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Multiple forms of maturation-promoting factor in unfertilized *Xenopus* eggs

(cell cycle/M phase induction/oocyte maturation/monoclonal antibody MPM-2)

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ABSTRACT **Maturation-promoting factor (MPF), which is functionally defined by its ability to induce frog oocyte maturation independent of protein synthesis, is hypothesized to be the mitotic inducer in eukaryotic cells. Previous studies have demonstrated that the cdc2 protein kinase complex ($p34^{cdc2}$–cyclin) meets the criteria for MPF. In the present study, we show that MPF activity in extracts of unfertilized *Xenopus* eggs can be resolved into three fractions by Q-Sepharose chromatography. Of the total MPF activity recovered, ≈20% was in the flow-through fraction that was accounted for by the cdc2 kinase complex, ≈40% was in the 0.2 M NaCl eluate, and the remaining ≈40% was in the 0.5 M NaCl eluate. Neither eluate contained cdc2 kinase, but each could activate cdc2 kinase upon microinjection into *Xenopus* oocytes. The MPF activity in the two eluates, but not in the flow-through fraction, could be depleted by the mitosis-specific monoclonal antibody MPM-2. This antibody has been shown to inhibit *Xenopus* oocyte maturation and deplete MPF activity from mature oocyte extract but does not recognize the cdc2 kinase complex. The three MPFs differed in apparent molecular size, H1 kinase activity, and stability at 4°C. We propose that MPF activity in unfertilized *Xenopus* eggs resides in at least three different molecular species, the combined activities of which may be required for autoamplification of MPF.**

In the last two decades, the study of mitotic or meiotic (M phase) induction in the eukaryotic cell cycle has focused on maturation-promoting factor (MPF) (1). MPF was originally defined as an activity in the cytoplasm of mature frog oocytes that could induce maturation (meiotic division) in immature frog oocytes, bypassing the natural process mediated by progesterone (2, 3). MPF exhibits several critical properties (4, 5). (*i*) MPF induces oocyte maturation independent of protein synthesis, which is required for progesterone-induced oocyte maturation. This property positions MPF activation downstream of the step requiring protein synthesis in the maturation-induction pathway. (*ii*) MPF amplifies itself upon microinjection into immature oocytes. This property of MPF suggests the existence of an autoactivation step during the maturation-induction pathway, in which a catalytic amount of active MPF generated via progesterone stimulation activates the entire pool of a preexisting latent form of MPF. (*iii*) MPF activity exists in M phase cells of all species tested, suggesting that it is highly conserved. (*iv*) MPF activity oscillates during the cell cycle in tandem with the onset and maintenance of M phase under both physiological and experimental conditions.

Almost 20 yr after its discovery, MPF was purified to near homogeneity from *Xenopus* and starfish oocytes (6, 7), although the recovery was low (≈1%). Significantly, as a result of this purification, one component of the purified MPF was discovered to be the 34-kDa gene product of *cdc2* ($p34^{cdc2}$) (7, 8), which, based on genetic studies in yeast, is essential for entry into mitosis and encodes a highly conserved kinase (9). $p34^{cdc2}$ is complexed with cyclin and is the major M phase-specific H1 kinase (10, 11). Consequently, MPF, originally defined as a biological activity, could now be associated with a specific molecule, namely $p34^{cdc2}$–cyclin or the cdc2 kinase complex.

In the present study, we addressed the question whether the cdc2 kinase complex is the only molecular species that meets the criteria for MPF. This question was raised for several reasons. For example, although Labbe *et al.* (7) showed that MPF copurified with H1 kinase in each of six successive chromatographic fractionations of starfish oocyte extract, Gerhart *et al.* (5) and Nguyen-Gia *et al.* (12) observed that MPF and histone kinase activities did not copurify during chromatography of *Xenopus* egg extract. In addition, we have shown that the mitosis-specific monoclonal antibody (mAb) MPM-2 inhibits *Xenopus* oocyte maturation and depletes MPF activity from both unfertilized *Xenopus* egg extract and mitotic HeLa cell extract (13) but does not recognize the cdc2 kinase complex (14). These facts suggested that MPF activity in the M phase extract might involve more than one factor and, thus, a more comprehensive investigation of MPF activity in M phase extract was required.

