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Tyrosine Phosphorylation and Activation of Homologous Protein Kinases during Oocyte Maturation and Mitogenic Activation of Fibroblasts

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Meiotic maturation of *Xenopus* and sea star oocytes involves the activation of a number of protein-serine/threonine kinase activities, including a myelin basic protein (MBP) kinase. A 44-kDa MBP kinase ($p44^{mpk}$) purified from mature sea star oocytes is shown here to be phosphorylated at tyrosine. Antiserum to purified sea star $p44^{mpk}$ was used to identify antigenically related proteins in *Xenopus* oocytes. Two tyrosine-phosphorylated 42-kDa proteins (p42) were detected with this antiserum in *Xenopus* eggs. *Xenopus* p42 chromatographs with MBP kinase activity on a Mono Q ion-exchange column. Tyrosine phosphorylation of *Xenopus* p42 approximately parallels MBP kinase activity during meiotic maturation. These results suggest that related MBP kinases are activated during meiotic maturation of *Xenopus* and sea star oocytes. Previous studies have suggested that *Xenopus* p42 is related to the mitogen-activated protein (MAP) kinases of cultured mammalian cells. We have cloned a MAP kinase relative from a *Xenopus* ovary cDNA library and demonstrate that this clone encodes the *Xenopus* p42 that is tyrosine phosphorylated during oocyte maturation. Comparison of the sequences of *Xenopus* p42 and a rat MAP kinase (*ERK1*) and peptide sequences from sea star $p44^{mpk}$ indicates that these proteins are close relatives. The family members appear to be tyrosine phosphorylated, and activated, in different contexts, with the murine MAP kinase active during the transition from quiescence to the G_1 stage of the mitotic cell cycle and the sea star and *Xenopus* kinases being active during M phase of the meiotic cell cycle.

Progesterone induces *Xenopus laevis* oocytes to mature—that is, to leave first meiotic prophase and progress part way through meiosis and arrest as eggs, in second meiotic metaphase. A burst in the phosphorylation of numerous proteins is triggered near the time of nuclear envelope (germinal vesicle) breakdown (GVBD) (46). Similarly, with sea star oocytes, 1-methyladenine induces both maturation and protein phosphorylation (19). Only a few of the phosphoproteins in maturing oocytes have been identified, such as the 40S ribosomal protein S6 (33, 38, 39, 51), nucleoplasmin (17), nuclear lamins (48), and possibly histone H1. Using these and other proteins as substrates, several maturation-activated protein-serine/threonine kinases have been characterized from sea star oocytes (6, 42, 52, 55) and *Xenopus* oocytes (12, 44, 47; reviewed in reference 53).

The major histone H1 kinases that are activated during *Xenopus* (20, 28) and sea star (6, 42) oocyte maturation are the amphibian and echinoderm homologs of the 34-kDa protein kinase encoded by the *Schizosaccharomyces pombe* mitotic control gene *cdc2*. Microinjection of active preparations of histone H1 kinase into immature oocytes is sufficient to induce GVBD (44). Two ribosomal S6 kinases (S6K-I and S6K-II) are also activated at GVBD in progesterone-treated *Xenopus* oocytes (12, 44, 47) or following microinjection of the frog homolog of $p34^{cdc2}$ (68). Both S6 kinases appear to be activated as a consequence of serine and threonine phosphorylation by unknown protein kinases in maturing oocytes (22).

Protein kinase activities with specificity for myelin basic protein (MBP) are also activated during oocyte maturation (12, 52). An MBP kinase has been purified from mature sea star oocytes (63). The purified kinase is a single silver-stained 44-kDa polypeptide on a sodium dodecyl sulfate (SDS)-polyacrylamide gel. The sea star kinase was designated $p44^{mpk}$ (*mpk* signifies MBP or meiosis-activated protein kinase). $p44^{mpk}$ contains phosphate in mature but not immature sea star oocytes (64). The properties of $p44^{mpk}$ suggest that it may be related to protein kinases activated during mitogenic stimulation of mammalian somatic cells.

