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Mitosis-specific monoclonal antibody MPM-2 inhibits *Xenopus* oocyte maturation and depletes maturation-promoting activity

(M-phase induction/meiosis/antibody injection/protein phosphorylation/cell cycle)

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ABSTRACT **MPM-2, a monoclonal antibody specific for cells in mitosis, recognizes a family of proteins that share a common phosphorylated epitope. In this study we have shown that during the maturation of *Xenopus laevis* oocytes induced by progesterone, phosphorylation of MPM-2 antigens coincided with the appearance of MPF activity. When MPM-2 (0.7–1.4 μg per oocyte) was injected into oocytes prior to progesterone stimulation, MPF activity failed to appear and induction of maturation was inhibited as judged by both germinal-vesicle breakdown and white-spot formation. Further, MPM-2 was able to neutralize as well as immunodeplete MPF activity from mitotic HeLa cell and mature oocyte extracts. These results suggest that MPM-2 recognizes either MPF itself or a protein(s) that regulates MPF activity and that the kinase that phosphorylates MPM-2 antigens may be a key component in the regulation of M-phase induction.**

The regulation of the initiation of the M phase (mitosis and meiosis) of the eukaryotic cell cycle has been studied intensively (reviewed in ref. 1). In particular, the identification and characterization of the maturation-promoting factor or M-phase-promoting factor (MPF) have drawn much attention (1). MPF is an activity present in M-phase cells of all species tested that upon microinjection induces immature oocytes to undergo meiotic division (maturation). This activity oscillates during the cell cycle and always correlates with the initiation and maintenance of the M phase (2). Therefore, MPF has been considered as the universal trigger of the interphase/M-phase transition.

MPF has been purified 3500-fold from mature oocytes of *Xenopus* (3). The purified MPF consists of two major proteins of 32 kDa and 45 kDa and exhibits protein kinase activity. The 32-kDa component of MPF has been demonstrated by immunocrossreactivity to be the *Xenopus* homologue of the *cdc2* gene product of *Schizosaccharomyces pombe* (4). Dunphy *et al.* (5) reached the same conclusion by using affinity depletion of the *cdc2* gene product. In both studies, the 45-kDa protein was associated with the 32-kDa protein. Thus, MPF is considered to be a protein complex expressing cdc2 kinase activity.

In *Xenopus* oocytes and in rapidly cleaving embryos, MPF is stored in a latent form during interphase (6–8) and is activated during the induction of M phase. *In vivo* activation of MPF requires protein synthesis both for oocyte maturation and for interphase/mitosis transition in rapidly cleaving embryos (6, 9), suggesting that the newly synthesized protein(s) might act as a positive regulator of MPF. The development of an *in vitro* system capable of activating latent MPF has led to the discovery of an inhibitor of MPF activation termed INH (8). Therefore, MPF is under both positive and negative control.

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Activation of MPF always correlates with a high level of protein phosphorylation (10–16). However, the exact roles of the M-phase-specific phosphorylations are not known. In this study, we have characterized the mitosis-specific phosphorylation detected by a mitosis-specific monoclonal antibody, MPM-2. MPM-2, an IgG antibody, reacts specifically with mitotic cells of different species by indirect immunofluorescence (17) and on immunoblots recognizes a family of phosphoproteins. When MPM-2 antigens are dephosphorylated *in vitro*, their antigenicity is lost (17). Thus, MPM-2 detects mitosis-specific phosphorylation of a group of proteins during the G_2/M transition. In this study, we have found that during progesterone-induced oocyte maturation, phosphorylation of MPM-2 antigens coincides with the appearance of MPF activity. MPM-2 inhibits oocyte maturation upon microinjection and neutralizes and immunodepletes MPF activity from M-phase extracts. These results indicate that MPM-2 recognizes a mitosis-specific phosphorylation of either MPF itself or a protein(s) that regulates MPF activity.

MATERIALS AND METHODS

Materials. Frogs (*Xenopus laevis*) were obtained from Nasco (Fort Atkinson, WI). Affi-Gel blue (100–200 mesh), Bio-Gel A-1.5m, alkaline phosphatase-conjugated goat anti-mouse IgG, and protein A MAPS II kit were from Bio-Rad. Centricon-30 concentrators were from Amicon. Most other reagents were from Sigma.