MATERIALS AND METHODS

Materials. Frogs (*Xenopus laevis*) were obtained from Nasco (Ft. Atkinson, WI). Bio-Gel A-1.5 m and Affi-Prep protein A are Bio-Rad products. Q-Sepharose Fast Flow was acquired from Pharmacia. Ultrogel AcA 34 came from IBF. Adenosine 5′-[γ-[^{35}S]thio]triphosphate (ATP[γS]) and protease inhibitors were obtained from Boehringer Mannheim, and the Centricon 30 concentrator was from Amicon. Most other reagents were purchased from Sigma.

Preparation of Unfertilized Egg Extracts. Unfertilized eggs were collected and dejellied as described (14). Egg extract was prepared according to Wu and Gerhart (15) with minor modifications. Briefly, the eggs were packed in cold extraction buffer (EB) consisting of 80 mM sodium β-glycerophosphate (pH 7.3), 20 mM EGTA, and 15 mM $MgCl_2$ and crushed by pipetting in an equal volume of EB containing 10 mM

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Abbreviations: MPF, maturation-promoting factor; EB, extraction buffer; CB, column buffer; QF, Q-Sepharose flow-through fraction; QE, Q-Sepharose eluate; QE1, QE at 0.2 M NaCl; QE2, QE at 0.5 M NaCl; MFD, maximal-fold dilution; ATP[γS], adenosine 5′-[γ-[^{35}S]thio]triphosphate; M phase, mitosis and meiosis phase; mAb, monoclonal antibody.

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dithiothreitol, and a mixture of protease inhibitors (13). One micromolar ATP or 2 mM ATP[γS] were added as described in text. The homogenate was centrifuged at 40,000 rpm with a Beckman Ti50 rotor for 1 hr at 4°C, and the material between the pellet and lipid cap was recovered. The protein concentration of this extract was ≈8–10 mg/ml.

Assay for MPF Activity. Samples were assayed for MPF as presented in detail earlier (14). One unit of MPF was defined as the amount of MPF in 70 nl that would cause maturation in 50% of the injected oocytes. The maximal-fold dilution (MFD) of the samples that allowed maturation of 50% of the injected oocytes was used to express relative MPF activity.

Assay of H1 Kinase Activity. H1 kinase activity in the solution was assayed by mixing 7.5 μl of sample with 2.5 μl of H1 kinase assay reaction mixture at 22°C for 30 min (14). H1 kinase activity of the immunocomplexes on the affinity beads was assayed by mixing 15 μl of beads with 5 μl of the reaction mixture at 22°C for 30 min. The proteins were then released from the beads by gel sample buffer. The samples were electrophoresed on a 12.5% polyacrylamide gel, and the phosphorylation of histone H1 was revealed by autoradiography.

Immunoblot Analysis. Sample proteins (10–40 μl per lane) were separated on a 12.5% polyacrylamide gel, transblotted onto nitrocellulose, and immunostained according to Kuang et al. (14). The PSTAIR polyclonal antiserum was from the laboratory of Paul Nurse (Oxford University). mAb MPM-2 ascites was produced as described (16).

Immunoabsorption. IgGs from ascites were immobilized to Affi-Prep protein A beads (Bio-Rad) using the binding buffer from the MAPS II kit (Bio-Rad). Anti-frog cyclin B2 beads, $p13^{suc1}$-Sepharose beads, and corresponding preimmune serum control beads were obtained from the laboratories of Tim Hunt (Cambridge University) and Marc Kirschner (University of California, San Francisco). The beads were washed with EB and mixed with 1.5–2 vol of sample by rotation at 4°C for 3–4 hr. The depleted samples were obtained by pelleting the beads and recovering the supernatants. The beads were washed as reported (14). The supernatants and/or beads were analyzed either for H1 kinase or by immunoblot.

Ultrogel AcA 34 Chromatography of Egg Extract. One milliliter of egg extract, which had an MFD of 4–6, was chromatographed on a 40-ml Ultrogel AcA 34 column (1.5 × 59 cm) preequilibrated with a column buffer (CB) that contained EB plus 1 mM dithiothreitol and 20 mM NaF, supplemented with either 1 mM ATP (CB–ATP) or 50 μM ATP[γS] (CB–ATP[γS]). One-milliliter fractions were collected at a flow rate of 0.3 ml/min. Each fraction was measured for its absorbance at 280 nm as a relative indicator of protein concentration. Fractions 12–37 were concentrated by a Centricon 30 concentrator to 40 μl for MPF assay.

