by $p13^{suc1+}$-precipitation of the kinase activity. In this paper we present results which indicate that the dynamics of histone H1 kinase activity in mouse oocytes differs

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TABLE 1

**INDUCTION OF GVBD AND CHROMATIN CONDENSATION AFTER FUSION OF AN IMMATURE GV
REPORTER OOCYTE TO A MATURING MOUSE OOCYTE**

	Treatment		n	Induced changes in the morphology of the reporter GV nucleus		
	of the maturing oocyte before cell fusion	of the oocyte hybrid after cell fusion (60-90 min)		GV	GVBD and chromatin ondensation	
					partial	complete
Group A	1-2 h control	dbcAMP	22			100%
Group B	4-5 h control	dbcAMP	22			100%
Group C	1-2 h cycloheximide	dbcAMP+ cycloheximide	86	51%	28%	21%
Group D	4-5 h cycloheximide	dbcAMP+ cycloheximide	73	92%	5%	3%
Group E	1-2 h control	dbcAMP+ cycloheximide	51		6%	94%
Group F	1-2 h cycloheximide	dbcAMP+ cycloheximide and 1 h dbcAMP	25			100%

Maturing mouse oocytes in control and cycloheximide-containing (100 µg/ml) medium underwent GVBD and chromatin condensation within 1 h after release from the follicle. One maturing mouse oocyte was fused to one immature GV-stage reporter oocyte to determine the MPF activity of the maturing oocyte as described in Materials and Methods. After fusion the oocyte hybrids were cultured for 60-90 min in dbcAMP (150 µg/ml) or dbcAMP and cycloheximide (100 µg/ml) containing medium. The oocyte hybrids in group F were cultured for 60-90 min in dbcAMP and cycloheximide containing medium and for a second consecutive 1 h period in only dbcAMP containing medium. All oocyte hybrids contained one set of condensed chromatin originating from the maturing test oocyte. Only induced morphological changes observed in the reporter nucleus are listed in this Table. Details of the various treatments used in groups A to F are described fully in the "Results" section. "n" stands for the number of oocytes listed.

markedly from that of MPF during the G_2- to M-phase transition and conclude therefore that the two activities should not be used in an interchangeable manner.

Results

MPF activity during mouse oocyte maturation

As outlined previously, biological MPF activity was assessed by measuring the capacity of oocytes at different stages of maturation to induce nuclear membrane breakdown and chromatin condensation when fused to a dbcAMP arrested immature fusion partner. Our results presented in Table 1 support those of Hashimoto and Kishimoto (1988) who used a starfish assay system for the analysis of MPF activity. The data show that MPF activity appeared at the time of GVBD and reached high levels within the first hour after breakdown of the nuclear membrane (Group A and B). In oocyte hybrids in which test oocytes were matured for 1-2 h or 4-5 h, 100% of reporter fusion partners had undergone nuclear membrane breakdown and chromatin condensation at the end of the post-fusion period. Mouse oocytes maintain their high level of MPF activity throughout MI stage, decrease it during AI/TI transition and raise it again at the TI to MII border (Fulka *et al.*, 1992).

Histone H1 kinase activity during mouse oocyte maturation

In order to determine whether the increase of MPF activity during the G_2- to M-phase transition in mouse oocytes is accompanied by a parallel increase in histone H1 kinase activity, we analyzed the histone H1 kinase activity in single mouse oocytes in an *in vitro* assay system (Fulka *et al.*, 1992). To verify the specificity of the histone H1 kinase assay system as a measure for the activity of the p34[cdc2] protein kinase complex phosphorylation activity was determined in unextracted oocyte homogenates, in p13[suc1+]-precipitated

and supernatant fractions. As shown in Fig. 1, the histone H1 kinase activity in unextracted homogenates (lanes 5,6,11, and 12) of mouse oocytes is nearly completely precipitable by p13[suc1+]-Sepharose (lanes 1,2,7, and 8). This was assayed in GV (lanes 1 to 6) and MI oocytes (lanes 7 to 12), respectively. We conclude from these results that the histone H1 kinase activities measured in our experiments reflect the activities of the p34[cdc2] protein kinase complex in mouse oocytes during maturation.