Purification of Monoclonal Antibodies. Three successive chromatographies were performed to purify antibodies from mouse hybridoma ascites. (*i*) The IgG fraction from the ascites was purified by protein A-mediated affinity chromatography using the Affi-Prep protein A MAPS II system. (*ii*) The eluate, containing mostly IgG, was subjected to gel filtration through a Bio-Gel A-1.5m column (1.5 × 46 cm) preequilibrated in Tris-buffered saline (TBS: 10 mM Tris·HCl/150 mM NaCl, pH 7.5). (*iii*) The IgG fractions from gel filtration were pooled, concentrated with Centricon-30, and subjected to HPLC on hydroxyapatite. Then the IgG fractions were pooled again and concentrated to 20 mg/ml in TBS with Centricon-30. The concentrated antibodies were stored at −20°C until used. MPM-2 and the control antibody (MPM-7) were purified in the same manner.

Assay for Inhibition of Oocyte Maturation by Antibodies. Purified antibodies (20 mg/ml) were diluted serially with TBS before injection. Seventy nanoliters of diluted antibodies or TBS was injected into at least 10 oocytes just prior to the exposure of oocytes to progesterone (10 μg/ml). When all TBS-injected oocytes had matured, as judged by white-spot

Abbreviations: MPF, maturation-promoting factor; GVBD, germinal-vesicle breakdown; WSF, white-spot formation; MFD, maximal fold dilution of MPF activity that causes maturation in 50% of injected oocytes; EB, extraction buffer; TBS, Tris-buffered saline.

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formation (WSF), antibody-injected oocytes were scored for both WSF and germinal-vesicle breakdown (GVBD) after dissection. These two events usually coincide in the normal maturation process, although GVBD can be induced without WSF under certain circumstances.

Preparation of Oocyte Extracts. For small-scale preparation of oocyte extracts (50 oocytes) in the time-course studies, oocytes were rinsed twice in cold extraction buffer (EB: 80 mM sodium β-glycerophosphate/20 mM EGTA/15 mM $MgCl_2$/1 mM dithiothreitol/1 mM ATP/1 mM phenylmethylsulfonyl fluoride, pH 7.4; ref. 18) and transferred into Eppendorf tubes (500 μl). After excess buffer was removed with a gentle spin, an equal volume of 2× EB containing a mixture of protease inhibitors [leupeptin, 5 μg/ml; L-7-amino-1-chloro-3-tosylamido-2-heptanone ("N^{α}-tosyl-L-lysine chloromethyl ketone"), 0.1 mM; phenylmethylsulfonyl fluoride, 1 mM; *N*-ethylmaleimide, 0.5 mM; α_2-macroglobulin, 1 μg/ml] was added. The oocytes were homogenized by pipetting and the homogenates were microcentrifuged at 15,000 × *g* for 10 min at 4°C. The material between the pellet and the lipid cap was recovered. An aliquot was diluted immediately with 3× $NaDodSO_4$/PAGE sample buffer. Microinjection of the extract into oocytes was done within 20 min to assay for MPF activity. For large-scale preparation of mature oocyte extracts, the unfertilized eggs were dejellied in 2% cysteine (pH 7.9). After three washes in 100 mM NaCl, the eggs were rinsed in cold EB. The eggs were crushed by centrifugation at 15,000 × *g* for 25 min and the cytoplasmic material was recovered. Pre-MPF was prepared from immature oocyte extract (22).

Preparation of Mitotic HeLa Cell Extract. HeLa cells were synchronized in mitosis as described (19). The mitotic cell extract was prepared by pelleting the cells at 600 × *g* for 5 min at 4°C, removing the excess medium, and suspending the cells at 4 × 10^7 per ml in 2× EB containing a mixture of protease inhibitors. Cells were disrupted by sonication and the lysates were centrifuged at 100,000 × *g* for 60 min at 4°C in a Beckman L5-50 ultracentrifuge. The supernatants were stored at −70°C until used. Because the extracts made in 1× EB and 2× EB showed similar MPF activity on microinjection, we chose to use 2× EB, as the extracts would be diluted with an equal volume of TBS in neutralization and immunodepletion studies.