Q-Sepharose Chromatography of Pooled MPF-Positive Fractions After Gel Filtration of Egg Extract on Ultrogel AcA 34. MPF-positive fractions (numbers 19–32) from Ultrogel AcA 34 chromatography of egg extract were pooled and loaded directly onto a 5-ml Q-Sepharose Fast Flow column preequilibrated in CB containing ATP[γS]. The column was then washed with 15 ml of CB–ATP[γS], supplemented with 25 mM NaCl to increase washing efficiency, and eluted in two steps—(*i*) with 20 ml of CB–ATP[γS] containing 0.2 M NaCl and (*ii*) with 10 ml of 0.5 M NaCl in CB–ATP[γS]. One-milliliter fractions were collected at a flow rate of 0.3 ml/min. After absorbance at 280 nm was determined for individual fractions, the flow-through (QF) and peak fractions of the 0.2 M NaCl (QE1) and 0.5 M NaCl (QE2) eluates were pooled separately. They were then dialyzed, concentrated, and assayed for MPF activity, H1 kinase activity (diluted 1:3 with EB), and PSTAIR antibody reactivity.

Bio-Gel A-1.5 m Chromatography of QF, QE1, and QE2. A volume of 0.5 ml containing pooled and concentrated QF, QE1, or QE2 from two-step Q-Sepharose chromatography (previously described) was chromatographed on a 20-ml Bio-Gel A-1.5 m column (0.9 × 53 cm) preequilibrated in CB (for QF) or CB–ATP[γS] (for QE1 and QE2). Fractions of 0.5 ml were collected at a flow rate of 0.15 ml/min. Thyroglobulin (670 kDa), mouse IgG (150 kDa), and bovine serum albumin (68 kDa) were used as molecular size standards to calibrate the column.

RESULTS

Stabilization of MPF. MPF is notoriously unstable, especially during the early stages of purification. To ensure that we would not lose any major species of MPF during the investigation, we initially sought to define conditions under which MPF activity could be quantitatively recovered from gel filtration of unfertilized egg extract.

When extract was prepared with EB (15), which stabilizes MPF activity in the extract for 24 hr at 4°C, little MPF activity could be recovered after gel filtration. We found, however, that MPF inactivation could be prevented by including 2 mM ATP[γS], shown to promote MPF stability (17), in preparation and fractionation of the extract. (*i*) When extract prepared with ATP[γS] was fractionated by Ultrogel AcA 34 gel filtration (exclusion limit, ≈750 kDa), 50% MPF activity could be recovered (Fig. 1*B*). (*ii*) When ATP[γS] was also included in CB, the recovered MPF activity was still 50% but was more stable (2–3 days at 4°C) (Fig. 1*C*). (*iii*) Storage of the extract at 4°C for 3 days further increased recovery of MPF activity to 75–90%, which was stable for 3–4 days (Fig. 1*D*). Therefore, the quantitative recovery of MPF could be achieved when thiophosphorylation was maximally favored. We consider the recovered MPF activity to be M phase-specific because no MPF activity could be recovered when immature oocyte extract was prepared and fractionated under similar conditions.

In the gel-filtration experiments described, the peak of recovered MPF activity was rather broad (Fig. 1*A*; fractions 18–34). Interestingly, when fresh extract was fractionated in CB-ATP, the highest level of MPF activity was in fractions 23–24 (Fig. 1*B*) but shifted to fractions 26–27 (Fig. 1*C*) when the column buffer contained ATP[γS] instead. However, when 3-day-old extract was fractionated in CB–ATP[γS], the maximum level of MPF activity extended from fractions 23–26 (Fig. 1*D*), including both high points. From these findings, it seemed that more than one factor might have contributed to the recovered MPF activity.

