Purification of Maturation-Promoting Factor, an Intracellular Regulator of Early Mitotic Events

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ABSTRACT Maturation-promoting factor causes germin al vesicle breakdown when injected into Xenopus oocytes and can induce metaphase in a cell-free system. The cell-free assay was used to monitor maturation-promoting factor during its purification from unfertilized Xenopus eggs. Ammonium sulfate precipitation and six chromatographic procedures resulted in a preparation purified >3000-fold that could induce germinal vesicle breakdown within 2 hr when injected into cycloheximide-treated oocytes. Proteins of 45 kDa and 32 kDa were correlated with fractions of highest activity in both assays. These fractions contained a protein kinase activity able to phosphorylate the endogenous 45-kDa protein, as well as histone H1, phosphatase inhibitor 1, and casein. The highly purified preparations described here should help to identify the mechanism of action of maturation-promoting factor and to elucidate the role of protein kinases in the induction of metaphase.

Two events mark the reproductive life of a eukaryotic cell; replication of the genetic material in the S phase and in the cell cycle and segregation of the replicated DNA to daughter cells during the mitotic phase (M phase). In most types of cells a G1 phase separates the completion of M phase from the beginning of S phase, whereas a G2 phase separates S phase from M phase. Two major control points in the cell cycle have been identified (for review, see refs. 1 and 2). One such control point is early in G1, and its regulation by serum and peptide growth factors has been well documented (1, 3). Another control point is late in G2. Certain cell division cycle mutants in yeast and other fungi are defective in gene products required for G2 → M transition (4–6), and several mammalian cell lines can be reversibly arrested in G2 by N+,O2-dibutyryl adenosine 3',5'-cyclic monophosphate (7, 8). Perhaps the best studied example of a cell naturally arrested in G2 is the fully grown amphibian oocyte, which, in response to hormones, enters M phase and matures into a metaphase-arrested unfertilized egg (for review, see refs. 9 and 10). Upon fertilization, eggs complete meiosis II and enter S phase. Thus, oocytes undergo a simple cell cycle comprising a G2 → M transition at maturation and then an M → G1/S transition at fertilization.

When cytoplasm from matured oocytes or eggs is injected into immature oocytes, the recipients undergo oocyte maturation, even in the absence of new protein synthesis (11, 12). The active component in the transferred cytoplasm has been called maturation-promoting factor (MPF). MPF has been detected both in oocytes and in mitotic cells from many species, ranging from yeast to man (10, 13). As MPF activity oscillates with the cell cycle, becoming detectable in late G2 and disappearing in early G1, it may be a fundamental regulator of M phase in eukaryotes. Despite the importance of MPF in the cell cycle, the protein(s) responsible for MPF activity has not been identified as yet.

A cell-free system from amphibian eggs has been developed in which nuclei can be induced to undergo early mitotic events by the addition of crude or partially purified preparations of MPF (14–16). Experiments in this cell-free system implicated protein phosphorylation in the mechanism of action of MPF (17), results consistent with the observations (18, 19) of increased protein phosphorylation when MPF appears during oocyte maturation or after MPF injection. The hypothesis that a protein kinase is involved in the regulation of the G2 → M transition is supported by observations of increased phosphorylation of histones, lamin, and other proteins during M phase (20, 21). Furthermore, in yeast at least two of the mutations that cause arrest in G2 affect genes encoding protein kinase activities (22–24). As genes encoding proteins of similar sequence can also be detected in mammalian cells (25), protein kinases may have a widespread importance in regulating the G2 → M transition. In fact, MPF may be a phosphoprotein since its extraction from cells is enhanced by the presence of phosphatase inhibitors (see ref. 13, and antibodies against thior-phosphate immunoprecipitates MPF from extracts prepared in the presence of adenosine 5'-[γ-thio]triphosphate (ATP[γ-S]) (26).

In this paper, six chromatographic steps were used to purify from Xenopus unfertilized eggs protein fractions that are able to induce M phase in the cell-free assay and when injected into immature, cycloheximide-treated Xenopus oocytes. Fractions able to induce germinal vesicle breakdown (GVBD) in injected oocytes possessed a protein kinase activity, although this kinase was either absent or greatly reduced in some fractions that could induce M phase in the cell-free system.