Assay for MPF Activity. Cell extracts were assayed for the ability to induce oocyte maturation upon microinjection and quantitated by the maximal fold dilution (MFD) that caused maturation in 50% of the injected oocytes. Samples were diluted serially with EB just prior to microinjection, and 70 nl of diluted sample was injected into each oocyte. At least 10 oocytes were injected with each sample. The injected oocytes were incubated for 2 hr in Barth's solution (20) at 22°C and scored for maturation by WSF at the animal pole and the absence of germinal vesicles after fixation in 5% (wt/vol) trichloroacetic acid and dissection. The MFD for maturation induction in 50% of oocytes was determined by plotting the inverse of the dilution against the percentage of oocytes showing GVBD.

Neutralization and Immunodepletion of MPF. In neutralization studies, purified antibodies (20 mg/ml) were diluted serially with TBS. The diluted antibodies were mixed with an equal volume of mitotic HeLa cell extract or meiotic *Xenopus* oocyte extract. After the mixture was incubated on ice for 2 hr, 70 nl was injected into each oocyte. Two hours later, the oocytes were fixed with 5% trichloroacetic acid and dissected to determine GVBD.

For immunodepletion studies, IgG from ascites was immobilized to protein A-conjugated beads (protein A-agarose or Affi-Prep protein A matrix) by using the MAPS II kit. The immunoaffinity beads were equilibrated and washed with TBS and then mixed with an equal volume of mitotic HeLa cell extract or meiotic *Xenopus* oocyte extract. After the mixture was rotated for 3–4 hr at 4°C, the beads were pelleted and the supernatants were assayed for MPF activity.

$NaDodSO_4$/PAGE and Immunoblot Analysis. Proteins were separated by $NaDodSO_4$/8% PAGE (21), electrophoretically transferred onto nitrocellulose (22), and stained with MPM-2 by using alkaline phosphatase-conjugated goat anti-mouse IgG as the second antibody (23).

RESULTS

Appearance of MPM-2 Antigens Coincides with the Appearance of MPF Activity During Oocyte Maturation. MPM-2 antigens are present in M phase and absent in interphase (17). To define more precisely when MPM-2 antigens appeared during the G_2/M transition, we monitored the appearance of the expression of MPM-2 antigens in relation to the appearance of MPF activity during the progesterone-induced maturation of *Xenopus* oocytes. Immature oocytes had few or no MPM-2 antigens (Fig. 1*A*). Some MPM-2 antigens of about 180 kDa began to appear at 50 min after the exposure of oocytes to progesterone, when MPF activity was still undetectable. By 150 min, when MPF activity was detectable, MPM-2 antigens were more numerous and were expressed at much higher levels. The immunoblot showed that two of the antigens (180 and 58 kDa) were the most abundant. At 250 min (i.e., between the first and second meiotic divisions), there was a significant decrease in MPF activity. This was associated with a noticeable decrease in the 58-kDa antigen. These results indicate that, in general, appearance of MPM-2 antigens correlates well with MPF activity during oocyte maturation, although expression of some MPM-2 antigens at a low level preceded the detection of MPF activity.

Protein Synthesis Is Required for the Appearance of MPM-2 Antigens in Progesterone-Stimulated Oocytes. During progesterone-stimulated maturation, protein synthesis is required