Relationship Between MPF and cdc2 Kinase Activity After Gel Filtration. After Ultrogel AcA 34 fractionation of egg extract, two prominent peaks of H1 kinase activity were detected, the first eluting near thyroglobulin (670 kDa) and the second near mouse IgG (150 kDa) (Fig. 1*E*). The MPF-positive fractions extended from the trailing edge of the first H1 kinase peak (fraction 18) to the trailing edge of the second H1 kinase peak (fraction 34). Recently, we have demonstrated that only the second H1 kinase peak is cdc2 kinase (14). The first peak, which does not contain $p34^{cdc2}$ or cyclin but is recognized by mAb MPM-2, is a different M phase-specific H1 kinase, designated MPM-2 kinase. Therefore, the recovered MPF activity includes, but does not copurify with, cdc2 kinase, supporting the possibility that more than one factor might contribute to MPF activity.

Resolution of MPF Activity into Three Fractions by Q-Sepharose Chromatography. Fractions 19–32 from gel filtration of egg extract, which contained 90% of the recovered MPF and cdc2 kinase activities but little MPM-2 kinase activity, were pooled. The pooled material was directly loaded onto a Q-Sepharose column. When we used a continuous gradient (0–0.5 M NaCl) to elute the bound proteins from Q-Sepharose, two peaks of MPF activity were detected in the eluate (data not shown). The first peak was eluted at 0.1–0.2 M

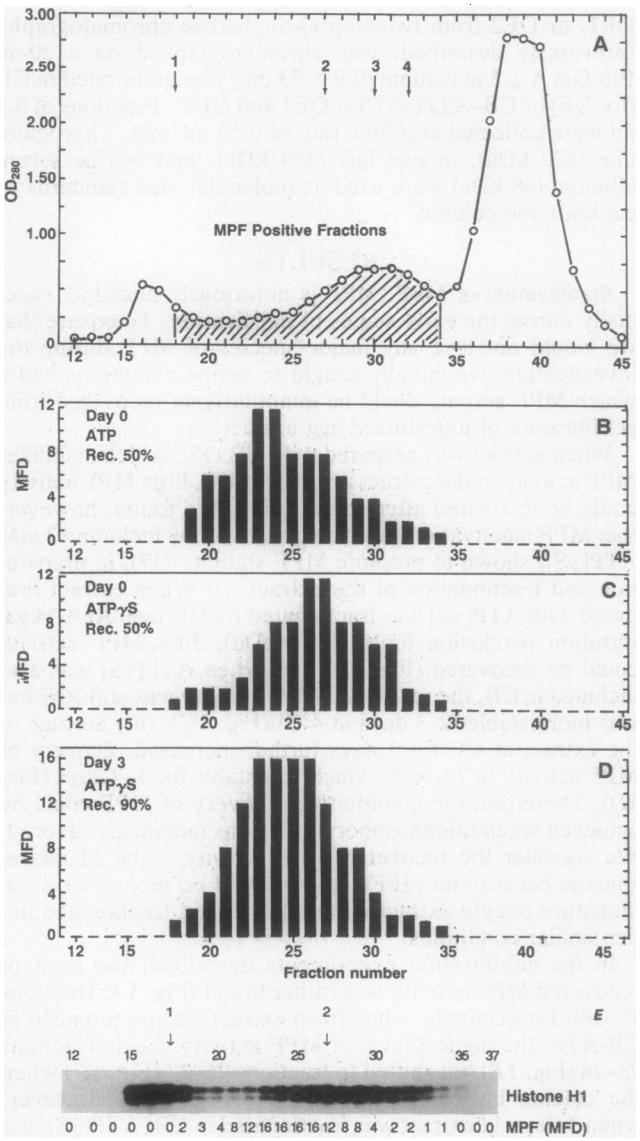

FIG. 1. Fractionation of *Xenopus* egg extract by Ultrogel AcA 34 chromatography. (*A*) Protein absorbance at 280 nm of individual fractions; hatched section indicates MPF-positive fractions. Elution positions of thyroglobulin (670 kDa) (arrow 1), mouse IgG (150 kDa) (arrow 2), chicken ovalbumin (44 kDa) (arrow 3), and horse myoglobin (17.8 kDa) (arrow 4) are indicated. Recovered (Rec.) MPF activity after gel filtration of fresh extract (0 days of storage) in CB–ATP (*B*), fresh extract in CB–ATP[γS] (*C*), and 3-day-old extract in CB–ATP[γS] (*D*). (*E*) Relationship between MPF and H1 kinase activity after gel filtration. Positions of thyroglobulin (670 kDa) (arrow 1) and mouse IgG (150 kDa) (arrow 2) are indicated. Maximal-fold dilution (MFD) was used to express the relative MPF activity of each concentrated fraction (40 μl).