In contradistinction to the results in lower species our results show that the dynamics of histone H1 kinase activity in mouse oocytes during GVBD differs markedly from that of MPF. It is evident from Fig. 2 that the levels of H1 kinase activity at GVBD as measured in single oocytes are low and do not differ significantly from those in G_2-arrested oocytes. The first significant increase in H1 kinase activity occurred after 6 h, only 5 after GVBD, and maximal levels were not recorded until after 8 h or 7 h post GVBD.

To exclude the possibility of a kinase inhibitory activity in oocytes before or immediately after GVBD (1 h) single GV oocytes or oocytes immediately after GVBD were homogenized with single MI oocytes (8 h) and histone H1 kinase activities were determined. The measured activities in both groups were not different from the simple additive activities of the individual oocytes (data not shown). These results indicate that there is no obvious histone H1 kinase inhibitory activity in mouse oocytes immediately before or after GVBD.

Thus, while the biological activity of MPF reaches a maximum within one hour of nuclear membrane breakdown the activation of histone H1 kinase lags 5-7 h behind that of the biological MPF activity required to induce GVBD. It is interesting that these two apparently disparate activities become synchronized thereafter with correspondingly low levels during the AI/TI transition and a parallel rise at the TI to MII border (Fulka *et al.*, 1992).
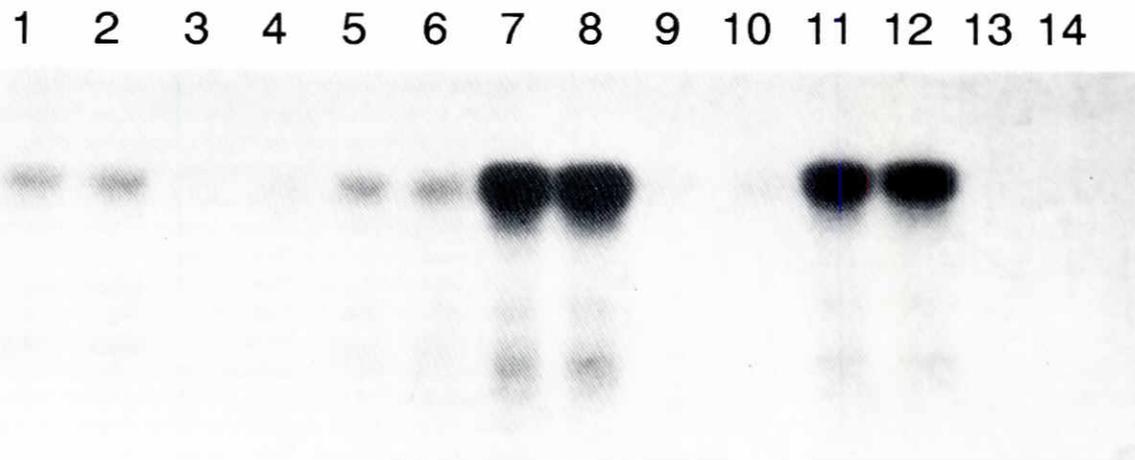
Fig. 1. Histone H1 kinase activity in mouse oocytes can be precipitated with p13^{suc1+}-Sepharose. *Oocytes were released from large antral follicles and matured in vitro. After 0 h (GV stage, **lanes 1 to 6**) or 8 h (MI stage, **lanes 7 to 12**) in vitro oocytes were homogenized and the p34^{cdc2} protein kinase complex was precipitated with p13^{suc1+}-Sepharose. Histone H1 kinase activity was measured in unextracted oocyte homogenates (lanes 5,6,11, and 12), p13^{suc1+}-precipitated (lanes 1,2,7, and 8) and supernatant fractions (lanes 3,4,9, and 10). As a control for unspecific non-enzymatic labeling of histone H1 during the assay complete kinase reactions were performed without oocytes* **(lanes 13 and 14)**.