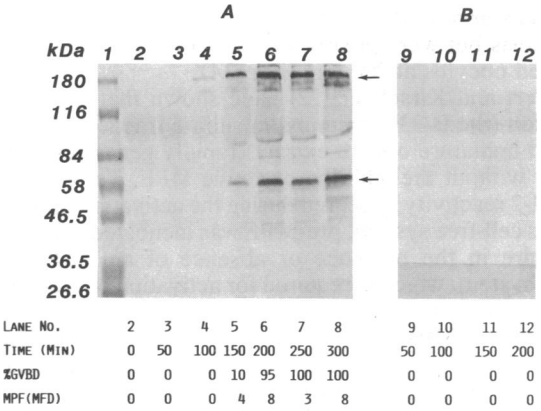

FIG. 1. Expression of MPM-2 antigens and the appearance of MPF activity during progesterone-induced oocyte maturation. Defolliculated *X. laevis* oocytes were incubated in Barth's solution in the absence (*A*) or presence (*B*) of cycloheximide (100 μg/ml). Progesterone was added to the medium at time zero. Every 50 min thereafter, oocytes were scored for WSF and GVBD, and a batch of oocytes was taken to make extracts. The extracts were analyzed for MPF activity by microinjection into immature oocytes and for the expression of MPM-2 antigens on immunoblots. Proteins from the extracts were separated by $NaDodSO_4$/8% PAGE, electrophoretically transferred to nitrocellulose sheets, and stained with MPM-2. The time of sampling, percent GVBD, and relative MPF activity (expressed as the MFD) are indicated below each lane. In cycloheximide-treated oocytes, neither MPM-2 antigens nor MPF activity was detectable (*B*). In oocytes not treated with cycloheximide (*A*), MPM-2 antigens of about 180 kDa appeared as early as 50 min after progesterone stimulation, whereas MPF activity was not detectable until 150 min. A 58-kDa band appeared with the detection of MPF activity. Lane 1 shows protein size standards.

for the appearance of MPF activity. To test whether protein synthesis is necessary for the expression of MPM-2 antigens, cycloheximide (100 μg/ml) was added to the culture medium 30 min before the exposure of oocytes to progesterone. When protein synthesis was inhibited, neither MPF activity nor MPM-2 antigens could be detected (Fig. 1*B*). These results suggest that appearance of MPM-2 antigens is an event downstream of the nascent protein synthesis required for oocyte maturation.

Appearance of MPM-2 Antigens Is Due to Phosphorylation of Preexisting Proteins. In HeLa cells, the proteins of MPM-2 antigens are synthesized in interphase and phosphorylated during mitosis induction (31). Since MPM-2 recognizes only the phosphorylated form of these proteins, the appearance of MPM-2 antigens in mitosis indicates their phosphorylation. To determine whether the appearance of MPM-2 reactivity during oocyte maturation represents phosphorylation of preexisting proteins, we analyzed the expression of MPM-2 antigens during MPF-induced maturation as well as activation of pre-MPF *in vitro*.

MPF can induce oocyte maturation by activating the endogenous pool of inactive MPF. During MPF-induced maturation, protein synthesis is not necessary for the induction of the first meiotic division but is required before the oocytes can progress into the second meiotic metaphase (8). We tested whether MPM-2 antigens appeared in MPF-injected oocytes in the absence of protein synthesis. Seventy nanoliters of mitotic HeLa cell extract (MFD = 3) was injected into oocytes with or without the inhibition of protein synthesis by cycloheximide (100 μg/ml). At intervals during the next 200 min, extracts were made and assayed for MPF activity and the expression of MPM-2 antigens (Fig. 2*A*). As expected (8), cycloheximide did not prevent the appearance of high MPF activity in the first meiotic cycle. However, in the absence of protein synthesis, the MPF-injected oocytes could not progress into the second meiotic metaphase, as indicated by a drop in MPF activity at 200 min. MPM-2 antigens appeared at similar levels with or without protein synthesis but were greatly decreased in the cycloheximide-treated oocytes at 200 min (Fig. 2*A*).

Cyert and Kirschner (22) have shown that the pre-MPF fraction (the 0–33% ammonium sulfate fraction of the high-speed immature oocyte extract) rapidly generates MPF activity without the addition of active MPF. To test whether MPM-2 reactivity appeared during the activation of pre-MPF in this cell-free system, pre-MPF was incubated at room temperature in the presence or absence of an ATP-regenerating system, which is required for activation of pre-MPF. In the absence of the ATP-regenerating system, neither MPF activity nor MPM-2 reactivity was detectable (Fig. 2*B*). In contrast, both MPF activity and MPM-2 antigens appeared when the ATP-regenerating system was present.

Thus, the expression of MPM-2 antigens correlates with the presence of MPF activity, as shown in progesterone-stimulated oocyte maturation. Further, since protein synthesis is not necessary for either MPF-induced maturation or activation of pre-MPF *in vitro*, we conclude that the appearance of MPM-2 antigens during oocyte maturation must be due to the phosphorylation of preexisting proteins.