NaCl, whereas elution of the second peak began one to two fractions later at 0.2–0.3 M NaCl. We could clearly separate the two peaks of MPF activity by two successive elutions—the first at 0.2 M NaCl (QE1) and the second at 0.5 M NaCl (QE2) (Fig. 2). QE1 and QE2 each contained 40% of the recovered MPF activity, whereas the flow-through fraction (QF) contained 20%. Interestingly, when QF was dialyzed into EB without ATP[γS], its MPF activity remained stable, whereas the stability of MPF in QE1 and QE2 required ATP[γS]. At 4°C QE1 lost all MPF activity within 24 hr, whereas the MPF activity in QE2 was stable for at least 3 days. When, however, QE1 and QE2 were coeluted by 0.5 M NaCl, the combined recovered MPF activity was as stable as that in QE2, suggesting that QE2 may stabilize the MPF in QE1.

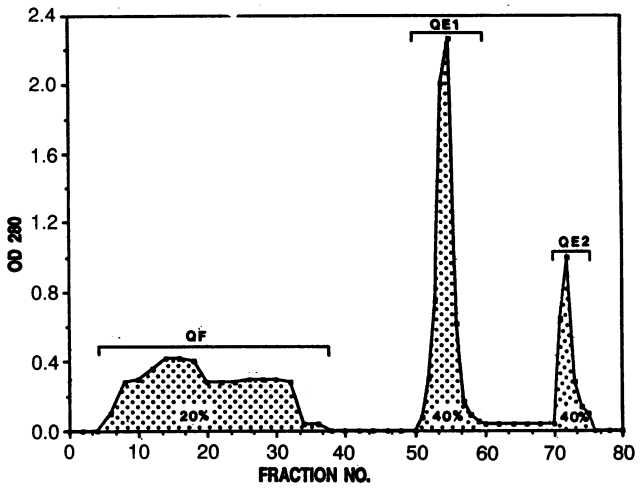

FIG. 2. Q-Sepharose chromatography of MPF-positive fractions from Ultrogel AcA 34 gel filtration of egg extract. The pooled MPF-positive fractions obtained from Ultrogel AcA 34 gel filtration of egg extract (see legend to Fig. 1; conditions are as described in *D*) were fractionated by Q-Sepharose chromatography using a two-step elution, with 0.2 M NaCl in the first elution and 0.5 M NaCl in the second. Protein absorbance at 280 nm of the individual fractions is indicated. Each eluate contained ≈40% of the total recovered MPF activity, whereas QF contained 20%.

QF, QE1, and QE2 were individually fractionated on a Bio-Gel A-1.5 m column, chosen for its wider separation range, to estimate the apparent molecular size of the MPF, which differed in each fraction: ≈90 kDa in QF, ≈150 kDa in QE1, and ≈250 kDa in QE2. This difference explains why MPF activity eluted as a broad peak when the crude extract was fractionated by gel filtration.

Identification of cdc2 Kinase–MPF. QF, QE1, and QE2 were assayed for H1 kinase activity and reactivity to the PSTAIR antibody, which recognizes the highly conserved domain of $p34^{cdc2}$. On the average, ≈90% of total H1 kinase activity was recovered in QF, ≈9% was recovered in QE1, and ≈1% was recovered in QE2 (Fig. 3*A*). The PSTAIR antibody-reactive protein was only detectable in QF (Fig. 3*B*). To exclude the possibility that QE1 and QE2 contained a level of the PSTAIR antibody-reactive protein too low for detection, we enriched $p34^{cdc2}$ by p13 adsorption of fractions that had been concentrated ≈20-fold and analyzed the immobilized proteins. Again, the PSTAIR antibody-reactive protein was only detectable in QF, suggesting that cdc2 kinase was only present in QF (Fig. 3*C*). In addition, anti-

