Histone H1 kinase activity, germinal vesicle breakdown and M phase entry in mouse oocytes

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SUMMARY

Meiotic reinitiation of the mouse oocyte is caracterized by a slow entry into metaphase I, beginning with germinal vesicle breakdown and ending with spindle formation. It is accompanied by a cascade of protein kinases and phosphatases increasing protein phosphorylation. The activation of histone H1 kinase and that of the mitogen-activated protein kinase p42 have been compared during spontaneous or okadaic acid-induced meiotic reinitiation.

In spontaneously maturing oocytes, histone H1 kinase activity increases before germinal vesicle breakdown (2-fold), in a protein synthesis-independent manner. It is associated with the disappearance of the upper migrating form of $p34^{cdc2}$, which, in our system, seems to represent the tyrosine phosphorylated form. Following germinal vesicle breakdown, histone H1 kinase activity culminates (8-fold) in metaphase I and requires protein synthesis. Activation by phosphorylation of $p42^{MAPK}$ is observed as a permanent shift upward-migrating form and by its myelin basic protein kinase activity. It occurs after germinal vesicle breakdown and depends on protein synthesis.

In contrast, no increase of histone H1 kinase is detectable in oocytes induced to reinitiate meiosis by a transient inhibition of okadaic acid-sensitive phosphatase(s), either before germinal vesicle breakdown or during the following 7 hours of culture. A slight increase is nevertheless evident after 17 hours, when oocytes are arrested with an abnormal metaphase I spindle. The upper migrating form of $p34^{cdc2}$ is present for 8 hours. The activation of $p42^{MAPK}$ begins before germinal vesicle breakdown. Furthermore, when okadaic acid is applied to oocytes that have had meiosis reinitiation prevented by $p13^{suc1}$ microinjection, neither increase of histone H1 kinase activity nor $p34^{cdc2}$ dephosphorylation is observed although germinal vesicle breakdown is induced; $p42^{MAPK}$ is activated.

Altogether these results suggest that germinal vesicle breakdown may or may not be associated with a detectable activation of histone H1 kinase, depending on the experimental conditions. Activation of $p34^{cdc2}$ and $p42^{MAPK}$ are separable events. The effect of okadaic acid on mouse oocyte maturation is positive and negative. It induces meiotic events (germinal vesicle breakdown) independently of histone H1 kinase activation, perhaps through premature activation of $p42^{MAPK}$. It interferes negatively with the meiotic post-germinal vesicle breakdown events, most probably through mechanisms inhibiting protein synthesis and preventing cell cycle progression. We propose that, in the mouse oocyte, okadaic acid might be able to activate an alternative pathway leading to germinal vesicle breakdown that is cdc2-independent and that involves $p42^{MAPK}$ activation ensuing maturation promoting factor-independent phosphorylations.

Key words: mouse oocyte, okadaic acid, cell cycle control, maturation-promoting factor, MAP kinase

INTRODUCTION

At the end of oogenesis, oocytes arrest in prophase of meiosis at the G_2/M border. The fully grown mouse oocyte is capable of resuming meiosis either in vivo, under the favorable effect of gonadotrophins, or spontaneously in vitro, when it is released from its follicular environment. The reinitiation of meiosis is manifested by the appearance of a cytoplasmic activity called maturation promoting factor (MPF), which leads to germinal vesicle breakdown (GVBD) and entry into metaphase I. In mammals, the course of meiotic resumption seems to be different from that in *Xenopus* and starfish oocytes: entry into metaphase I proceeds particularly slowly (7-8 hours).

The meiotic cell cycle progression is regulated by a network of kinases and phosphatases. Among the proteins coded by cell-cycle-controlling genes is the MPF, which is active in M phase of meiotic and mitotic cells of all species (Dorée, 1990; Masui and Markert, 1971; reviewed by Nurse, 1990). This factor is known to be a heterodimer of a B-type cyclin and the

$p34^{cdc2}$ kinase subunit that undergoes an association-dissociation cycle involving phosphorylations and dephosphorylations according to a well-documented pattern (reviewed by Lewin, 1990). Its activity oscillates during the cell cycle, it is monitored by its histone H1 kinase activity and correlates with the initiation and maintenance of the M phase (Arion et al., 1988; Gautier et al., 1988; reviewed by Hunt, 1989; Labbé et al., 1989b; Lohka, 1989; Murray, 1993; Pines and Hunter, 1990). The burst of H1 kinase activity is generated by the cyclin abundance cycle (Murray et al., 1989; Murray and Kirchner, 1989; Solomon et al., 1990). Meiotically arrested oocytes are known to contain a stockpile of cyclin B already complexed with cdc2 (Meijer et al., 1989; Minshull et al., 1991; Westendorf et al., 1989). This complex is kept inactive (pre-MPF) by inhibitory phosphorylations (Atherton-Fessler et al., 1993; Cyert and Kirschner, 1988; Gautier and Maller, 1991; Morla et al., 1989; Norbury et al., 1991; Pondaven et al., 1990) on the residues corresponding to tyrosine 15 and threonine 14 of cdc2 (Krek and Nigg, 1991). Dephosphorylation of cdc2 is carried out when the oocyte reinitiates meiosis before germinal vesicle breakdown, through a homolog of the fission yeast phosphatase cdc25 (Clarke et al., 1993; Devault et al., 1991; Dunphy and Kumagai, 1991; Gautier et al., 1991; Izumi et al., 1992; Kumagai and Dunphy, 1992; Lee et al., 1992; reviewed by Millar and Russell, 1992; Strausfeld et al., 1991). Although cdc2 dephosphorylation is thought to proceed via an autocatalytic amplification loop involving cdc25 (Hoffmann et al., 1993; Jessus and Beach, 1992; Labbé et al., 1991) it is not clear what triggers this loop during oocyte maturation, and whether it is triggered during okadaic acid (OA)-induced meiosis. Another protein implicated in the regulation of $p34^{cdc2}$ is a 13 kDa protein (p13) encoded by the $suc1^{+}$ gene (Brizuela et al., 1987; Hayles et al., 1986; Hindley et al., 1987). When microinjected into oocytes, it inhibits MPF activation preventing meiotic maturation (GVBD), presumably by interfering with $p34^{cdc2}$ activation (Dunphy et al., 1988; Dunphy and Newport, 1989; Jessus et al., 1990; Picard et al., 1990). The presence of MPF always correlates with a high level of protein phosphorylation and with the presence of a group of characteristic mitosis-specific phosphoproteins detected by a monoclonal antibody MPM-2 (Kuang et al., 1989). This antibody recognizes a discrete set of polypeptides that are synthesized during interphase and phosphorylated during the G_2/M transition (Davies et al., 1983).

It is not yet known whether activation of cdc25 is the only trigger for turning on MPF. Other candidates include mos and microtubule-associated protein kinase (MAPK). In fully grown mouse oocytes (germinal vesicle (GV) stage oocytes), the mos protein is present and is involved in the cascade of events leading to meiotic reinitiation (Paules et al., 1989; Zhao et al., 1991). MAP kinases are known to be activated during hormone-induced meiotic maturation of frog and starfish oocytes (Ferrell et al., 1991; Haccard et al., 1990; Posada and Cooper, 1992; Sanghera et al., 1991), during fertilization of clam oocytes (Shibuya et al., 1992) and during mouse oocyte maturation (Sobajima et al., 1993; Verlhac et al., 1993). $p44^{MAPK}$ and $p42^{MAPK}$ pre-exist in the oocyte and are activated through phosphorylation. It has been reported that, in *Xenopus* oocyte extracts, purified MPF can activate MAP kinase (Nebreda and Hunt, 1993), whereas in clam oocytes MAP kinase is activated shortly before the major burst of MPF

activity (Shibuya et al., 1992). The *mos* protooncogene protein kinase turns on and maintains the activity of MAP kinase through stimulation of a physiological MAP kinase kinase (Posada et al., 1993). A number of proteins are substrates for both $p34^{cdc2}$ and MAP kinases. MAP kinases indeed phosphorylate nuclear lamins and display sequence specificity overlapping that of the mitotic protein kinase $p34^{cdc2}$ (Peter et al., 1992). $p42^{MAPK}$ phosphorylates in vitro the microtubule-associated protein 2 (MAP-2) (Ray and Sturgill, 1987).