MATERIALS AND METHODS

Materials. Xenopus laevis were obtained from Xenopus I (Ann Arbor, MI). ATP[γ-S], α-naphthyl phosphate, and 4',6-diamidino-2-phenylindole were from Boehringer Mannheim, and Brij 35 was from Pierce. Bovine serum albumin (BSA; Pentex, fraction V) was from Miles. All other reagents were from Sigma. Green A Matrix gel was from Amicon. DEAE- Sephadex, heparin-Sepharose, TSK 3000SW (0.8 cm × 30 cm), Mono Q HR 5/5, and Mono S HR 5/5 columns were from Pharmacia-LKB. [γ-32P]ATP was prepared by the procedure of Johnson and Walsh (27). The heat-stable inhibitor of cAMP-dependent protein kinase (PKI) was pu-

Abbreviations: MPF, maturation-promoting factor; GVBD, germinal vesicle breakdown; PKI, heat-stable inhibitor of cAMP-dependent protein kinase; BSA, bovine serum albumin; ATP[γ-S], adenosine 5'-[γ-thio]triphosphate.

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rified from rabbit skeletal muscle by the method of Walsh et al. (28). NaDodSO₄/PAGE was performed as described by Laemmli (29).

Assay of MPF Activity in a Cell-Free System. Extracts able to cause nuclear formation in vitro were prepared as described (15), and demembranated sperm nuclei were incubated in these extracts for 1 hr. Then 25 μl of the extract was added to 50 μl of the sample to be assayed for MPF activity, and the mixture was incubated for 2 hr at 19°C. Five to 10 μl of the mixture was removed after 1 or 2 hr, mixed with an equal volume of a 4',6-diamine-2-phenylindole solution (5 μg/ml), and observed by phase-contrast and fluorescence microscopy.

A unit of MPF activity was defined as the minimum amount that causes at least 20% of the pronuclei to enter M phase during a 2-hr incubation. To assess the amount of activity, a sample was serially diluted 1:1 with 100 mM sodium β-glycerophosphate/20 mM Hepes, pH 7.5/15 mM MgCl₂/5 mM EGTA/1 mM dithiothreitol. The reciprocal of the highest dilution in which activity could be detected defined the amount of activity initially present in 50 μl of the sample.

Assay of MPF Activity by Microinjection of Xenopus Oocytes. After incubation for 30 min in OR-2 medium (37) containing cycloheximide (0.5 μg/ml) at a concentration that inhibits both progesterone- and PKI-induced maturation, groups of 5–10 oocytes (>1.2 mm in diameter) were microinjected with 100 nl of sample. Injected oocytes were incubated for 2 hr at room temperature, and the fraction that underwent GVBD was determined by scoring for "white spot" formation. GVBD was confirmed by manually dissecting oocytes after fixation in 10% (wt/vol) trichloroacetic acid.

Preparation of 250,000 × g Supernatants from Xenopus Eggs. Sixty to 80 ml of dejellied eggs were washed with 400 ml of cold modified extraction buffer (MEB, ref. 19) consisting of 80 mM sodium β-glycerophosphate, 50 mM NaF, 20 mM EGTA, 15 mM MgCl₂, 20 mM Hepes (pH 7.5), 1.5 mM dithiothreitol, leupeptin (3 μg/ml), and 300 μM phenylmethylsulfonyl fluoride. The MEB was decanted, and eggs were suspended in 50 ml of MEB/20 mM α-naphthyl phosphate. The eggs were poured into 30-ml centrifuge tubes containing 0–15 ml of MEB/20 mM α-naphthyl phosphate. After excess MEB was withdrawn, eggs were crushed by centrifugation at 15,000 × g for 15 min. Crude cytoplasmic fractions were pooled, and ATP[y-S] and cytochalasin B were added to 1 mM and 10 μg/ml, respectively. The crude cytoplasmic fraction was again centrifuged for 15 min. The material between the pellet and lipid cap was centrifuged for 4 hr at 50,000 rpm in a Beckman SW 55 rotor. Usually 60 ml of dejellied eggs produced ~30 ml of the crude cytoplasmic fraction, from which ~20 ml of particle-free supernatant was obtained.

Purification of MPF. A 0–34% (NH₄)₂SO₄ fraction from the supernatant was prepared by the addition of 0.6 vol of 3.8 M (NH₄)₂SO₄ dissolved in 80 mM sodium β-glycerophosphate/15 mM MgCl₂/20 mM EGTA/20 mM Hepes, pH 7.5/1 mM dithiothreitol. The mixture was incubated on ice for 30 min, and the precipitate was collected by centrifugation. The precipitate was resuspended in 6 ml of dialysis buffer (DB: 100 mM sodium β-glycerophosphate/15 mM MgCl₂/5 mM EGTA/20 mM Hepes, pH 7.5/100 μM phenylmethylsulfonyl fluoride/1 mM dithiothreitol), dialyzed against 80–100 ml of DB for 4–6 hr, and stored at ~70°C.