Protein synthesis dependence of MPF during mouse oocyte maturation

Having established that the dynamics of biological MPF and histone H1 kinase activities in mouse oocytes are entirely different during the crucial G$_2$- to M-phase transition, we next evaluated the need for protein synthesis in the activation of biological MPF and H1 kinase function, respectively. In accord with previous reports, protein synthesis inhibition by cycloheximide did not block GVBD or chromatin condensation in cultured mouse oocytes but instead arrested meiosis before spindle formation and the formation of the definitive metaphase I plate (Wassarman et al., 1976; Hashimoto and Kishimoto, 1988). To determine MPF activity after protein synthesis inhibition, GV-stage reporter oocytes in dbcAMP-containing medium were fused to cycloheximide-treated mouse oocytes obtained at 1-2 h (Table 1, Group C) or 4-5 h (Group D) after explantation, i.e. 0-1 h or 3-4 h after GVBD, respectively. The fused hybrids were cultured thereafter in dbcAMP to prevent spontaneous meiotic progression in the GV-reporter partner. After cycloheximide inhibition, 21% of the immature reporter oocytes in Group C (1-2 h cycloheximide) and 3% in Group D (4-5 h cycloheximide) had similarly undergone full GVBD including complete nuclear membrane disassembly and chromatin condensation (Fig. 3a). Of the remainder, 28% in Group C and 5% in Group D showed partial membrane breakdown and some chromatin condensation (Fig. 3b) leaving 51% and 92%, respectively, arrested in the GV-stage (Fig 3c). Thus, residual MPF activity persists in oocytes whose protein synthesis has been inhibited for 1-2 h, whereas almost no MPF activity exists in oocyte cultured for 4-5 h in cycloheximide. Removal of the cycloheximide-induced protein synthesis block led to the acquisition of maximum MPF activity within 1 h (Table 1, Group E and F).

Protein synthesis dependence of histone H1 kinase activity during mouse oocyte maturation

Fig. 1 shows the effect of protein synthesis inhibition on histone H1-kinase activity. From these results it is clear that the activity of histone H1 kinase does not increase above basal levels in oocytes whose protein synthesis is inhibited by cycloheximide. After release from a 3 h period of cycloheximide inhibition (100 µg/ml), histone H1 kinase activity increases 5 to 7 h thereafter (data not shown) to attain the same maximal levels as those reported for control oocytes (see Fig. 1). Thus, the inhibition of protein synthesis in mouse oocytes results in a rapid and complete suppression of histone H1 kinase activity. After the resumption of protein synthesis, histone H1 kinase activity increases with a slow dynamic similar to that of the normally maturing oocyte. By contrast, MPF activity is merely reduced by a short period of protein synthesis inhibition and is only completely suppressed after a prolonged period of inhibition. On the other hand, its recovery is very rapid and within 1 h of release from protein synthesis inhibition maximal MPF activity is restored (see Table 1, Group F).

Discussion

The p34^{cdc2} kinase is found in all eukaryotes and controls onset of M-phase (Nurse, 1990). p34^{cdc2} phosphorylates histone H1 *in vitro* (Arion et al., 1988; Brizuela et al., 1989; Labbé et al., 1989; Meijer et al., 1989; Pines and Hunter, 1989; Reed and Wittenberg, 1990). The identification of p34^{cdc2} and B-type cyclins (Gautier et al., 1988, 1990; Labbé et al., 1989) as the principle components of highly purified MPF (Lohka et al., 1988; Labbé et al., 1989) provides biochemical evidence that MPF is a histone H1 kinase that acts as an M-phase inducer. Therefore histone H1 kinase activity is widely accepted as a biochemical indicator of MPF activity. The p34^{cdc2} protein kinase complex is specifically precipitated by the yeast gene product p13^{suc1+} (Meijer et al., 1989).