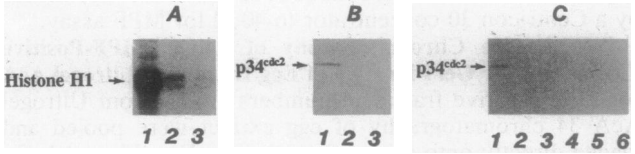

FIG. 3. Assay of QF, QE1, and QE2 for H1 kinase activity and reactivity to mAb PSTAIR. Concentrated QF, QE1, and QE2 fractions were obtained by Q-Sepharose chromatography of MPF-positive fractions from Ultrogel AcA 34 chromatography of egg extract. (*A*) H1 kinase activity of each fraction. H1 kinase activity was quantitated by scintillation counting of the histone H1 bands. Amount of H1 kinase activity in QF was ≈90% (lane 1), QE1 was ≈90% (lane 2), and QE2 was ≈1% (lane 3) of total H1 kinase activity recovered. (*B*) mAb PSTAIR staining of QF (lane 1), QE1 (lane 2), and QE2 (lane 3). (*C*) The PSTAIR antibody staining of proteins from QF, QE1, and QE2 immobilized onto p13 Sepharose beads. Lanes: 1, QF immobilized on p13-Sepharose; 2, QF immobilized onto control Sepharose; 3, QE1 immobilized onto p13-Sepharose; 4, QE1 immobilized onto control Sepharose; 5, QE2 immobilized onto p13-Sepharose; and 6, QE2 immobilized onto control Sepharose.

cyclin B2 affinity beads immobilized a high level of H1 kinase activity from QF, confirming that $p34^{cdc2}$ is complexed with cyclin (data not shown).

To show that the MPF activity in QF was due to cdc2 kinase, QF was fractionated by Bio-Gel A-1.5 m chromatography, and the fractions were assayed for H1 kinase activity, MPF activity, and the PSTAIR antibody-reactive protein. As illustrated in Fig. 4, the recovered MPF activity copurified with both H1 kinase activity and PSTAIR antibody-reactive protein. These results indicate that the MPF activity in QF is accounted for by $p34^{cdc2}$–cyclin.

Identification of MPM-2-Reactive MPF. MPM-2 is a mitosis-specific mAb that recognizes a family of phosphoproteins in M phase cells (16). The fact that mAb MPM-2 immunodepletes MPF activity from egg extract but does not recognize the cdc2 kinase complex (14) suggested that mAb MPM-2 might recognize the MPF in QE1 or QE2. To explore this possibility, QF, QE1, and QE2 were incubated with mAb MPM-2 or control antibody (RDA-1) affinity beads for 3 hr, and the supernatants were assayed for MPF activity. Table 1 shows that while QF depleted by mAb MPM-2 contained similar levels of MPF activity as the control, QE1 and QE2 contained no detectable MPF activity after mAb MPM-2 depletion. Therefore, we conclude the MPF in both QE1 and QE2 is recognized by mAb MPM-2.

Proteins immobilized on the mAb MPM-2 immunoaffinity beads were assayed for their ability to phosphorylate histone H1 *in vitro*. mAb MPM-2-adsorbed protein from QF or QE1 showed no H1 kinase activity. However, a low level of H1 kinase activity was detected on mAb MPM-2 affinity beads after the adsorption of QE2 (data not shown). These results indicate that although the MPF in QE1 is not an H1 kinase, the MPF in QE2, which contained only 1% of total H1 kinase activity (see Fig. 3*A*), could be weak H1 kinase. Finally, anticyclin beads depleted no MPF activity from either QE1 or QE2, indicating that cyclin B2 is unlikely to be a component of the MPF in QE1 or QE2.