The dialyzed (NH₄)₂SO₄ fraction from 40 ml of particle-free supernatant (~15 ml) was thawed, diluted with 45 ml of buffer A (5 mM MgCl₂/2 mM EGTA/20 mM Tris-HCl, pH 7.0/1 mM dithiothreitol), and centrifuged for 15 min at 15,000 × g. The supernatant was applied to a DEAE-Sephacel column (2.5 cm × 4.0 cm) that had been equilibrated with buffer A/25 mM NaCl/1 mM 3-[3-cholamidopropyl]dimethylammonio]-1-propanesulfonate, and the column was washed with buffer A/25 mM NaCl. Proteins were eluted with buffer A/200 mM NaCl, and the eluate was applied directly to a heparin-Sepharose column (2.5 cm × 4.0 cm) equilibrated with buffer A/300 mM NaCl. The heparin-Sepharose column was washed with buffer A/400 mM NaCl, and proteins were eluted with buffer A/400 mM NaCl. The eluate (~45 ml) was collected, diluted with 1/3 vol of buffer A, and applied to a Green A Matrex gel column (1.1 cm × 4.0 cm) equilibrated with buffer A/300 mM NH₄Cl. Proteins were eluted with buffer A/600 mM NH₄Cl and precipitated by the addition of an equal volume of 3.8 M (NH₄)₂SO₄ solution. The precipitate was resuspended in 200 μl of TSK buffer (DB except phenylmethylsulfonyl fluoride was omitted and 0.01% Brij 35 was added), loaded onto an LKB Ultrochrom GTi HPLC, and subjected to gel filtration at a flow rate of 0.05 ml/min through two TSK 3000SW columns coupled in tandem. Fractions (0.5 ml) were collected and assayed in the cell-free extracts. Active TSK fractions from two purifications were pooled and diluted with 3 vol of buffer A/0.01% Brij 35. The sample was applied to a Mono Q column equilibrated with buffer B (buffer A/25 mM NaCl/0.01% Brij 35), and the column was washed with buffer B. Proteins were eluted with a 30-min gradient to 275 mM NaCl in buffer B. Fractions (1 ml) were collected, dialyzed overnight against 1 liter of DB, and assayed in the cell-free system. Fractions with activity were pooled and diluted with 3 vol of buffer C (buffer A except Tris-HCl was replaced by 10 mM Hepes, pH 7.0/0.01% Brij 35). The sample was applied to a Mono S column that had been equilibrated with buffer C/25 mM NaCl, and the column was washed with buffer C/25 mM NaCl. Proteins were eluted with a 30-min gradient to 600 mM NaCl in buffer C. Fractions (1 ml) were collected, dialyzed, and assayed.

Active Mono S fractions were divided into 50- or 100-μl samples and stored at ~70°C. Preparations varied in their stability after freezing and thawing, and in some cases 100 μM ATP (from a 1 mM stock at pH 7.0) was added to help preserve MPF activity.

Determination of Protein Concentration. Protein concentration was determined by the method of Bradford (30) for all samples except the Mono S fractions. The amount of protein in the Mono S fractions was determined by a modification of the procedure of Graziani et al. (31). Samples (40, 20, and 10 μl) of each fraction were separated by NaDodSO₄/PAGE on 10% polyacrylamide gels. Adjacent lanes contained 10–60 ng of BSA. The gels were silver stained (32) and scanned by using a Quick Scan R & D densitometer that was equipped with an integrator pen and a pin-point slit (Helena Laboratories, Beaumont, TX). Two procedures were used to estimate the total protein.

(i) The maximum absorbance was set to 60 ng of BSA, and a standard curve was obtained. Under these conditions the absorbance of 10, 15, and 20 ng of BSA was linear, and the line extrapolated to zero. Mono S fractions were scanned, and the amount of protein in each band was determined from the linear portion of the standard curve. The total protein in each fraction was calculated as the sum of the protein in each band. By the use of this procedure the total protein in the three active Mono S fractions in Table 1 was estimated to be 4.3 μg. However, with this maximal absorbance setting, the sensitivity of the densitometer was too low to detect a few minor proteins that could be detected visually. Therefore, an alternative method was also used to estimate the amount of protein.