In this paper we present data showing first that the activation of biological MPF and histone H1 kinase in mouse oocytes occurs with markedly different kinetics during the G$_2$- to M-phase transition. Our results confirm an earlier report (Hashimoto and Kishimoto, 1988) using marine invertebrate oocytes as reporter cells to assess MPF activity of mouse oocytes. High MPF activities are recorded immediately before or at GVBD. This is in accordance with the finding that p34^{cdc2} is dephosphorylated within the first 2 h of oocyte maturation
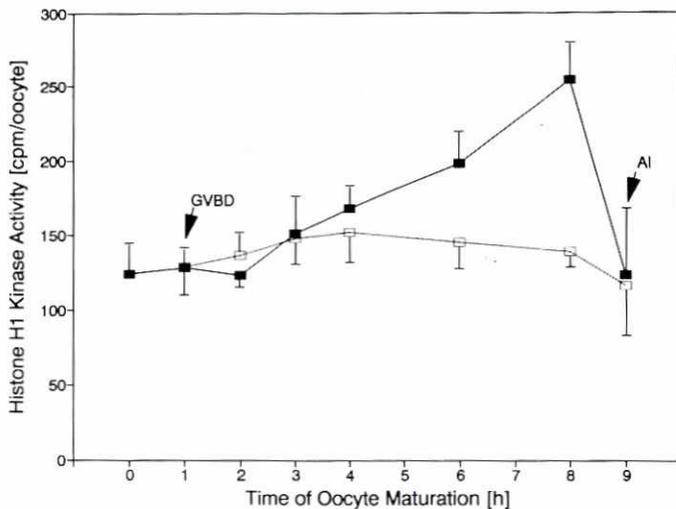
Fig. 2. Time course of histone H1 kinase activation during mouse oocyte maturation. *Fully grown mouse oocytes were removed from their antral follicles and cultured in vitro in the absence (filled symbols) or presence of cycloheximide (100 µg/ml) (open symbols). Kinase activity was determined in single mouse oocytes using histone H1 as the exogenous substrate. Data (x± SEM) are corrected for unspecific non-enzymatic labeling of histone H1 and for background and are shown in cpm [³²P] incorporated into histone H1 during a 30 min incubation by single mouse oocytes. Experiments were performed four times with 2 oocytes per time and treatment group in each experiment. Arrows marked GVBD or AI show the time of GVBD in control and cycloheximide-treated oocytes or the time of anaphase I in control oocytes, respectively.*

in the mouse and that the phosphorylation state thereafter is unchanged up to the metaphase II stage (Choi *et al.*, 1991). However, activation of histone H1 kinase lags behind GVBD and therefore the activation of MPF by 5-7 h. The measured H1 kinase activity in mouse oocytes is clearly related to the p34^{cdc2} protein kinase complex as shown by p13^{suc1+}-precipitation (Fig. 1). A similar slow increase in histone H1 kinase activity in p34^{cdc2}-immunopreciptates during mouse oocyte maturation was shown by Choi and co-workers (1991).

In Table 1 we present evidence that low MPF activity is detected in oocytes whose protein synthesis has been inhibited for 1-2 h. However, almost no MPF activity remains after block of protein synthesis for 4-5 h. Removal of the block leads to acquisition of high MPF activity within 1 h. These findings are in contrast to those of an earlier report (Hashimoto and Kishimoto, 1988) in which MPF activity was detected in mouse oocytes treated with cycloheximide for up to 8 h. However, the MPF assay itself was done with starfish oocytes as reporter cells and in the absence of cycloheximide. Our results using a homologous assay system with a continual inhibition of protein synthesis clearly show that high MPF activities are restored within 1 h after release from inhibition (Table 1, Groups E and F). The MPF assays described in both reports lasted for about 1 h. We conclude therefore that the inhibition of MPF activity in cultured mouse oocytes as measured in an homologous system is dependent upon continual suppression of new protein synthesis.

Histone H1 kinase activity in mouse oocytes is completely dependent on protein synthesis (Fig. 2). However, release from a protein synthesis block restores the ability of oocytes to activate the M-phase associated protein kinase with a similar dynamic than in

control oocytes, i.e. a significant increase is detectable 5-7 h after release. This indicates that histone H1 kinase activity in mouse oocytes and biological MPF activity are completely dependent on protein synthesis. However, the difference in the dynamics of the activities between the two might reside in differing half lifes of their active and/or activating principles. We conclude therefore that the two activities should not be used in an interchangeable manner.

We interpret the results presented in this paper as showing first that the activation of MPF and histone H1 kinase in mouse oocytes occur with markedly different kinetics during the G$_2$- to M-phase transition. In this species MPF activity has a close temporal relationship to nuclear membrane breakdown while the formation of the definitive first metaphase plate is associated both with high MPF and histone H1 kinase activity. As to whether either of these activities is causally related to chromatin condensation and bivalent formation is unproven. That a direct causal relationship may indeed not exist is suggested by evidence from pig oocytes and somatic cells. Our results from experiments on porcine oocytes show that both condensation and pairing of chromosomes occur before any increase in either MPF or histone H1 kinase activity is detectable (Christmann *et al.*, 1993). Indeed, it is well established that chromatin condensation in this species occurs in the absence of new protein synthesis even though both MPF and histone H1 kinase activity are abolished by the addition of inhibitors of protein synthesis (Moor *et al.*, 1992). In experiments on isolated somatic cell nuclei, Peter *et al.* (1990) reported that the addition of activated p34^{cdc2} kinase induced little or no chromatin condensation and more recently cAMP-dependent protein kinase has been shown to be one of the kinases responsible for chromatin condensation in human fibroblast cells (Lamb *et al.*, 1991). Taken together, we postulate that the intracellular regulator of membrane disassembly and that of the metaphase I plate formation differ. Second, our present results show that histone H1 kinase activity cannot be used as a measure of the biological activity of MPF in mouse oocytes.