MPM-2 Inhibits GVBD and WSF upon Microinjection. The appearance of MPF activity concurrently with the appearance of MPM-2 antigens (i.e., the phosphorylation of MPM-2 antigens) suggests that the phosphorylation detected by MPM-2 might be involved in the induction of M phase. Therefore, we tested whether MPM-2, upon microinjection, could inhibit progesterone-stimulated oocyte maturation. We used another mitosis-specific monoclonal antibody (MPM-7) as a control. Both MPM-2 and MPM-7 were purified by successive protein A affinity chromatography, gel filtration, and hydroxyapatite HPLC. Oocytes were injected with antibodies or buffer just before they were exposed to progesterone. MPM-2-injected oocytes were scored after the buffer- or MPM-7-injected oocytes matured, as judged by WSF and GVBD. MPM-2 inhibited both WSF and GVBD in a dose-dependent manner (Fig. 3). In all experiments, 70 nl of the antibody was injected into each oocyte. Inhibition of GVBD required higher concentration of the antibody (10–20 mg/ml), and the inhibition was not complete (80%). In contrast, complete inhibition of WSF occurred at a much lower concentration (0.4 mg/ml). When the incubation of MPM-2-injected oocytes was continued after all the control oocytes had matured, the percentage of the oocytes that underwent GVBD sometimes increased, ranging from 0% to 50%, indicating that in certain cases MPM-2 caused a delay in oocyte maturation rather than a complete block. We also observed some variability among oocytes from different frogs with regard to the percentage of inhibition and the lowest doses needed for inhibition (Table 1). Nevertheless, MPM-2 consistently showed an inhibitory effect on oocyte maturation.

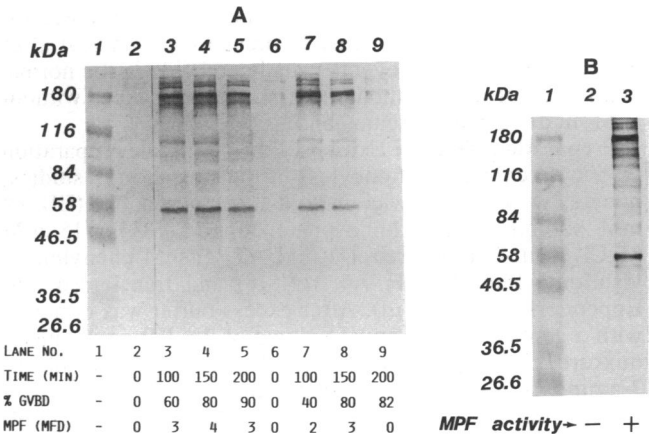

FIG. 2. (*A*) Appearance of MPM-2 antigens and MPF activity in oocytes injected with mitotic HeLa cell extract in the presence or absence of protein synthesis. Defolliculated *X. laevis* oocytes were incubated in Barth's solution in the presence (lanes 6–9) or absence (lanes 2–5) of cycloheximide (100 μg/ml) for 50 min. Seventy nanoliters of mitotic HeLa cell extract (MFD = 3) was injected into oocytes at time zero. At 100, 150, and 200 min, oocytes were scored for GVBD as judged by WSF. Fifty oocytes were taken for each treatment at each time point to make extracts. The extracts were then assayed for MPF activity by microinjection into immature oocytes upon serial dilution. The expression of MPM-2 antigens was analyzed on immunoblots stained with MPM-2 antibody. For time zero (lanes 2 and 6) the oocyte extracts were mixed with corresponding amounts of mitotic HeLa cell extract (70 nl per oocyte). (*B*) Appearance of MPM-2 antigens during *in vitro* activation of pre-MPF. The 0–33% ammonium sulfate fraction of a high-speed extract of immature oocytes was incubated at room temperature for 30 min in the presence (lane 3) or absence (lane 2) of an ATP-regenerating system (1 mM ATP, 50 μg of creatine kinase per ml, and 10 mM creatine phosphate). Both samples were then assayed for MPF activity by microinjection into immature oocytes and for MPM-2 reactivity by immunoblot analysis.