(ii) The sensitivity of the densitometer was increased by adjusting the absorbance so that 20 ng of BSA gave a maximal value. However, at this sensitivity a linear standard curve could not be obtained. Therefore, to provide an estimate of the maximum amount of protein in a band, the absorbance of the band in various dilutions of the sample was compared to
that of 10 ng of BSA. Each band with an absorbance equal to or less than that of 10 ng of BSA was designated to contain 10 ng of protein. By the use of this technique, the total protein in the three active fractions in Table 1 was estimated to be 8.7 μg. The value obtained in this manner was an overestimate of the actual amount of protein, since the absorbance of many bands was less than one-half that of 10 ng of BSA. We have used the mean of the values obtained by these two methods as the total protein shown for Mono S fractions in Table 1.

**Protein Kinase Assays.** Phosphorylation of endogenous substrates in the dialyzed Mono S fractions was performed by mixing 45 μl of sample with 5 μl of DB containing 1 mM ATP, 10^8 cpm of [γ-32P]ATP, and 0.17 μg of PKI. Reactions were incubated for 15 min at 30°C and terminated by the addition of 1/4 vol of 5 × electrophoresis sample buffer (29). Proteins were separated by NaDodSO4/PAGE on 10% gels, silver-stained, and processed for autoradiography. Reactions with histone H1 (type IIIS, 0.5 mg/ml) were carried out for 15 min at 30°C in a final volume of 25 μl containing 2.5 μl of sample, 100 mM sodium β-glycerophosphate, 15 mM MgCl2, 5 mM EGTA, 20 mM Hepes (pH 7.5), 100 μM ATP, 10^8 cpm of [γ-32P]ATP, 1 mM dithiothreitol, and 0.17 μg of PKI. The reactions were terminated as described above, and proteins were separated on 15% polyacrylamide gels.

**RESULTS**

Initial experiments involved efforts to optimize the extraction and stabilization of MPF activity from eggs. Preliminary experiments indicated that β-glycerophosphate, NaF, and α-naphthyl phosphate, putative phosphatase inhibitors, helped to increase recovery, and the addition of ATP[γ-S] was essential for stability during further purification. However, both α-naphthyl phosphate and NaF interfered with the assay of MPF activity in the cell-free extracts and had to be removed by dialysis prior to assay. In contrast, β-glycerophosphate markedly increased the ability to detect MPF in the assay, since activity often could not be detected without prior dialysis against a β-glycerophosphate buffer. However, β-glycerophosphate alone was unable to induce M phase. These results suggest that β-glycerophosphate may have additional properties besides phosphatase inhibition that help to stabilize MPF activity. Even with the addition of ATP[γ-S] to extracts and the use of β-glycerophosphate in the assay, MPF activity was highly unstable.

MPF could be eluted from DEAE-Sephalcel, heparin-Sepharose, and Green A Matrix gel by sequential step gradients. The 0–34% ammonium sulfate fraction was applied to a DEAE-Sephalcel column, and material was eluted with 200 mM NaCl. This step did not result in significant purification but removed lipid and yolk components that interfered with later steps. The eluate from DEAE-Sephalcel was applied directly to heparin-Sepharose, which was washed with 300 mM NaCl, and material was eluted with 400 mM NaCl. Although much protein was removed from the preparation by this step, much activity was also lost, even though none could be detected in the breakthrough fractions. Nevertheless, the heparin-Sepharose step was necessary to remove proteins that could not be removed by other columns and to lower the total protein concentration to a level suitable for later application onto the HPLC and FPLC columns. The heparin-Sepharose eluate was applied to a Green A Matrix gel column, and material was eluted with 600 mM NH4Cl. The sequential step gradients through DEAE-Sephalcel, heparin-Sepharose, and Green A Matrix gel removed >99% of the protein from the starting material and resulted in an ~25-fold purification with a 10–15% yield (Table 1). The pooled Green A Matrix gel eluate was precipitated with ammonium sulfate, resuspended, and applied to a TSK 3000SW column. As shown in Fig. 1A, MPF activity eluted as a broad peak with an apparent molecular mass of 200 kDa. In some experiments active fractions were pooled and frozen at −70°C at this point, prior to combination with a TSK eluate from another preparation. Active TSK fractions were then chromatographed on a Mono Q column (Fig. 1B), where MPF activity eluted at a NaCl concentration of 150 mM. Active Mono Q fractions were pooled and applied to a Mono S column, where MPF activity eluted at 250 mM NaCl (Fig. 1C). The final preparation was purified >3000-fold with a recovery of 1% as shown in Table 1. In practice, the behavior of MPF on the TSK 3000SW and Mono Q columns was highly reproducible, allowing us to pool fractions solely on the basis of their position in the protein UV-absorbance profile, without dialysis and assay. With this protocol only the Mono S fractions were dialyzed and assayed, as were samples of the eluates from all of the previous columns. The use of step gradients and the ability to predict the behavior of MPF on the HPLC and FPLC columns enabled us to subject the 0–34% ammonium sulfate sample to six chromatographic steps with only a single assay within 54 hr. The speed of this procedure may have helped to overcome the inherent instability of MPF during purification.