In the mouse oocyte, the function of $p34^{cdc2}$ kinase, the critical regulator of mitosis, has not yet been directly linked to GVBD. We examined, therefore, the changes in the histone H1 kinase activity and $p34^{cdc2}$ phosphorylation in relation to $p42^{MAPK}$ activation and MPM-2 antigens expression, during spontaneous or okadaic acid (OA)-induced meiotic reinitiation. OA is an inhibitor of phosphatases 1 and 2A (Bialojan and Takai, 1988; reviewed by Cohen et al., 1990), which has been reported to induce meiotic reinitiation in frog, starfish, mouse, pig and cattle oocytes (Alexandre et al., 1991; Gavin et al., 1991; Goris et al., 1989; Kalous et al., 1993; Picard et al., 1989; Picard et al., 1991; Pondaven et al., 1989; Rime and Ozon, 1990; Schwartz and Schultz, 1991). This phosphatases inhibitor induces a premature mitosis-like state in BHK cells with activation of cdc2/H1 kinase (Yamashita et al., 1990). Transient applications of OA induce the formation of an abnormal spindle of meiotic metaphase I in mouse oocytes (Gavin et al., 1991), as well as in LCC-PK cells, where they induce a mitotic metaphase (Vandré and Wills, 1992). OA is furthermore reported to induce (hyper)phosphorylation of many proteins in different systems and to interfere sometimes with protein synthesis (Redpath and Proud, 1989). It is also known to activate $p42^{MAPK}$ (Casillas et al., 1993; Gotoh et al., 1990; Miyasaka et al., 1990; Sobajima et al., 1993).

In this study, we show that GVBD is not necessarily associated with an increase in histone H1 kinase activity. OA, although inducing GVBD and metaphase I entry, does not increase histone H1 kinase activity, at least for many hours following GVBD, contrary to what has been reported in other systems. We and others (Sobajima et al., 1993; Verlhac et al., 1993) showed that $p42^{MAPK}$ is involved in the regulation of mouse oocyte maturation before or immediately after GVBD, depending on the meiotic inducing conditions. In OA-treated oocytes, although histone H1 kinase activity is not increased, $p42^{MAPK}$ is activated before GVBD. Studying the biochemical events that underlie the activity of OA in mouse oocytes, we can show that it takes a pathway that is independent of $p34^{cdc2}$, bypassing its $p13^{suc1}$ inhibition, and that leads to $p42^{MAPK}$ activation.

MATERIALS AND METHODS

Oocyte collection and microinjection

Fully grown, meiotically competent oocytes were harvested from ovaries of 20- to 28-day-old randomly bred Swiss albino mice. The follicles were punctured with fine forceps in minimal essential medium (MEM; Earle's salts) supplemented with pyruvate (100 µg/ml), polyvinylpyrrolidone (PVP; 3 mg/ml), and 10 mM Hepes, pH 7.2 (MEM/PVP); the medium also contained the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine (IBMX) (200 µM) to prevent the resumption of meiosis (Cho et al., 1974). Oocytes were freed of

attached follicular cells. They were cultured in a drop of medium, at 37°C, in a humidified atmosphere of 5% CO_2 in air, under paraffin oil. GVBD was scored by examination with an Olympus stereomicroscope at 100-fold magnification.

Pressure microinjection into zona-intact oocytes was performed as described previously (Gavin et al., 1991), using a 1 μm diameter micropipette (BB-CH Mecanex) and a Nikon Diaphot inverted microscope equipped with Nomarski optics (300-fold magnification). Typical injection volume was 5-20 pl, corresponding to 2.5-10% of total oocyte volume.

p13[suc1] was expressed in an *Escherichia coli* host and purified as described elsewhere (Labbé et al., 1989a).

Treatment with okadaic acid or cycloheximide

Since in mammals, meiotic reinitiation is spontaneous in vitro, meiotic arrest has to be maintained by adding either membrane-permeable analogs of cyclic AMP (cAMP) or inhibitors of phosphodiesterase (PDE) to the culture medium (Cho et al., 1974). Okadaic acid (OA) was used to bypass the PDE inhibition in mouse oocytes.

A stock solution of 10 mM OA was made in dimethyl sulfoxide (DMSO). OA was added to the medium at a final concentration of 2.5 μM. In most experiments, oocytes, collected as described above, were transferred into OA-containing medium and cultured for 60 minutes (unless otherwise noted). Thereafter they were washed and transferred into OA-free, IBMX-containing medium for the remaining culture period. Such a brief treatment induced meiotic reinitiation (GVBD) and enabled spindle formation (Gavin et al., 1992). In some experiments, the time of incubation in OA lasted for up to 3 hours as referred to in the text, a treatment that is known to prevent spindle formation (Gavin et al., 1992).

Cycloheximide was directly dissolved in the culture medium at a final concentration of 100 μg/ml. This protein synthesis inhibitor was present during the whole incubation time.

Histone H1 and myelin basic protein (MBP) kinases assays

Forty-five oocytes were denuded from their zona pellucida by a brief exposure to 0.5% Pronase in MEM/PVP. They were washed three times in MEM/PVP and once in homogenization buffer (HB) containing 60 mM β-glycerophosphate, 15 mM *p*-nitrophenylphosphate, 15 mM $MgCl_2$, 0.1 mM EDTA, 15 mM EGTA, 1 mM DTT, 0.1 mM sodium orthovanadate, 20 μg/ml leupeptin, 10 μg/ml aprotinin, 10 μg/ml pepstatin, 10 μg/ml chymostatin, 2 mM phenylmethylsulfonyl fluoride (PMSF) and 25 mM Hepes, pH 7.2. They were finally lysed in 10 μl HB. The reaction was started by the addition of 10 μl of a solution, containing 10 mM $MgCl_2$, 20 μM cAMP-dependent protein kinase inhibitor (PKI), 0.2 mM ATP, 20 μg/ml each of pepstatin, chymostatin, aprotinin, 40 μg/ml leupeptin, 0.4 mM sodium-orthovanadate, 5 μCi of [^{32}P]ATP (Amersham, 10 mCi/ml) and 5 μg histone H1 (Boehringer Mannheim) and/or 10 μg MBP (Sigma). Reaction was performed at 37°C for 20 minutes and stopped by boiling for 3 minutes after the addition of 20 μl of 2-fold concentrated Laemmli sample buffer (Laemmli, 1970). The samples were subjected to 12 or 15% SDS-PAGE. The gels were stained with Coomassie Brilliant Blue, and dried on filter paper. The bands corresponding to histone H1 were excised from the dried gel. Radioactivity was measured by liquid scintillation counting. Histone H1 kinase activity was normalized as % versus control GV oocytes (100%).

Immunoblotting

A hundred oocytes were lysed in 10 μl of lysis buffer (Izumi et al., 1992) and 10 μl of 2-fold concentrated Laemmli sample buffer was added immediatly. After boiling for 3 minutes, the samples were subjected to 8 or 15% SDS-PAGE as referred to in the text. After transfer to a nitrocellulose membrane, MPM-2 antigens, MAP kinases and p34[cdc2] were detected by immunoblotting with an anti-MPM-2 monoclonal antibody (generously supplied by P. Rao), a monoclonal

anti-ERK1/2 antibody (no. 691, Santa Cruz Biotechnology, Inc.) and a polyclonal anti-PSTAIRE antibody (Santa Cruz Biotechnology, Inc.), respectively, using a Blotting Chemiluminescence Detection Kit (Amersham, UK) according to the manufacturer's instructions.