Materials and Methods

Oocyte collection and culture

Mouse oocytes were released from large antral follicles of PMSG-stimulated F1 CFLP a/a females and cultured in MEM containing pyruvate (0.22 mM), Gentamycin (25 µg/ml) and bovine serum albumin (3 mg/ml) at 37°C in an atmosphere of 5%CO$_2$ in air (Fulka *et al.*, 1992). Oocytes were collected hourly from a single pool and used for histone H1 kinase activity assays (see below). For cell fusion experiments one partner (reporter oocyte) was maintained in the GV-stage of meiosis by continuous culture in dbcAMP (150 µg/ml, Cho *et al.*, 1974). The second partner (test oocyte) was matured for periods ranging from 0-9 h in either the absence or presence of cycloheximide (100 µg/ml). Details of the various reporter-test oocyte fusion combinations are described fully in the Results section.

Cell fusion assay for biological MPF assay

In order to determine MPF activity during oocyte maturation in the mouse, maturing oocytes were fused to GV-arrested mouse reporter oocytes. MPF activity was assessed as the capacity to induce membrane breakdown and chromatin condensation in the immature reporter. Immediately before fusion zonae pellucidae were removed by pronase treatment (0.5%). Contact between the oocyte partners was achieved in PBS containing phytohemagglutinin (PHA, 300 µg/ml) using a narrow-bore pipette. Fusion was induced by polyethylene glycol (M$_r$ 1000) as described earlier (Fulka, 1985). After fusion the oocyte hybrids were cultured for 60-90 min in dbcAMP (150 µg/ml) or dbcAMP and cycloheximide (100 µg/ml) supplemented medium. Thereafter the oocyte hybrids were fixed and stained.
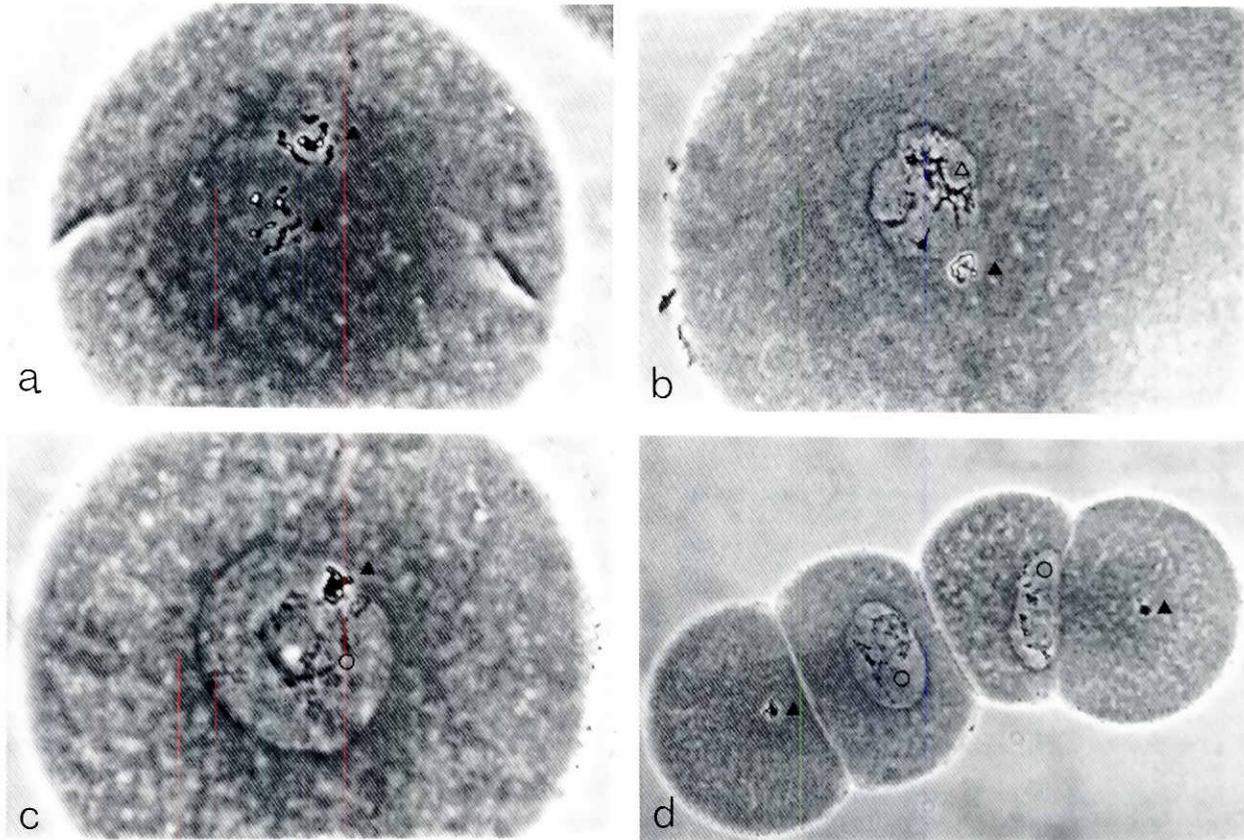
Fig. 3. Changes in nuclear morphology after fusion of a maturing mouse oocyte to a reporter GV-stage oocyte. *Oocytes were treated as described in Table 1. The following changes in nuclear morphology were recorded. (a) Induction of complete GVBD and chromatin condensation. These oocyte hybrids contain two sets of condensed chromatin (▲), one originating from the maturing oocyte and the other from the reporter GV-nucleus. This configuration indicates high levels of MPF activity in the maturing oocyte. (b) Induction of partial GVBD and chromatin condensation. Beside one set of condensed chromatin (▲) inherited from the maturing oocyte, there are the remnants of a GV-nucleus with condensed chromatin within the collapsed membrane (△), indicating intermediate levels of MPF activity. (c) Unchanged GV-nucleus in an oocyte hybrid. The intact GV-nucleus (○) from the immature reporter oocyte persists beside a set of condensed chromatin (▲) originating from the maturing mouse oocyte, indicating either very low or no MPF activity in the maturing oocyte. (d) Unfused mouse oocytes. In all unfused oocytes a similar pattern of chromatin configuration has been observed. Maturing oocytes contained condensed chromatin (▲), while immature reporter oocytes showed an intact GV-nucleus (○).*