Fig. 2A shows a silver-stained polyacrylamide gel of the fractions eluted from the Mono S column. Similar profiles have been observed in six independent purifications. Several proteins are visible in minor amounts in fractions with MPF activity, but two proteins of 45 kDa and 32 kDa were consistently observed in every preparation. These two proteins fractionated with activity both when the Mono S column followed the Mono Q column, as described above, and when the order of these columns was reversed (data not shown). The 45-kDa protein has a very close correlation with ability to induce nuclear envelope breakdown and chromosome condensation in the cell-free system, and the amount of this

<table>
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<th>Step</th>
<th>Total protein, mg</th>
<th>Total activity, units × 10^-3</th>
<th>Specific activity, units/mg</th>
<th>Recovery, %</th>
<th>Purification, fold</th>
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<tr>
<td>Supernatant (250,000 × g)</td>
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<td>5.2</td>
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<tr>
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<td>13</td>
<td>82</td>
<td>2.5</td>
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<tr>
<td>DEAE-Sephalcel</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
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<tr>
<td>Heparin-Sepharose</td>
<td>31</td>
<td>1.7</td>
<td>55</td>
<td>15</td>
<td>11</td>
</tr>
<tr>
<td>Green A Matrix gel</td>
<td>9.9</td>
<td>1.3</td>
<td>130</td>
<td>12</td>
<td>25</td>
</tr>
<tr>
<td>TSK 3000SW</td>
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<td>0.48</td>
<td>280</td>
<td>4.4</td>
<td>54</td>
</tr>
<tr>
<td>Mono Q</td>
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<td>2,900</td>
<td>1.8</td>
<td>560</td>
</tr>
<tr>
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<td>0.12</td>
<td>18,000</td>
<td>1.1</td>
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</table>

Total protein of samples from the 250,000 × g supernatant through the TSK 3000SW step was the sum of that in two separate preparations. After the TSK 3000SW step both preparations were applied to the Mono Q column. The DEAE-Sephalcel eluate was not analyzed as it was applied directly onto the heparin-Sepharose column.

*Only fractions 11–13 of Fig. 1C, which induced M phase in the cell-free system and GVBD in oocytes, are represented.*
protein is greatest in fractions that induce GVBD in cycloheximide-treated *Xenopus* oocytes. The 32-kDa protein also correlated with fractions having the greatest MPF activity in both assay systems but was absent from some side fractions that had activity only in the cell-free assay (Fig. 2A).

In view of the evidence that protein phosphorylation is involved in MPF action and the hypothesis that MPF may be a protein kinase, fractions from the Mono S column were examined for protein kinase activity. As shown in Fig. 2B, the 45-kDa protein was the only protein in the purified prepara-

**DISCUSSION**

The microinjection of cytoplasm or its extracts into immature oocytes of echinoderms or amphibians has been the assay for MPF activity for many years. In these systems MPF activity is detected by its ability to induce the recipient to undergo GVBD, which in cycloheximide-treated *Xenopus* oocytes occurs within 2 hr. We and others have shown that partially purified preparations of MPF can act in vitro to induce M phase in nuclei incubated in extracts of *Xenopus* eggs (14–16). In this paper, the response of interphase pronuclei in the cell-free extracts was used to assay MPF during its purification. The final purified preparations were able to induce both M phase in cell-free extracts and GVBD in injected *Xenopus* oocytes. Several purified proteins including the regulatory subunit of cAMP-dependent protein kinase (9), PK1 (9), and the human HRAS protein (33) have been shown to induce GVBD when injected into oocytes. Although these proteins can sometimes act almost as rapidly as MPF, none is able to induce GVBD in cycloheximide-treated oocytes. The preparations that we have obtained were able to induce GVBD within 2 hr in cycloheximide-treated oocytes, suggesting that genuine MPF activity has been purified.
Studies have indicated (34) that surf clam egg cyclin A mRNA can induce GVBD in Xenopus oocytes. However, cyclins have not been identified as yet in Xenopus eggs, and the relationship between cyclins and MPF is unknown.