RESULTS

Meiotic reinitiation triggers changes in histone H1 kinase activity and in the expression of MPM-2 antigens

As shown in Fig. 1, during spontaneous meiotic reinitiation, histone H1 kinase activity increases faintly (2- to 3-fold) up to GVBD, rises dramatically (8-fold) up to metaphase I (8 hours), drops at 9 hours and increases again (metaphase II). Under our conditions, 81% (1351/1659) of the oocytes lose their germinal vesicle within 1-2 hours and 95% (286/301) of them extrude their polar body at 10-11 hours. These results obtained on crude extracts of mouse oocytes are similar to those reported by Choi et al. (1991) using immunoprecipitated fractions of oocytes obtained by a cdc2 C-terminal peptide antibody. Therefrom, we assume that the histone H1 kinase activities here reported can be mostly ascribed to cdc2.

During OA-induced meiotic reinitiation, histone H1 kinase level does not increase for several hours (7 hours), unless slightly (3-fold) following 17 hours of culture. Under our conditions 78% (731/933) of the oocytes lose their germinal vesicle by 2 hours and only 6% (5/90) extrude their polar body by 17 hours. This unexpected result shows that GVBD may appear, in mouse oocytes, independently of histone H1 kinase activation.

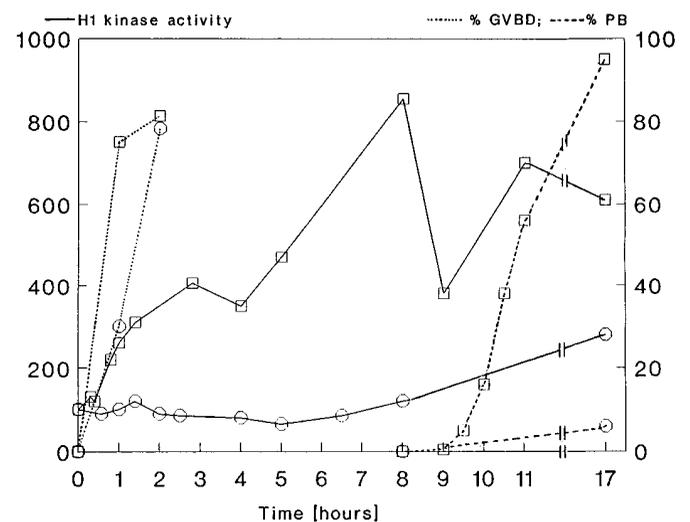OA was then applied on oocytes spontaneously reinitiating

Fig. 1. Changes in histone H1 kinase activity during the meiotic cell cycle. Oocytes bathed in IBMX-containing medium were induced to reinitiate meiosis either by transfer to IBMX-free medium (spontaneous maturation) or by 1 hour exposure to OA and further cultured in the presence of IBMX only. Time course of GVBD (··), first polar body extrusion (---) and histone H1 kinase activity (−) following spontaneous (□) or OA-induced (○) meiotic reinitiation. For each point, 45 oocytes were lysed and examined for histone H1 kinase activity as described in Materials and Methods.

meiosis (bathed in IBMX-free medium). As shown in Fig. 2, it does not interfere with the increase of histone H1 kinase activity associated with GVBD during spontaneous meiotic resumption, but prevents its subsequent rise accompanying entry into metaphase. This leads to the hypothesis that the continuous rise of histone H1 kinase activity, following GVBD, is positively regulated by an OA-sensitive phosphatase. Therefore, we analyzed the effects of the incubation time in OA (1, 2 or 3 hours) on histone H1 kinase activities, following 20 hours of culture. As shown on Fig. 3, histone H1 kinase activity is reduced with the length of OA treatments. Again, these results argue for OA-sensitive phosphatases positively involved in the cell cycle progression after GVBD. Whether metaphase entry or spindle assembly requires histone H1 kinase activation has not yet been demonstrated. In conclusion, OA exerts pleiotropic activities during the progression towards metaphase; it induces GVBD and interferes with further steps implicated in spindle formation.

Expression of MPM-2 antigens is usually associated with M phase and with MPF activation (Kuang et al., 1989). Their expression was analyzed in correlation with histone H1 kinase activity. Fig. 4 shows that GV-oocytes express few MPM-2 antigens when histone H1 kinase activity is low. Following spontaneous meiotic resumption, numerous MPM-2 antigens begin to be expressed around the time of GVBD (2 hours), whose full expression is reached only at metaphase I. During the metaphase I to II transition, MPM-2 antigens are expressed at a high level, although histone H1 kinase activity drops at 9 hours (data not shown).

The expression of MPM-2 antigens in OA-treated oocytes confirms that OA is inducing mitosis-specific phenomena. It exhibits the same profile as mentioned for spontaneously maturing oocytes. Nevertheless the premature appearance of most antigens and the level of stronger intensity of a protein around 65 kDa have to be noted. Although histone H1 kinase

activity is not increased, MPM-2 antigens are continuously expressed.

Activation of p34^{cdc2} and of p42^{MAPK} are separable events during meiotic reinitiation

Disassembly of the nuclear lamina in vivo might implicate p34^{cdc2} (Dessev et al., 1991; Lüscher et al., 1991) and/or p42^{MAPK} (Peter et al., 1992). We studied (Fig. 5) the electrophoretic mobilities of p42^{MAPK} and p34^{cdc2} kinases by immunoblotting, in relation to histone H1 and MBP kinases activities. Spontaneous and OA-induced meiotic reinitiation were compared using the same protocol as in Fig. 1. Immunoblotting of GV oocytes (Fig. 5A) reveals three different migrating forms of p34^{cdc2} using an anti-PSTAIRE antibody; the upper band seems to be the tyrosine phosphorylated form (using an anti-phosphotyrosine antibody, data not shown). In spontaneously maturing oocytes, the p34^{cdc2} upper band almost completely disappears by the time of GVBD. The low-migrating form is continuously present, whereas the middle one begins to decrease at 1 hour and disappears at around 3 hours. In OA-maturing oocytes, the three migrating forms are continuously detectable up to 5 hours of culture, the upper band decreasing from 3 hours onwards and disappearing around 17 hours. The middle- and the low-migrating forms remain detectable at least up to 17 hours.

Mouse oocytes contain 42 and 44 kDa proteins that are recognized by immunoblotting using an anti-ERK/MAP kinase antibody (Fig. 5B). On some immunoblots (Figs 5B, 6B), a band migrating more slowly than the 44 kDa protein is detectable. It may represent either a conformational change in the 44 kDa or a 45 kDa MAPK protein. However, this band does not change during the course of meiotic reinitiation and is not always detectable, depending on electrophoretic conditions. Spontaneously maturing oocytes show the appearance, following 2 hours of culture, of a slow-migrating band of
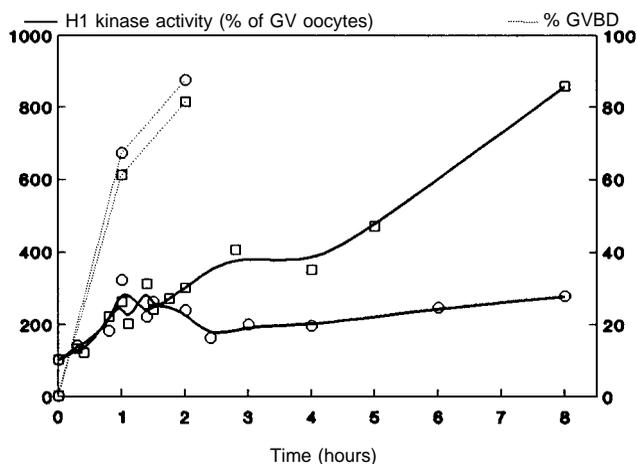