Control experiments were done with electric field (1.5 kV, 50 μsec, 3 pulses) induced cell fusion. Results with both cell fusion systems were identical.

After fusion oocytes were mounted on slides, fixed in aceto-ethanol (1:3), stained with aceto-orcein (45% acetic acid, 1% orcein) and evaluated under phase-contrast.

Histone H1 kinase assay

Single mouse oocytes were homogenized and kinase reactions were performed as described (Fulka *et al.*, 1992). In short, the kinase reactions were done in 45 mM ß-glycerophosphate, 12 mM p-nitrophenylphosphate, 20 mM MOPS-KOH (pH 7.2), 12 mM $MgCl_2$, 12 mM EGTA, 0.1 mM EDTA, 0.8 mM DTT, 2.3 mM Na_3VO_4, 2 mM NaF, 0.8 mM PMSF, 15 μg/ml leupeptin, 30 μg/ml aprotinin, 1 mg/ml PVA, 1 mg/ml histone H1, 2.2 μM PKI (TTYANFIASGRTGRRNAIHN) and 0.2 μM [γ-^{32}P]-ATP (37 TBq/mmol) for 30 min at 37°C. The reaction mixtures were subjected to SDS-PAGE, the bands corresponding to histone H1 were excised and incorporated. [^{32}P] was measured by liquid scintillation counting. The data given in Fig. 3 have been corrected for unspecific non-enzymatic labeling of histone H1 and for background. Unspecific non-enzymatic labeling was measured by perform-

ing the complete kinase reaction without oocytes. All experiments were repeated four times with 2 oocytes per time and treatment group. An analysis of variance demonstrates that the levels of histone H1 kinase activity at 6 and 8 h are significantly greater ($p \leq 0.05$ or $p \leq 0.01$, respectively) than those at 0, 1 and 2 h.