MPM-2 Inhibits the Appearance of MPF Activity in Progesterone-Stimulated Oocytes. Oocytes were injected with MPM-2 before they were exposed to progesterone. The appearance of MPF activity and the expression of MPM-2 antigens were then analyzed in MPM-2- and buffer-injected oocytes. MPF activity was not detectable in MPM-2-injected oocytes at a time when buffer-injected oocytes showed high MPF activity (Fig. 4). This indicates that at least one MPM-2 antigen is needed for the appearance of MPF activity. Furthermore, expression of MPM-2 antigens was also greatly decreased in MPM-2-injected oocytes, with the 58-kDa antigen being completely absent and the 180-kDa antigen being expressed at a much reduced level. This suggests that the MPM-2

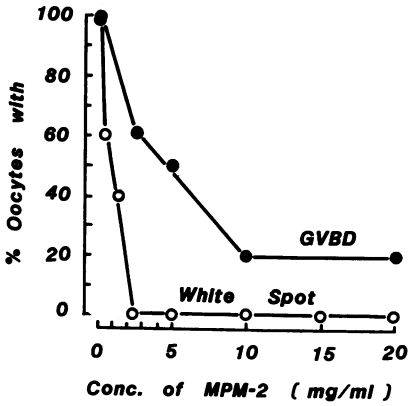

FIG. 3. Effect of MPM-2 on WSF and GVBD in progesterone-stimulated oocytes. MPM-2 at various concentrations (70 nl) was injected into batches of 20 oocytes, which were then immediately exposed to progesterone. When all the control oocytes injected with TBS or MPM-7 exhibited white spots, the MPM-2-injected oocytes were scored for WSF, fixed, and dissected for GVBD scoring. Oocytes injected with TBS or MPM-7 at corresponding concentrations matured normally.

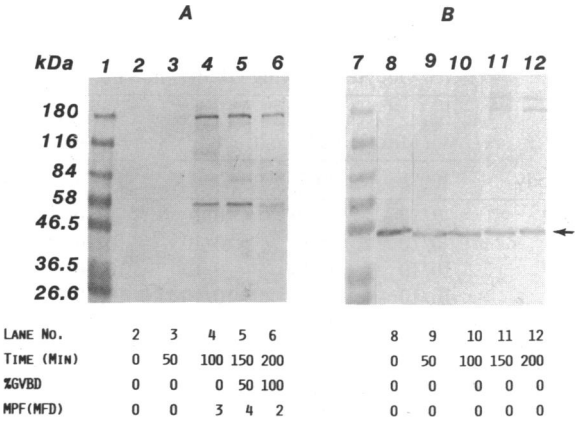

FIG. 4. Effect of MPM-2 on the appearance of MPF activity and MPM-2 antigen expression in progesterone-stimulated oocytes. *Xenopus* oocytes were injected with 70 nl of MPM-2 (20 mg/ml) or TBS and then immediately exposed to progesterone (10 μg/ml). Time-course studies were performed as described in the legend to Fig. 1. The time of sampling, percent GVBD, and MPF activity (MFD) are indicated below each lane. (*A*) Oocytes injected with TBS. (*B*) Oocytes injected with MPM-2. The dark band indicated by an arrow in *B* is the heavy chain of the injected antibody.

antigen involved in the appearance of MPF activity may in turn affect the phosphorylation of other MPM-2 antigens.