Fig. 2. Effect of OA on histone H1 kinase activity during spontaneous meiotic resumption. Time course of histone H1 kinase activity up to metaphase I. Oocytes were cultured in control medium; OA was present (○) or absent (□) during the first hour. For each point, lysates of 45 oocytes were examined for histone H1 kinase activity (−) as described in Materials and Methods. In parallel, the respective time courses of GVBD are shown (···).
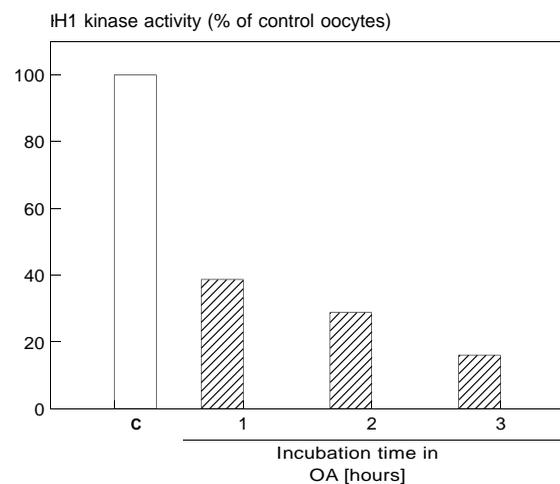

Fig. 3. Effects of exposure times to OA on histone H1 kinase activity. Oocytes were cultured during 20 hours in IBMX-containing medium. OA was present during the first, the first 2 or the first 3 hours of culture. Spontaneously maturing oocytes were used as controls. For each point, 45 oocytes were lysed and examined for histone H1 kinase activity after 20 hours. H1 kinase activity was expressed and normalized as % versus control oocytes.
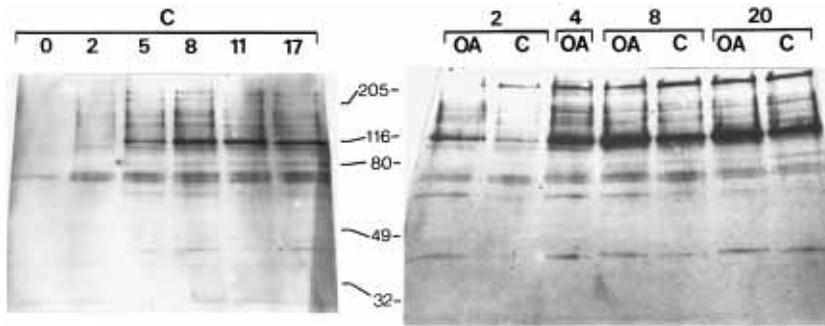
Fig. 4. MPM-2 antigen expression during spontaneous or OA-induced meiotic reinitiation. Oocytes bathed in IBMX-containing medium were induced to reinitiate meiosis either by transfer to IBMX-free medium (spontaneous maturation; C) or by a 1 hour exposure to OA (OA). Proteins of whole cell lysates were separated on 8% SDS-PAGE, transferred to nitrocellulose and probed for the presence of MPM-2 antigens. Numbers above each lane indicate the hours after transfer to control or OA-containing medium; 100 oocytes were used per lane.

$p42^{MAPK}$ (see Fig. 5B), which increases thereafter and which represents the active phosphorylated form of the enzyme (Anderson et al., 1990). Indeed, this upwards shift correlates with the increase of MBP kinase activity shown in Fig. 5C. The MBP kinase activity increases only slightly (2-fold) up to GVBD (1 hour), while the H1 kinase activity rises 3-fold. Thereafter, MBP kinase activity increases strongly (6-fold). In OA-treated oocytes, the complete conversion of $p42^{MAPK}$ to the slow migrating form appears earlier, before GVBD (Fig. 5B). Such results are confirmed by the MBP kinase assay (Fig. 5C). Indeed, this kinase activity increases dramatically before GVBD (10-fold); it is maintained in the absence of $p34^{cdc2}$ activation.

Effects of cycloheximide on histone H1 kinase activity during spontaneous meiotic resumption: interference with histone H1 and MAP kinases activities

In order to determine whether protein synthesis is required to activate histone H1 kinase during spontaneous GVBD, oocytes were treated with cycloheximide. As can be seen in Fig. 6A, the addition of cycloheximide to the culture medium does not

interfere either with the rise of histone H1 kinase, associated with spontaneous GVBD, or with the accompanying disappearance of the upper form of $p34^{cdc2}$ (data not shown). The pattern of the three migrating forms of $p34^{cdc2}$ is similar to that of spontaneously maturing oocytes (data not shown). However, from the third hour on, no further rise of histone H1 kinase activity is detectable (Fig. 6A), the first meiotic M phase being prevented. Later on, the histone H1 kinase activity drops. $p42^{MAPK}$ activation is prevented as revealed by the absence of the upwards shift (Fig. 6B).

Okadaic acid induces GVBD and MAP kinase activation in $p13^{suc1}$-microinjected oocytes

The $p34^{cdc2}$-binding protein, $p13^{suc1}$, is important for cdc2 activation. $p13^{suc1}$ might play a crucial regulatory role in mitosis through direct interaction with $p34^{cdc2}$ in vivo. Histone H1 kinase activity was analyzed in $p13^{suc1}$-microinjected oocytes treated with OA. $p13^{suc1}$ prevents the disappearance of the upper-migrating form of $p34^{cdc2}$ (Fig. 7A) and seems to decrease the histone H1 kinase activity even below that of prophase-blocked oocytes (Fig. 7C). We have previously shown that OA bypasses the inhibition of GVBD by $p13^{suc1}$
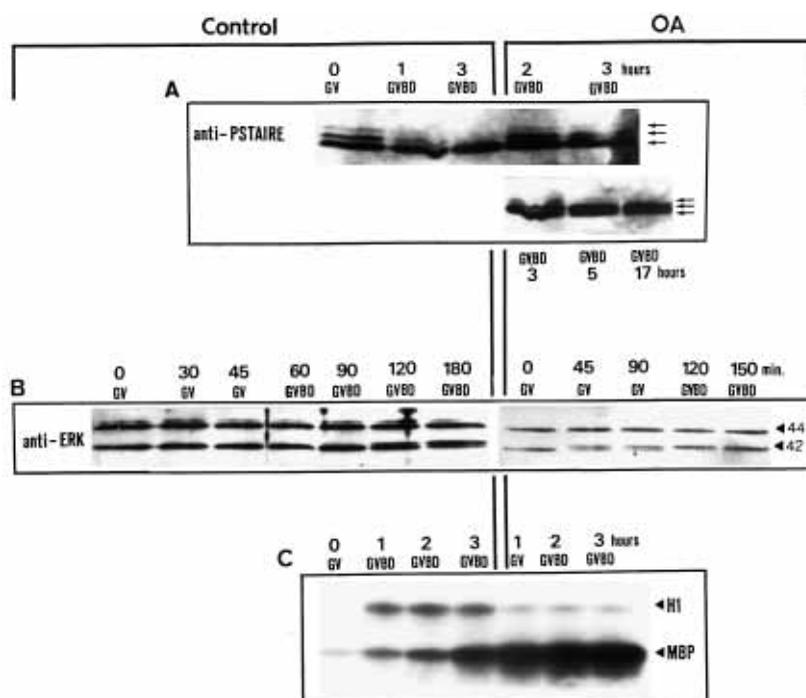