Characterization of MPF in QF, QE1, and QE2. We found that QF, QE1, and QE2 each could induce oocyte maturation in the presence of cycloheximide and amplify MPF activity in recipient oocytes through successive transfers. We then tested whether each of the three fractions could simultaneously activate endogenous H1 kinase and MPF and cause expression of MPM-2 antigens, as is normally seen at the onset of M phase. QF, QE1, and QE2, as well as egg extract (positive control), were microinjected into *Xenopus* oocytes. Extracts were prepared from injected oocytes at 20-min intervals and assayed for H1 kinase activity, MPF activity, and MPM-2 antigens. At time 0, we detected no MPF activity, no MPM-2 antigens, and little or no H1 kinase activity (Fig. 5). At 20 min after injection of QF, QE2, and egg extract and 40 min after injection of QE1, MPF activity appeared concurrently with expression of MPM-2 antigens as well as high levels of H1 kinase activity. Thus, within the limits of resolution in this time course study, each fraction could induce the simultaneous activation of H1 kinase and MPF and the expression of MPM-2 antigens. As illustrated in Fig. 5, the levels of MPF activity induced in oocytes injected with QF, QE1, or QE2 were similar to that induced by egg extract, so that all three MPFs can activate the entire pool of latent forms of MPF in immature oocytes.

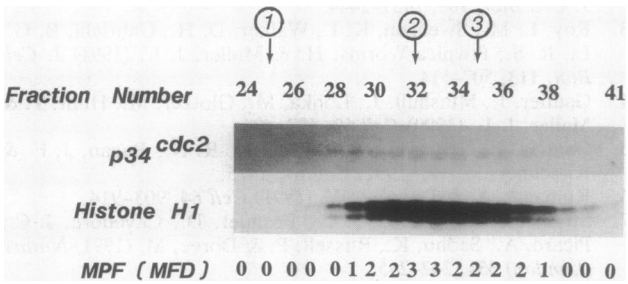

FIG. 4. Copurification of MPF activity with H1 kinase activity and the PSTAIR antibody-reactive protein during gel filtration of QF by Bio-Gel A-1.5 m chromatography. Concentrated QF was fractionated on a Bio-Gel A-1.5 m column in EB plus 1 mM dithiothreitol. From each fraction, 7.5 μl was assayed for H1 kinase activity, 20 μl was analyzed for the PSTAIR antibody-reactive protein, and the rest was concentrated to 40 μl for assay of MPF activity (MFD). Elution positions of thyroglobulin (670 kDa) (arrow 1), mouse IgG (150 kDa) (arrow 2), and bovine serum albumin (68 kDa) (arrow 3) are indicated.

Table 1. MPF activity in QF, QE1, and QE2 after MPM-2 immunoabsorption

	MPF activity*								
	QF			QE1			QE2		
mAb	Und	1:2	1:4	Und	1:2	1:4	Und	1:1	1:4
MPM-2	10	0	0	0	0	0	0	0	0
RDA-1	10	0	0	10	5	0	10	6	0

Immunoabsorption was done as described. RDA-1 was the control antibody. Und, undiluted.
*MPF activity represented as number of oocytes showing germinal vesicle breakdown per 10 oocytes injected.

DISCUSSION

In this study, we developed a procedure by which MPF activity can be quantitatively recovered after gel filtration of unfertilized egg extract. The recovered MPF activity, which occurred as a rather broad peak, could be resolved into three fractions by Q-Sepharose chromatography (two-step elution). All three fractions can induce oocyte maturation independent of protein synthesis, activate endogenous H1 kinase and MPF, and induce the appearance of MPM-2 antigens in the oocyte. However, the MPF in each fraction differs in apparent molecular mass, H1 kinase activity, the presence of the PSTAIR antibody-reactive protein, and stability at 4°C.

The MPF activity in QF is due to the cdc2 kinase complex. QF contains most of the H1 kinase activity, which can be immobilized by anti-cyclin B2 affinity beads and is strongly reactive to the PSTAIR antibody. When QF was fractionated by gel filtration, the recovered MPF activity copurified with both H1 kinase activity and the PSTAIR antibody-reactive