MPM-2 Neutralizes and Immunodepletes MPF Activity. The most plausible explanation for our results is that one of the MPM-2 antigens might be MPF itself or a protein required to activate MPF. Therefore, we tested the ability of MPM-2 to neutralize MPF upon mixing and to immunodeplete MPF activity from M-phase cell extracts. In neutralization studies, meiotic extracts from *Xenopus* oocytes or mitotic extracts from HeLa cells were mixed *in vitro* with MPM-2 or the control antibody MPM-7 (final concentration of antibodies, 5 mg/ml). After a 2-hr incubation at 0°C, the samples were assayed for MPF activity with or without dilution with an equal volume of EB. The concentrations of the unbound antibodies in the mixture were <5 mg/ml without dilution or 2.5 mg/ml with dilution, which were not high enough to inhibit the induction of GVBD in 50% of progesterone-treated oocytes. Whereas M-phase extracts mixed with MPM-7 or TBS were able to induce GVBD even upon dilution, the MPM-2-mixed extracts did not induce GVBD with or without dilution (Table 2). The results indicate that MPF activity was neutralized by MPM-2 but not by MPM-7 or TBS. To exclude the possibility that the failure to detect MPF activity in MPM-2-mixed M-phase extracts was attributable to the presence of unbound antibody instead of the inactivation of MPF in M-phase extracts, immunodepletion studies in which antibodies were immobilized to protein A beads were performed. In addition to MPM-7, mouse IgG or RDA-1 (nucleolus-specific monoclonal antibody) was also used as a control. The immunoaffinity beads were mixed with an equal volume of M-phase extract. After mixing at 4°C for 3 hr, the beads were pelleted by centrifugation and the supernatants were assayed for MPF activity. Only MPM-2 was able to deplete MPF activity from M-phase extracts (Table 3). When the depleted extracts were analyzed on immunoblots, the MPM-2-depleted extracts showed very little of the MPM-2 antigens, in contrast to the extracts depleted by control antibodies (Fig. 5). Some bands disappeared completely and others were barely perceptible. These results indicate that MPM-2 can deplete MPF activity from both mitotic and meiotic cell extracts.

Table 1. MPM-2 inhibits progesterone-induced maturation of *Xenopus* oocytes

Experiment	$GVBD_{50}$,* hr	% GVBD	
		MPM-2	MPM-7
1	7.0	10	100
2	6.0	20	100
3	3.0	20	100
4	3.0	30	100
5	4.0	10	100
6	6.0	0	100
7	3.0	30	100
8	3.0	20	100

In all the experiments 70 nl of the antibody (MPM-2 or MPM-7) at 20 mg/ml was injected into each oocyte. MPM-2-injected oocytes were scored for GVBD when MPM-7-injected oocytes had matured.
*Time required for 50% of the control (MPM-7-injected) oocytes to mature after exposure to progesterone. This time varies among oocytes from different frogs.

DISCUSSION

MPM-2, a mitosis-specific monoclonal antibody raised in our laboratory, recognizes a family of phosphoproteins in mitotic cells (17). In HeLa cells, these proteins are synthesized in interphase and phosphorylated in M phase. The antibody recognizes only their phosphorylated form in M phase. In this study, we have attempted to define the role that the phosphorylation of MPM-2 antigens plays in the M-phase induction pathway. We have shown that during progesterone-stimulated oocyte maturation, there is a general correlation between the expression of MPM-2 antigens and the appearance of MPF activity, although expression of some MPM-2 antigens at a low level preceded the detection of MPF activity. When protein

Table 2. Neutralization of MPF activity by *in vitro* mixing of MPM-2 with mitotic HeLa cell extract or mature oocyte extract

Antibody	Oocytes showing GVBD, no./no. injected			
	Mitotic HeLa extract		Mature oocyte extract	
	Undiluted	Diluted	Undiluted	Diluted
MPM-2	0/10	0//10	0/10	0/10
MPM-7	10/10	7/10	10/10	5/10
None (TBS)	10/10	7/10	10/10	3/10

Five microliters of the extract (10 mg/ml) was mixed with 5 μl of antibody (MPM-7 or MPM-2, 10 mg/ml) or TBS in an Eppendorf tube. The mixture was incubated on ice for 3 hr (HeLa cell extract) or 2 hr (oocyte extract) and then injected, with or without 1:1 dilution with EB, and scored for GVBD 2 hr after injection.