Fig. 5. Activation of $p34^{cdc2}$ and of $p42^{MAPK}$ kinases during the first hours of spontaneous or OA-induced meiosis. Oocytes bathed in IBMX-containing medium were induced to reinitiate meiosis either by transfer to IBMX-free medium (spontaneous maturation, Control) or by 1 hour exposure to OA in IBMX-containing medium (OA). (A) Immunoblots of whole cell lysates with anti-PSTAIRE antibody. Numbers above each lane indicate hours after meiotic reinitiation. 100 oocytes were used per lane. (B) Immunoblots with anti-ERK1/2 antibody. Numbers above each lane indicate minutes after meiotic reinitiation. 100 oocytes were used per lane. (C) Histone H1 and MBP double kinases assay during spontaneous or OA-induced meiotic reinitiation. Numbers above each lane indicate hours after meiotic reinitiation. For each point, 45 oocytes were lysed and examined for kinases activities as described in Materials and Methods.
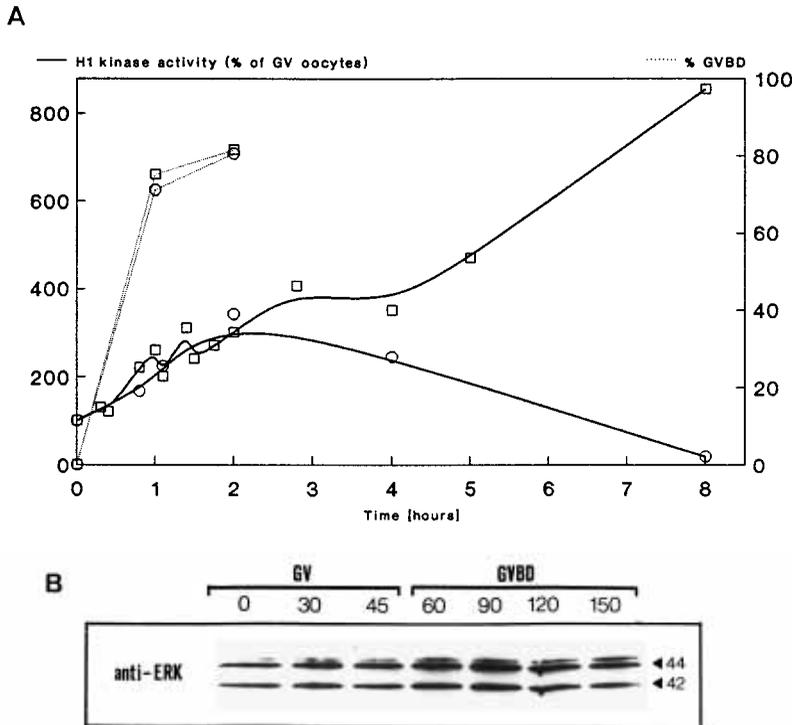
A

B

Fig. 6. Effects of cycloheximide on histone H1 kinase activity and phosphorylation state of p42^{MAPK} during the first 8 hours of meiotic resumption. Oocytes bathed in IBMX-containing medium were transferred either into IBMX-free medium (□) or into cycloheximide-containing medium (○). (A) Time courses of GVBD (⋯) and histone H1 kinase activity (−). For each point, 45 oocytes were lysed and examined for histone H1 kinase activity as described in Materials and Methods. (B) Immunoblots of whole cell lysates with anti-ERK1/2 antibody. Proteins were separated on 15% SDS-PAGE, transferred to nitrocellulose and probed with anti-ERK1/2 antibody. Numbers above each lane indicate the time (minutes) after meiotic reinitiation; 100 oocytes were used per lane.

(Gavin et al., 1992). When OA is applied to p13^{suc1}-injected oocytes, the upper-migrating form of p34^{cdc2} is still present (Fig. 7A) and histone H1 kinase activity reaches a level comparable to that of prophase-blocked oocytes (Fig. 7C). Nevertheless, GVBD is induced. In contrast, a shift of p42^{MAPK} band to its slow-migrating form is detected (Fig. 7B), correlating with a marked increase in MBP kinase activity (Fig. 7C). The activation of p42^{MAPK} appears to be linked to the process of GVBD, since it is not detected in p13^{suc1}-injected oocytes. These results lend support to the hypothesis that OA induces GVBD through a pathway not involving cdc2 kinase but in p42^{MAPK} activation.

DISCUSSION

Histone H1 kinase activation is not always required for the appearance of GVBD

The timing of M phase entry is governed by a cytoplasmic mechanism that generates periodic H1 kinase activity (MPF) inducing meiosis- or mitosis-specific phosphorylation of proteins, leading to nuclear membrane dissolution and chromatin condensation. In order to correlate MPF activity to subcellular processes, we analyzed histone H1 kinase activity in mouse oocytes during the period extending from meiotic reinitiation until metaphase I. At GVBD, histone H1 kinase is only faintly activated (contrary to other oocyte systems) and MPM-2 antigens are not fully expressed. These antigens are known as mitotic phosphoproteins associated with microtubule-organizing centers (MTOCs) in mitotic and meiotic cells and are used as mitotic markers.

OA has been used in oocyte maturation and cell cycle progression to infer the involvement of serine/threonine protein phosphatases. This inhibitor has been claimed to activate MPF
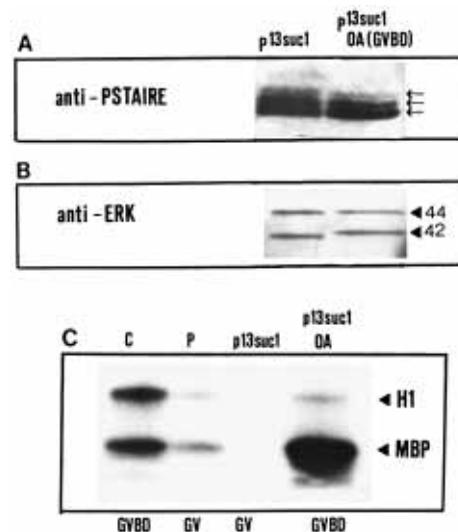
A

B

C

Fig. 7. Effects of OA on MBP and histone H1 kinases activities in p13^{suc1}-microinjected oocytes. p13suc1-injected oocytes, were either induced to reinitiate meiosis by OA (p13suc1/OA) or cultured in OA-free medium (p13suc1) as described in Materials and Methods. They were lysed after 3 hours of culture. (A) Immunoblot of whole cell lysates with anti-PSTAIRE antibody. Proteins were separated on 15% SDS-PAGE, transferred to nitrocellulose and probed with anti-PSTAIRE antibody; 100 oocytes were used per lane.
(B) Immunoblot with anti-ERK1/2 antibody. Proteins were separated on 15% SDS-PAGE, transferred to nitrocellulose and probed with anti-ERK1/2 antibody; 100 oocytes were used per lane.
(C) Histone H1 and MBP double kinases assay. For each point, 45 oocytes were lysed and examined for histone H1 and MPB kinases activities as described in Materials and Methods. C and P refer to spontaneously maturing and to prophase-blocked oocytes, respectively.