Relative low concentrations of ATP were used in the histone H1 kinase assay to avoid unspecific non-enzymatic phosphorylation of H1 (Abdel-Ghany *et al.*, 1988). To verify the specificity of the histone H1 kinase assay as a measure for the activity of the p34^{cdc2} protein kinase complex phosphorylation activity was determined after p13^{suc1+}-precipitation as described by Meijer *et al.* (1989). Oocytes (n= 40) were homogenized and precipitation was done with 2 μl p13^{suc1+}-Sepharose bead suspension (1:1) in 100 μl bead buffer (50 mM Tris-HCl, pH 7.4, 5 mM NaF, 250 mM NaCl, 5 mM EDTA, 5 mM EGTA, 0.1% NP-40, 10 μg/ml leupeptin, 10 μg/ml aprotinin, 100 μM benzamidine and 100 μM PMSF) for 1 h. Unextracted oocyte homogenate, supernatant and precipitate (after three times washing with bead buffer) were collected and H1 kinase activity was determined as described for single mouse oocytes. All chemicals used in these experiments were purchased from Sigma and the ^{32}P-labeled compound from ICN.

References

ABDEL-GHANY, M., RADEN, D., RACKER, E. and KATCHALSKI-KATZIR, E. (1988). Phosphorylation of synthetic random polypeptides by protein kinase P and other protein-serine (threonine) kinases and stimulation or inhibition of kinase activities by microbial toxins. *Proc. Natl. Acad. Sci. USA 85:* 1408-1411.

ARION, D., MEIJER, L., BRIZUELA, L. and BEACH, D. (1988). cdc2 is a component of the M phase-specific histone H1 kinase: evidence for identity with MPF. *Cell 55:* 371-378.

BALAKIER, H. (1978). Induction of maturation in small oocytes from sexually immature mice by fusion with meiotic or mitotic cells. *Exp. Cell Res. 112:* 137-141.

BRIZUELA, L., DRAETTA, G. and BEACH, D. (1989). Activation of human CDC2 protein as a histone H1 kinase is associated with complex formation with the p62 subunit. *Proc. Natl. Acad. Sci. USA 86:* 4362-4366.

CHO, W.K., STERN, S. and BIGGERS, J.D. (1974). Inhibitory effect of dibutyryl cAMP on mouse oocyte maturation *in vitro. J. Exp. Zool. 187:* 383-386.

CHOI, T., AOKI, F., MORI, M., YAMASHITA, M., NAGAHAMA, Y. and KOHMOTO, K. (1991). Activation of p34^{cdc2} protein kinase activity in meiotic and mitotic cell cycles in mouse oocytes and embryos. *Development 113:* 789-795.

CHRISTMANN, L., JUNG, T. and MOOR, R.M. (1994). MPF components and meiotic competence in growing pig oocytes. *Mol. Reprod. Dev.* (In press).

CICIRELLI, M.F., PELECH, S.L. and KREBS, E.G. (1988). Activation of multiple kinases during the burst in protein phosphorylation that precedes the first meiotic division in *Xenopus* oocytes. *J. Biol. Chem. 263:* 2009-2019.

DORÉE, M. (1990). Control of M-phase by maturation-promoting factor. *Curr. Opin. Cell Biol. 2:* 269-273.

DORÉE, M., PEAUCELLIER, G. and PICARD, A. (1983). Activity of maturation-promoting factor and the extent of protein phosphorylation oscillate simultaneously during meiotic maturation of starfish oocytes. *Dev. Biol. 99:* 489-501.

DUNPHY, W.G., BRIZUELA, L., BEACH, D. and NEWPORT, J. (1988). The *Xenopus* cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell 54:* 423-431.

FULKA, J., Jr. (1985). Maturation-inhibiting activity in growing mouse oocytes. *Cell Differ. 17:* 45-48.

FULKA, J., Jr., JUNG, T. and MOOR, R.M. (1992). The fall of biological maturation promoting factor (MPF) and histone H1 kinase activity during anaphase and telophase in mouse oocytes. *Mol. Reprod. Dev. 32:* 378-382.