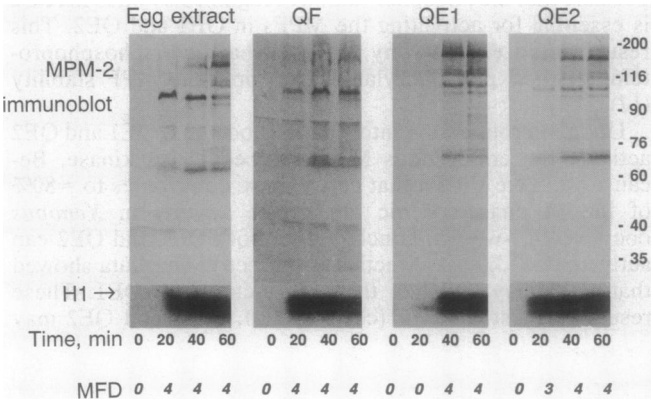

FIG. 5. Activation of MPF and H1 kinase and expression of MPM-2 antigens in QF-, QE1-, and QE2-injected oocytes. *Xenopus* oocytes were injected with 70 nl (MFD = 2) of QF, QE1, QE2, or egg extract at time 0. Every 20 min thereafter, 30 oocytes were taken to make extracts as described, except that oocytes were crushed in 2 vol of extraction buffer and ultracentrifugation was replaced by a 10-min microcentrifuge spin.

protein. These results are consistent with previous findings that cdc2 kinase is a form of MPF.

Although the biochemical nature of the MPFs in QE1 and QE2 remains to be established, they are not cdc2 kinase. Although QE1 and QE2 contain 80% of the recovered MPF activity, they contain no detectable amount of the PSTAIR antibody-reactive protein. Secondly, ATP[γS] is required for the stability of the MPF activity in QE1 and QE2 but is not required for cdc2 kinase. Furthermore, although cdc2 kinase prefers histone H1 as an *in vitro* substrate, the MPF in QE1 is not an H1 kinase and the MPF in QE2 is, at most, a weak H1 kinase. Finally, anticyclin B2 does not deplete any MPF activity from either QE1 or QE2. These results might explain the recent finding that when unfertilized egg extract is treated with okadaic acid, which causes the degradation of cyclin and inactivation of cdc2 kinase, that extract can still induce maturation when injected into immature oocytes (18). The MPF activity in the okadaic acid-treated extract is probably due to the MPF present in QE1 and/or QE2.

Although the product of the protooncogene c-*mos* can induce oocyte maturation (19, 20), it is not a likely candidate for MPF in QE1 or QE2 because it is newly synthesized during progesterone-induced oocyte maturation, whereas the full activation of MPF in immature oocytes can occur in the absence of c-mos protein synthesis (2, 17). It is, therefore, likely that the c-mos protein functions as an upstream regulator of MPF and contributes little, if any, to the MPF activity in unfertilized eggs. *Xenopus* cyclins also can induce maturation (21–23) and are a component of cdc2 kinase (24). However, cyclin B2 is probably not a component of the MPF from QE1 or QE2 based on our immunodepletion studies. Whether other cyclins are components of the MPF in QE1 or QE2 remains to be established. Recently, the *cdc25* gene product has been shown to have MPF activity (25) and be able to activate cdc2 kinase *in vitro* via dephosphorylation (26, 27), which might account for the MPF in QE1 or QE2.

We have demonstrated that the mitosis-specific mAb MPM-2 can deplete the MPF activity from QE1 and QE2 but not from QF. This result may explain our previous finding that mAb MPM-2 depletes MPF activity from *Xenopus* egg extract (13), although it does not recognize cdc2 kinase (14). Additionally, as described by Davis *et al.* (16), mAb MPM-2 reacts with a distinct set of proteins once they are phosphorylated during the G_2/M transition. Therefore, mAb MPM-2 recognition of the MPFs in QE1 and QE2 indicates that they have undergone this mitosis-specific phosphorylation. It is possible that the phosphorylation recognized by mAb MPM-2 is essential for activating the MPFs in QE1 and QE2. This result would explain why reagents that favor phosphoproteins in their phosphorylated state promote MPF stability (17).

Upon microinjection into *Xenopus* oocytes, QE1 and QE2 activate the endogenous M phase-specific H1 kinase. Because we have shown that cdc2 kinase contributes to ≈80% of the M phase-specific H1 kinase activity in *Xenopus* oocytes (14), we can conclude that both QE1 and QE2 can activate cdc2 kinase directly or indirectly. Our data showed that QE2 may stabilize the MPF activity in QE1. These results suggest that QF (cdc2–cyclin), QE1, and QE2 may form a regulatory loop that together can account for MPF activity.

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