(Félix et al., 1990; Goris et al., 1989; Picard et al., 1989; Pondaven et al., 1989; Solomon et al., 1990; Yamashita et al., 1990), indirectly inducing the tyrosine dephosphorylation of p34^{cdc2} by cdc25 activation. Our results show that OA induces a number of M-phase-specific events such as chromosome condensation, GVBD, appearance of MPM-2 antigens, without detectable histone H1 kinase activation. In this context, although OA bypasses the inhibitory effect of p13^{suc1} on GVBD, it does not antagonize the inhibitory phosphorylation of p34^{cdc2} maintained by p13^{suc1}. We suggest that, in mouse oocytes, GVBD may be induced either through bypassing p34^{cdc2} activation or through alternative biochemical mechanism(s) not implicating p34^{cdc2}. However, spindle assembly may require a sustained level of histone H1 kinase activity. Indeed, it has been reported that brief exposures to OA or its microinjection into oocytes enables the assembly of an abnormal spindle; under such conditions, histone H1 kinase activity is slightly increased (3-fold) at 17 hours. Long-term incubations arrested oocyte meiotic resumption at a step prior to spindle assembly (Gavin et al., 1992); histone H1 kinase activity is not increased at 20 hours. These results suggest that a threshold level of histone H1 kinase may be required for metaphase spindle formation.

p42^{MAPK} is activated during early steps of meiotic reinitiation and may function as an alternative trigger for GVBD

Oocyte stimulation induces activation of a pre-existing p42^{MAPK} and cyclin B/cdc2 complexes, known as pre-MPF, leading to resumption of the cell cycle. The MAP kinase plays an important role in controlling M phase events, it might mediate the mitotic disassembly of the nuclear lamina in vivo, and modulate microtubule reorganization (Gotoh et al., 1991). We and others (Sobajima et al., 1993; Verlhac et al., 1993) show that p42^{MAPK} is activated slightly after GVBD during spontaneous meiotic reinitiation. In contrast, a premature and stronger activation is observed even before GVBD under OA treatment, in the absence of histone H1 kinase activation. The expression of MPM-2 antigens correlates with this premature activation.

Since it has been shown that MAP kinase displays substrate specificities overlapping that of p34^{cdc2} (Peter et al., 1992), the possibility that some of the substrates of cdc2 are physiological MAP kinase substrates may explain our results. We suggest that MAP kinase might be able to substitute for MPF and propose that it may be either downstream of p34^{cdc2} kinase, in the same cascade of phosphorylation, or belong to an alternative pathway leading to GVBD. In *Xenopus* oocyte extracts, *mos* protooncogene protein kinase has been shown to turn on the activity of MAP kinase, but not that of MPF, through a cascade of kinases positively regulated by phosphorylation (Nebreda and Hunt, 1993). mos protein being already present in GV mouse oocyte (Paules et al., 1989), OA might interfere at any level of this cascade.

Protein synthesis is only required for the late activation of H1 kinase following GVBD

Mouse oocyte contains the mos protein (Paules et al., 1989). This may explain why cycloheximide prevents neither the GVBD nor the histone H1 kinase increase that is associated with the loss of the upper and middle migrating forms of p34^{cdc2}. In contrast, it prevents p42^{MAPK} activation. During the slow entry into metaphase I, only the low-migrating form of p34^{cdc2} is still detectable after 3 hours, even though histone H1 kinase activity rises continuously until the 8th hour. The biochemical mechanism of this late increase remains unknown. Our results show that it necessitates protein synthesis and is OA-sensitive. Indeed, the negative effect of OA on post-GVBD events may be correlated with that of cycloheximide, suggesting a role for OA-sensitive protein phosphatases in protein synthesis (Gavin et al., 1992; Schwartz and Schultz, 1991). In other species, it has been reported that protein synthesis inhibition may lead to the destruction of the majority of endogeneous cyclin B, preventing p34^{cdc2} kinase activity (Kobayashi et al., 1991) and to the depletion of mos protein. It has been indeed suggested that mos can be responsible for reorganizing the microtubular cytoskeleton and regulating both the function of the meiotic spindle and the chromosomes/microtubules affinity in maturing mouse oocytes (Zhao et al., 1991). For the oocyte to proceed to spindle formation and metaphase entry, either a sustained synthesis of protein (mos) is required and/or new protein(s) have to be neosynthesized.

Alternative pathways depending either on histone H1 and/or on MAP kinase activation may lead to GVBD

In mouse oocytes, depending on the experimental conditions, GVBD occurs through alternative pathways: (i) MPF-dependent and MAP kinase-associated (spontaneously maturing oocytes), (ii) MPF-dependent and MAP kinase-independent (cycloheximide-treated oocytes) or (iii) MPF-independent, but possibly MAP kinase-dependent (OA induced oocytes). The activation of p42^{MAPK} in p13^{suc1} microinjected oocytes under OA treatment (GVBD oocytes) without histone H1 kinase activation, lends support to this last hypothesis.

Altogether our results suggest that, in mouse oocytes, OA-sensitive phosphatases regulate meiotic reinitiation time-dependently. They negatively regulate prophase release through alternative pathways (depending or not on cdc2) but involving p42^{MAPK}. Later on, following GVBD, they positively regulate metaphase I entry, probably through a protein synthesis-dependent mechanism.

REFERENCES

Alexandre, H., Van Cauwenberge, A., Tsukitani, Y. and Mulnard, J. (1991). Pleiotropic effect of okadaic acid on maturing mouse oocytes. *Development* **112**, 971-980.

Anderson, N. G., Maller, J. L., Tonks, N. K. and Sturgill, T. W. (1990). Requirement for integration of signals from two distinct phosphorylation pathways for activation of MAP kinase. *Nature* **343**, 651-653.

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.

Atherton-Fessler, S., Parker, L. L., Geahlen, R. L. and Piwnica-Worms, H. (1993). Mechanisms of p34^{cdc2} regulation. *Mol. Cell. Biol.* **13**, 1675-1685.

Bialojan, C. and Takai, A. (1988). Inhibitory effect of a marine-sponge toxin, okadaic acid, on protein phosphatases. *Biochem. J.* **256**, 283-290.

Brizuela, L., Draetta, G. and Beach, D. (1987). p13^{suc1} acts in the fission yeast cell division cycle as a component of the p34^{cdc2} protein kinase. *EMBO J.* **6**, 3507-3514.

Casillas, A. M., Amaral, K., Chegini-Farahani, S. and Nel, A. E. (1993). Okadaic acid activates p42 mitogen-activated protein kinase (MAP kinase; ERK-2) in B-lymphocytes but inhibits rather than augments cellular proliferation: Contrast with phorbol 12-myristate 13-acetate. *Biochem. J.* **290**, 545-550.

Cho, W. K., Stern, S. and Biggers, J. D. (1974). Inhibitory effect of dibutyrylcAMP 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.

Clarke, P. R., Hoffmann, I., Draetta, G. and Karsenti, E. (1993). Dephosphorylation of cdc25-C by a type-2A protein phosphatase: Specific regulation during the cell cycle in Xenopus egg extracts. *Mol. Biol. Cell* **4**, 397-411.

Cohen, P., Holmes, C. F. B. and Tsukitani, Y. (1990). Okadaic acid: A new probe for the study of cellular regulation. *Trends Biochem. Sci.* **15**, 98-102.

Cyert, M. S. and Kirschner, M. W. (1988). Regulation of MPF activity in vitro. *Cell* **53**, 185-195.

Davies, F. M., Tsao, T. H., Fowler, S. K. and Rao, P. N. (1983). Monoclonal antibodies to mitotic cells. *Proc. Nat. Acad. Sci. USA* **80**, 2926-2930.

Dessev, G., Iovcheva-Dessev, C., Bischoff, J. R., Beach, D. and Goldman, R. (1991). A complex containing p34^{cdc2} and cyclin B phosphorylates the nuclear lamin and disassembles nuclei of clam oocytes in vitro. *J. Cell Biol.* **112**, 523-533.

Devault, A., Cavadore, J. C., Fesquet, D., Labbé, J. C., Lorca, T., Picard, A., Strausfeld, U. and Dorée, M. (1991). Concerted roles of cyclin A, cdc25$^+$ mitotic inducer, and type 2A phosphatase in activating the cyclin B/cdc2 protein kinase at the G$_2$/M phase transition. *Cold Spring Harbor Symp. Quant. Biol.* **56**, 503-513.

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

Dunphy, W. G., Brizuela, L., Beach, D. and Newport, J. W. (1988). The Xenopus cdc2 protein is a component of MPF, a cytoplasmic regulator of mitosis. *Cell* **54**, 423-431.

Dunphy, W. G. and Newport, J. W. (1989). Fission yeast p13 blocks mitotic activation and tyrosine dephosphorylation of the Xenopus cdc2 protein kinase. *Cell* **58**, 181-191.