GAUTIER, J., MINSHULL, J., LOHKA, M., GLOTZER, M., HUNT, T. and MALLER, J.L. (1990). Cyclin is a component of maturation-promoting factor from *Xenopus. Cell 60:* 487-494.

GAUTIER, J., NORBURY, C., LOHKA, M., NURSE, P. and MALLER, J. (1988). Purified maturation-promoting factor contains the product of a *Xenopus* homolog of the fission yeast cell cycle control gene cdc2+. *Cell 54:* 433-439.

GERHART, J., WU, M. and KIRSCHNER, M. (1984). Cell cycle dynamics of an M-phase-specific cytoplasmic factor in *Xenopus laevis* oocytes and eggs. *J. Cell Biol. 98:* 1247-1255.

HASHIMOTO, N. and KISHIMOTO, T. (1988). Regulation of meiotic metaphase by a cytoplasmic maturation-promoting factor during mouse oocyte maturation. *Dev. Biol. 126:* 242-252.

KISHIMOTO, T. and KANATANI, H. (1976). Cytoplasmic factor responsible for germinal vesicle breakdown and meiotic maturation of starfish oocyte. *Nature 260:* 321-322.

LABBÉ, J.C., CAPONY, J.P., CAPUT, D., CAVADORE, J.C., DERANCOURT, J., KAGHAD, M., LELIAS, J.M., PICARD, A. and DORÉE, M. (1989). MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cyclin B. *EMBO J. 8:* 3053-3058.

LAMB, N.J.C., CARADORE, J.C., LABBÉ, J.C., MAURER, R.A. and FERNANDEZ, A. (1991). Inhibition of cAMP-dependent protein kinase plays a key role in the induction of mitosis and nuclear envelope breakdown in mammalian cells. *EMBO J. 10:* 1523-1533.

LOHKA, M.J., HAYES, M.K. and MALLER, J.L. (1988). Purification of maturation-promoting factor, an intracellular regulator of early mitotic events. *Proc. Natl. Acad. Sci. USA 85:* 3009-3013.

MALLER, J.L. (1990). *Xenopus* oocytes and the biochemistry of cell division. *Biochemistry 29:* 3157-3166.

MASUI, Y. and MARKERT, C.L. (1971). Cytoplasmic control of nuclear behaviour during meiotic maturation of frog oocytes. *J. Exp. Zool. 177:* 129-146.

MEIJER, L., ARION, D., GOLDSTEYN, R., PINES, J., BRIZUELA, L., HUNT, T. and BEACH, D. (1989). Cyclin is a component of the sea urchin egg M-phase specific histone H1 kinase. *EMBO J. 8:* 2275-2282.

MEIKRANTZ, W. and SCHLEGEL, R.A. (1992). M-phase-promoting factor activation. *J. Cell Sci. 101:* 475-481.

MOOR, R.M., JUNG, T., MIYANO, T. and BARKER, P. (1993). Cyclin and p34^{cdc2} kinase activity in pig oocytes during the G_2- to M-phase transition. In *Gonadal Development and Function* Vol. 94 (Ed. S.G. Hillier). Serono Symposium Publications, Raven Press, pp. 85-98.

NURSE, P. (1990). Universal control mechanism regulating onset of M-phase. *Nature 344:* 503-508.

PETER, M., NAKAGAWA, J., DORÉE, M., LABBÉ, J.C. and NIGG, E.A. (1990). *In vitro* disassembly of the nuclear lamina and M-phase specific phosphorylation of lamins by cdc2 kinase. *Cell 61:* 591-602.

PINES, J. and HUNTER, T. (1989). Isolation of a human cyclin cDNA: evidence for cyclin mRNA and protein regulation in the cell cycle and for interaction with p34^{cdc2}. *Cell 58:* 833-846.

REED, S.I. and WITTENBERG, C. (1990). Mitotic role for the cdc28 protein kinase of *Saccharomyces cerevisiae. Proc. Natl. Acad. Sci. USA 87:* 5697-5701.

WASSARMAN, P.M., JOSEFOWICZ, W.J. and LETOURNEAU, G.E. (1976). Meiotic maturation of mouse oocytes *in vitro:* inhibition of maturation at specific stages of nuclear progression. *J. Cell Sci. 22:* 531-545.