In earlier work, partially purified preparations of MPF from Xenopus eggs were shown by Coomassie blue staining of NaDODSO\textsubscript{4}/polyacrylamide gels to have proteins of 62 kDa, 53 kDa, 49 kDa, 39 kDa, and 37 kDa (35). Preparations from mitotic Hela cells were enriched in a 50-kDa protein (36). In both cases only the final pooled fractions were examined. We have used the more sensitive technique of silver-staining NaDODSO\textsubscript{4}/polyacrylamide gels to visualize proteins eluting from the Mono S column and have followed the appearance and disappearance of proteins with changes in MPF activity. In the preparations described here proteins of 45 kDa and 32 kDa consistently fractionated with MPF activity. The 45-kDa protein was invariably correlated with the ability to induce M phase in the cell-free assay, and proteins in neighboring fractions without the 32-kDa protein, were also active in this assay. However, the presence of both proteins was correlated with fractions able to induce GVBD in oocytes. Since the 45-kDa protein was also more abundant in these fractions, the ability of only the peak fractions to induce GVBD would indicate that the cell-free assay is more sensitive than the microinjection assay. The cell-free assay may be able to detect lower levels of MPF activity because samples that are tested make up 67% of the volume of the reaction mixture, whereas they comprise only 5–10% of the oocyte volume after microinjection. However, the results are also consistent with the possibility that the 45-kDa and 32-kDa proteins are required to induce GVBD, whereas the 45-kDa protein is sufficient to induce M phase in the cell-free assay. (If the 32-kDa protein was already present in the egg extracts, but not in immature oocytes, then the addition of the 45-kDa protein alone might be sufficient to induce M phase.)

Upon gel filtration through TSK 3000SW columns, MPF eluted as a broad peak of ≈200 kDa. This behavior was observed in seven consecutive experiments, and all data presented in this paper were from such preparations. However, in other experiments MPF activity eluted with a lower apparent molecular mass. Nevertheless, in such preparations MPF behavior on Mono Q and Mono S remained the same as shown in Fig. 1B and C, and the 45-kDa and 32-kDa proteins fractionated with MPF activity.

As described above, there is considerable evidence suggesting that protein phosphorylation is involved in the mechanism of action of MPF. In this regard, it is of great interest that protein kinase activity coeluted with MPF activity. Phosphorylation of the 45-kDa protein was observed only with fractions in which protein kinase activity against other substrates was also detectable, suggesting that this protein may be a substrate. It is not yet clear whether the protein kinase activity or phosphorylation of the 45-kDa protein is essential for MPF action. The first Mono S fractions with activity in the cell-free assay elute ahead of the protein kinase activity, suggesting that the protein kinase is not necessary for the induction of M phase in cell-free extracts. On the other hand, fractions that are able to induce GVBD also have the highest protein kinase activity. One might speculate that the induction of GVBD requires the protein kinase activity in addition to the 45-kDa protein. Since the 32-kDa protein correlated with the protein kinase activity, it may be a second component required to induce GVBD.

Regardless of whether the protein kinase that elutes with MPF is necessary for GVBD, increases in protein phosphorylation in M phase are well documented. The nuclear laminins, vimentin, and ribosomal protein S6, among others, are known to be phosphorylated in maturing oocytes and mitotic cells. As these proteins do not appear to be substrates for the protein kinase in our preparation, MPF must be able to act indirectly to control the phosphorylation of these substrates. The purified preparations of MPF that we have described may be useful in elucidating the mechanisms regulating the transition from G\textsubscript{2} to M phase in dividing cells and in defining the role of protein phosphorylation in this transition.

We are grateful to Eleanor Erikson for helpful suggestions and numerous stimulating discussions and to Dr. Charles McHenry (Department of Biochemistry) for use of his gel scanning densitometer. This research was supported by grants to J.L.M. from the National Institutes of Health (GM 26743) and the American Cancer Society (CD-279). J.L.M. was supported in part during this work by an established investigatorship from the American Heart Association.