Dunphy, W. G. and Kumagai, A. (1991). The cdc25 protein contains an intrinsic phosphatase activity. *Cell* **67**, 189-196.

Félix, M. A., Cohen, P. and Karsenti, E. (1990). cdc2 H1 kinase is negatively regulated by a type 2A phosphatase in the Xenopus early embryonic cell cycle: Evidence from the effects of okadaic acid. *EMBO J.* **9**, 675-683.

Ferrell, J. E., Jr, Wu, M., Gerhart, J. C. and Martin, G. S. (1991). Cell cycle tyrosine phosphorylation of p34^{cdc2} and a microtubule-associated protein kinase homolog in Xenopus oocytes and eggs. *Mol. Cell. Biol.* **11**, 1965-1971.

Gautier, J. and Maller, J. L. (1991). Cyclin B in Xenopus oocytes: implications for the mechanism of pre-MPF activation. *EMBO J.* **10**, 177-182.

Gautier, J., Norbury, C., Lohka, M., Nurse, P. and Maller, J. L. (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.

Gautier, J., Solomon, M. J., Booher, R. N., Bazan, J. F. and Kirschner, M. W. (1991). cdc25 is a specific tyrosine phosphatase that directly activates p34^{cdc2}. *Cell* **67**, 197-211.

Gavin, A. C., Tsukitani, Y. and Schorderet-Slatkine, S. (1991). Induction of M-phase entry of prophase-blocked mouse oocytes through microinjection of okadaic acid, a specific phosphatase inhibitor. *Exp. Cell Res.* **192**, 75-81.

Gavin, A. C., Vassalli, J. D., Cavadore, J.-C. and Schorderet-Slatkine, S. (1992). Okadaic acid and p13^{suc1} modulate the reinitiation of meiosis in mouse oocytes. *Mol. Reprod. Dev.* **33**, 287-296.

Goris, J., Hermann, J., Hendrix, P., Ozon, R. and Merlevede, W. (1989). Okadaic acid, a specific protein phosphatase inhibitor, induces maturation and MPF formation in Xenopus laevis oocytes. *FEBS Lett.* **245**, 91-94.

Gotoh, Y., Nishida, E. and Sakai, H. (1990). Okadaic acid activates microtubule-associated protein kinase in quiescent fibroblastic cells. *Eur. J. Biochem.* **193**, 671-674.

Gotoh, Y., Nishida, E., Matsuda, S., Shiina, N., Kosako, H., Shiokawa, K.,

Akiyama, T., Ohta, K. and Sakai, H. (1991). In vitro effects on microtubule dynamics of purified Xenopus M phase-activated MAP kinase. *Nature* **349**, 251-254.

Haccard, O., Jessus, C., Cayla, X., Goris, J., Merlevede, W. and Ozon, R. (1990). In vivo activation of a microtubule-associated protein kinase during meiotic maturation of the Xenopus oocyte. *Eur. J. Biochem.* **192**, 633-642.

Hayles, J., Aves, S. and Nurse, P. (1986). suc1 is an essential gene involved in both the cell cycle and growth in fission yeast. *EMBO J.* **5**, 3373-3379.

Hindley, J., Phear, G. A., Stein, M. and Beach, D. (1987). suc1+ encodes a predicted 13 kilodalton protein that is essential for cell viability and directly involved in the division cycle of Schizosaccharomyces pombe. *Mol. Cell. Biol.* **7**, 504-511.

Hoffmann, I., Clarke, P. R., Jesus Marcote, M., Karsenti, E. and Draetta, G. (1993). Phosphorylation and activation of human cdc25-C by cdc2-cyclin B and its involvement in the self-amplification of MPF at mitosis. *EMBO J.* **12**, 53-63.

Hunt, T. (1989). Maturation promoting factor, cyclin and the control of M-phase. *Curr. Opin. Cell Biol.* **1**, 268-274.

Izumi, T., Walker, D. H. and Maller, J. L. (1992). Periodic changes in phosphorylation of the Xenopus cdc25 phosphatase regulate its activity. *Mol. Biol. Cell* **3**, 927-939.

Jessus, C., Ducommun, B. and Beach, D. (1990). Direct activation of cdc2 with phosphatase: identification of p13^{suc1}-sensitive and insensitive steps. *FEBS Lett.* **266**, 4-8.

Jessus, C. and Beach, D. (1992). Oscillation of MPF is accompanied by periodic association between cdc25 and cdc2-cyclin B. *Cell* **68**, 323-332.

Kalous, J., Sutovsky, P., Rimkevicova, Z., Shioya, Y., Lie, B. L. and Motlik, J. (1993). Pig membrana granulosa cells prevent resumption of meiosis in cattle oocytes. *Mol. Reprod. Dev.* **34**, 58-64.

Kobayashi, H., Minshull, J., Ford, C., Golsteyn, R., Poon, R. and Hunt, T. (1991). On the synthesis and destruction of A- and B-type cyclins during oogenesis and meiotic maturation in Xenopus laevis. *J. Cell Biol.* **114**, 755-765.

Krek, W. and Nigg, E. A. (1991). Differential phosphorylation of vertebrate p34^{cdc2} kinase at the G1/S and G2/M transitions of the cell cycle: identification of major phosphorylation sites. *EMBO J.* **10**, 305-316.

Kuang, J., Zhao, J. Y., Wright, D. A., Saunders, G. F. and Rao, P. N. (1989). Mitosis-specific monoclonal antibody MPM-2 inhibits Xenopus oocyte maturation and depletes maturation-promoting activity. *Proc. Nat. Acad. Sci. USA* **86**, 4982-4986.

Kumagai, A. and Dunphy, W. G. (1992). Regulation of the cdc25 protein during the cell cycle in Xenopus extracts. *Cell* **70**, 139-151.

Labbé, J. C., Capony, J. P., Caput, D., Cavadore, J. C., Derancourt, J., Kaghad, M., Lelias, J. M., Picard, A. and Dorée, M. (1989a). MPF from starfish oocytes at first meiotic metaphase is a heterodimer containing one molecule of cdc2 and one molecule of cycline B. *EMBO J.* **8**, 3053-3058.

Labbé, J. C., Picard, A., Peaucellier, G., Cavadore, J. C., Nurse, P. and Dorée, M. (1989b). Purification of MPF from starfish: identification as the H1 histone kinase p34^{cdc2} and a possible mechanism for its periodic activation. *Cell* **57**, 253-263.

Labbé, J. C., Cavadore, J. C. and Dorée, M. (1991). M phase-specific cdc2 kinase: preparation from starfish oocytes and properties. *Meth. Enzymol.* **200**, 291-301.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature* **227**, 680-685.

Lee, M. S., Ogg, S., Xu, M., Parker, L. L., Donoghue, D. J., Maller, J. L. and Piwnica-Worms, H. (1992). cdc25$^+$ encodes a protein phosphatase that dephosphorylates p34^{cdc2}. *Mol. Biol. Cell* **3**, 73-84.

Lewin, B. (1990). Driving the cell cycle: M phase kinase, its partners, and substrates. *Cell* **61**, 743-752.

Lohka, M. J. (1989). Mitotic control by metaphase-promoting factor and cdc proteins. *J. Cell Sci.* **92**, 131-135.

Lüscher, B., Brizuela, L., Beach, D. and Eisenman, R. N. (1991). A role for the p34^{cdc2} kinase and phosphatases in the regulation of phosphorylation and disassembly of lamin B2 during the cell cycle. *EMBO J.* **10**, 865-875.

Masui, Y. and Markert, C. L. (1971). Cytoplasmic control of nuclear behavior during maturation of frog oocytes. *J. Exp. Zool.* **177**, 129-146.

Meijer, L., Arion, D., Golsteyn, 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.