Table 3. MPM-2 immunodepletion of MPF activity from mitotic HeLa cell and mature oocyte extracts

	Oocytes showing GVBD, no./no. injected				
	Mitotic HeLa extract			Mature oocyte extract	
Antibody	100%	50%	25%	100%	50%
MPM-2	2/10	0/10	0/10	1/10	0/10
	0/10	0/10	0/10	0/10	0/10
MPM-7	10/10	10/10	0/10	10/10	1/10
	10/10	10/10	10/10	10/10	2/10
RDA-1	10/10	10/10	0/10	10/10	2/10
Mouse IgG	10/10	10/10	0/10	10/10	3/10

Immunodepletion was performed as described in *Materials and Methods*. The depleted extracts were assayed for MPF activity without dilution (100%) or after dilution with 1 volume (50%) or 3 volumes (25%) of EB. Oocytes were scored for GVBD 2 hr after injection. The results are from two representative experiments.

synthesis was inhibited by cycloheximide, MPM-2 antigens did not appear and MPF activity was not detected. The appearance of MPM-2 antigens coincided with activation of MPF in MPF-injected oocytes as well as in *in vitro* activation of pre-MPF. In neither case was protein synthesis required. Since MPM-2 only recognizes a phosphorylated epitope (31), we conclude that the appearance of MPM-2 antigens was due to the phosphorylation of proteins already present in immature oocytes. These results suggest that the phosphorylation of MPM-2 antigens is an event downstream of the nascent protein synthesis required for progesterone-stimulated maturation and coincides with the activation of MPF.

MPM-2 neutralized and immunodepleted MPF activity from M-phase cell extracts. These results suggest that the MPM-2 antigen involved in the appearance of MPF activity might be MPF itself or a regulator of MPF present in M-phase extracts. If MPM-2 could recognize MPF itself, then it could inactivate MPF directly. However, if MPM-2 recognizes a regulatory protein that is necessary to keep MPF in its active form, it could inactivate MPF indirectly. Consequently, the inactivation of either MPF or the regulator of MPF would give the same result—the loss of MPF activity. Thus, we conclude that MPM-2 recognizes either MPF or a regulator of MPF. Further studies would be necessary to distinguish between these two possibilities.

In earlier studies (24), MPM-2 was unable to inhibit the induction of GVBD and to immunodeplete MPF activity. We believe that this was due to the low levels of MPM-2 used in those experiments. In the present work, the inhibition of GVBD by MPM-2 required very high doses, i.e., 70 nl of the antibody at 10–20 mg/ml. These doses are 5–10 times higher than the doses used in the earlier studies (70 nl of 2 mg/ml). We also noticed that the amount of MPM-2 was critical for immunodepletion of MPF activity. If the amount of antibody was only enough to remove a portion of the antigens, the reduction in MPF activity was less than that of the MPM-2 antigens. When the depleted extracts were analyzed on immunoblots, some MPM-2 antigens disappeared completely while others were slightly depleted. These results indicate that different MPM-2 antigens may have different binding affinities for the antibody. It is possible that in the earlier studies, although MPM-2 was able to deplete a significant portion of its antigens from mitotic cell extracts, the antigen involved in MPF activity was still mainly free.

It has been proposed that MPF is activated by protein phosphorylation (8). However, the specific phosphorylation that is responsible for the activation of MPF has not been identified. This study has raised an interesting possibility: that the phosphorylation detected by MPM-2 might be responsible for the activation of either MPF or a regulator of MPF. If this is the case, the kinase that phosphorylates MPM-2 antigens should be one of the key components in the regulation of M-phase induction.

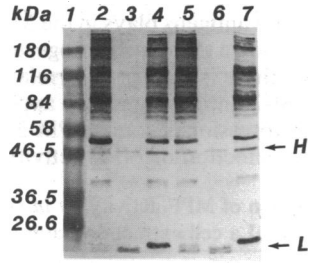

FIG. 5. Immunoblot of mitotic HeLa cell extracts immunodepleted with MPM-2 or control antibodies and stained with MPM-2. Mitotic HeLa cell extract (100 μl) was mixed with an equal volume of protein A beads to which antibody was bound. After rotating at 4°C for 3 hr, the beads were pelleted and the supernatant was assayed for MPF activity. Thirty-three microliters of each supernatant was mixed with 3× $NaDodSO_4$/PAGE sample buffer for immunoblot analysis with MPM-2. Lanes: 1, molecular mass markers; 2, nucleolus-specific monoclonal antibody RDA-1; 3, MPM-2; 4, MPM-7; 5, mouse IgG; 6, MPM-2; and 7, MPM-7. Arrows indicate the heavy (H) and light (L) chains of IgG, which are probably due to trace amounts of immunoaffinity beads in the supernatant.

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