Millar, J. B. A. and Russell, P. (1992). The cdc25 M-phase inducer: An unconventional protein phosphatase. *Cell* **68**, 407-410.

Minshull, J., Murray, A., Colman, A. and Hunt, T. (1991). Xenopus oocyte maturation does not require new cyclin synthesis. *J. Cell Biol.* **114**, 767-772.

Miyasaka, T., Miyasaka, J. and Saltiel, A. R. (1990). Okadaic acid stimulates the activity of microtubule associated protein kinase in PC-12 pheochromocytoma cells. *Biochem. Biophys. Res. Commun.* **168**, 1237-1243.

Morla, A. O., Draetta, G., Beach, D. and Wang, J. Y. J. (1989). Reversible tyrosine phosphorylation of cdc2: Dephosphorylation accompanies activation during entry into mitosis. *Cell* **58**, 193-203.

Murray, A. W. and Kirschner, M. W. (1989). Cyclin synthesis drives the early embryonic cell cycle. *Nature* **339**, 275-280.

Murray, A. W., Solomon, M. J. and Kirschner, M. W. (1989). The role of cyclin synthesis and degradation in the control of maturation promoting factor activity. *Nature* **339**, 280-286.

Murray, A. W. (1993). Turning on mitosis. *Curr. Biol.* **3**, 291-293.

Nebreda, A. R. and Hunt, T. (1993). The c-mos proto-oncogene protein kinase turns on and maintains the activity of MAP kinase, but not MPF, in cell-free extracts of Xenopus oocytes and eggs. *EMBO J.* **12**, 1979-1986.

Norbury, C., Blow, J. and Nurse, P. (1991). Regulatory phosphorylation of the p34^{cdc2} protein kinase in vertebrates. *EMBO J.* **10**, 3321-3329.

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

Paules, R. S., Buccione, R., Moschel, R. C., Vande Woude, G. F. and Eppig, J. J. (1989). Mouse Mos protooncogene product is present and functions during oogenesis. *Proc. Nat. Acad. Sci. USA* **86**, 5395-5399.

Peter, M., Sanghera, J. S., Pelech, S. L. and Nigg, E. A. (1992). Mitogen-activated protein kinases phosphorylate nuclear lamins and display sequence specificity overlapping that of mitotic protein kinase p34^{cdc2}. *Eur. J. Biochem.* **205**, 287-294.

Picard, A., Capony J. P., Brautigan, D. L. and Dorée, M. (1989). Involvement of protein phosphatases 1 and 2A in the control of M phase-promoting factor activity in starfish. *J. Cell Biol.* **109**, 3347-3354.

Picard, A., Cavadore, J. C., Lory, P., Bernengo, J. C., Ojeda, C. and Dorée, M. (1990). Microinjection of a conserved peptide sequence of p34^{cdc2} induces a Ca^{2+} transient in oocytes. *Science* **247**, 327-329.

Picard, A., Labbé, J. C., Barakat, H., Cavadore, J. C. and Dorée, M. (1991). Okadaic acid mimics a nuclear component required for cyclin B-cdc2 kinase microinjection to drive starfish oocytes into M phase. *J. Cell Biol.* **115**, 337-344.

Pines, J. and Hunter, T. (1990). p34^{cdc2}: the S and M kinase. *New Biol.* **2**, 389-401.

Pondaven, P., Meijer, L. and Bialojan, C. (1989). La microinjection d'acide okadaique, un inhibiteur de phosphoprotéine phosphatases, induit la maturation de l'ovocyte de l'étoile de mer. *C. R. Acad. Sci. Paris* **309**, 563-569.

Pondaven, P., Meijer, L. and Beach, D. (1990). Activation of M-phase-specific histone H1 kinase by modification of the phosphorylation of its p34^{cdc2} and cyclin components. *Genes Dev.* **4**, 9-17.

Posada, J. and Cooper, J. A. (1992). Requirements for phosphorylation of MAP kinase during meiosis in Xenopus oocytes. *Science* **255**, 212-215.

Posada, J., Yew, N., Ahn, N. G., Vande Woude, G. F. and Cooper, J. A. (1993). Mos stimulates MAP kinase in Xenopus oocytes and activates a MAP kinase kinase in vitro. *Mol. Cell. Biol.* **13**, 2546-2553.

Ray, L. B. and Sturgill, T. W. (1987). Rapid stimulation by insulin of a serine/threonine kinase in 3T3-L1 adipocytes that phosphorylates microtubule-associated protein 2 in vitro. *Proc. Nat. Acad. Sci. USA* **84**, 1502-1506.

Redpath, N. T. and Proud, C. G. (1989). The tumour promoter okadaic acid inhibits reticulocyte-lysate protein synthesis by increasing the net phosphorylation of elongation factor 2. *Biochem. J.* **262**, 69-75.

Rime, H. and Ozon, R. (1990). Protein phosphatases are involved in the in vivo activation of histone H1 kinase in mouse oocyte. *Dev. Biol.* **141**, 115-122.

Sanghera, J. S., McNabb, C. K., Tonks, N. and Pelech, S. L. (1991). Tyrosyl phosphorylation and activation of the myelin basic protein kinase p44^{mpk} during sea star oocyte maturation. *Biochim. Biophys. Acta* **1095**, 153-160.

Schwartz, D. A. and Schultz, R. M. (1991). Stimulatory effect of okadaic acid, an inhibitor of protein phosphatases, on nuclear envelope breakdown and protein phosphorylation in mouse oocytes and one-cell embryos. *Dev. Biol.* **145**, 119-127.

Shibuya, E. K., Boulton, T. G., Cobb, M. H. and Ruderman, J. V. (1992). Activation of p42 MAP kinase and the release of oocytes from cell cycle arrest. *EMBO J.* **11**, 3963-3975.

Sobajima, T., Aoki, F. and Kohmoto, K. (1993). Activation of mitogen-activated protein kinase during meiotic maturation in mouse oocytes. *J. Reprod. Fertil.* **97**, 389-394.

Solomon, M. J., Glotzer, M., Lee, T. H., Philippe, M. and Kirschner, M. W. (1990). Cyclin activation of p34^{cdc2}. *Cell* **63**, 1013-1024.

Strausfeld, U., Labbé, J. C., Fesquet, D., Cavadore, J.-C., Picard, A., Sadhu, K., Russell, P. and Dorée, M. (1991). Dephosphorylation and activation of a p34^{cdc2}/cyclin B complex in vitro by human CDC25 protein. *Nature* **351**, 242-245.

Vandré, D. D. and Wills, V. L. (1992). Inhibition of mitosis by okadaic acid: Possible involvement of a protein phosphatase 2A in the transition from metaphase to anaphase. *J. Cell Sci.* **101**, 79-91.

Verlhac, M. H., De Pennart, H., Maro, B., Cobb, M. H. and Clarke, H. J. (1993). MAP kinase becomes stably activated at metaphase and is associated with microtubule-organizing centers during meiotic maturation of mouse oocytes. *Dev. Biol.* **158**, 330-340.

Westendorf, J. M., Swenson, K. I. and Ruderman, J. V. (1989). The role of cyclin B in meiosis I. *J. Cell Biol.* **108**, 1431-1444.

Yamashita, K., Yasuda, H., Pines, J., Yasumoto, K., Nishitani, H., Ohtsubo, M., Hunter, T., Sugimura, T. and Nishimoto, T. (1990). Okadaic acid, a potent inhibitor of type 1 and type 2A protein phosphatases, activates cdc2/H1 kinase and transiently induces a premature mitosis-like state in BHK21 cells. *EMBO J.* **9**, 4331-4338.

Zhao, X., Singh, B. and Batten, B. E. (1991). The role of c-mos proto-oncoprotein in mammalian meiotic maturation. *Oncogene* **6**, 43-49.