In quiescent Swiss 3T3 cells, mitogenic stimulation with epidermal growth factor involves the activation of numerous serine kinase activities, including two MBP kinases (4). These MBP kinase activities resemble a mitogen-activated protein (MAP) kinase purified from insulin-stimulated 3T3L1 adipocytes (57, 58) in substrate specificity and physical properties. Similar kinase activities have been reported to be involved in a variety of different cellular processes (7, 35, 36, 60). For instance, a MAP kinase is activated in postmitotic adrenal chromaffin cells stimulated to secrete catecholamines (21). In PC-12 cells, neuronal differentiation induced by nerve growth factor results in activation of MAP kinase (49). In adipocytes, the protein-serine phosphatase inhibitor okadaic acid activates MAP kinase (34).

Activation of MAP kinase in insulin-stimulated adipocytes is accompanied by tyrosine phosphorylation of an approximately 40-kDa protein (59). Purification of MAP kinase to apparent homogeneity yielded a 42-kDa protein that contained both phosphotyrosine and phosphothreonine (21, 61). Using a renaturation assay, Ferrell and Martin (24) have also shown that protein-serine kinase activity comigrates with a 42-kDa phosphotyrosine-containing protein. Experiments

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with phosphatases indicate that MAP kinase activity requires both tyrosine and serine/threonine phosphorylation (3, 5, 70). Two-dimensional gel analysis shows that the major form of MAP kinase comigrates with a phosphotyrosine-containing protein, p42 (21, 61). Phosphorylated p42 was previously detected in quiescent cells stimulated with diverse mitogens such as phorbol ester, platelet-derived growth factor (PDGF), and epidermal growth factor (15, 40, 41, 72). Unphosphorylated p42 is present in resting cells and becomes phosphorylated on serine, threonine, and tyrosine residues following stimulation (16).

In *Xenopus* oocytes, progesterone-induced entry into meiosis is similarly accompanied by the appearance of phosphate on tyrosine and serine residues of a 42-kDa protein (Xp42), which lacks phosphate in parthogenetically activated eggs (39, 45, 51). Partial proteolytic cleavage analysis suggests that Xp42 is structurally related to mouse fibroblast p42 (14). These data suggest that Xp42 could be a murine MAP kinase relative and predict that it should phosphorylate MBP when activated.

A partial clone for a MAP kinase relative, ERK1, has recently been isolated from a rat brain cDNA library (8). This clone contains many peptide sequences found in a MAP kinase purified from insulin-stimulated Rat-1 cells. ERK1 shares greater than 50% identity with two yeast kinases, KSS1 and FUS3, which regulate entry into G_1 following arrest by pheromone (18). In addition, the MAP kinase gene, *ERK1*, is expressed in many tissues, with highest expression in the brain and spinal cord. Preliminary data suggest that ERK1 may be one of a small family of related protein kinases (8).

Given the strikingly similar properties of the sea star $p44^{mpk}$, Xp42, and murine MAP kinase, we attempted to determine the relatedness of these three kinases by using anti-$p44^{mpk}$ antibodies. We found that Xp42 is antigenically related to sea star $p44^{mpk}$ and copurifies over one ion-exchange column with MBP kinase activity. Therefore, we used the *ERK1* cDNA sequence to clone a related cDNA from a *Xenopus* ovary library and obtained evidence that the clone encodes Xp42. The sequence of this cDNA confirms that Xp42 and murine MAP kinase are homologous protein-serine kinases.

MATERIALS AND METHODS

Animals and materials. *Pisaster ochraceus* were obtained from the beaches in the Vancouver area. Female, adult, gonadotropin-primed *X. laevis* were brought from Xenopus I (Ann Arbor, Michigan). $p44^{mpk}$ was purified to homogeneity from maturing sea star oocytes (63). Rabbit polyclonal anti-$p44^{mpk}$ antibodies that were affinity purified on a $p44^{mpk}$-agarose column were prepared as described previously (64). [γ-^{32}P]ATP was from New England Nuclear. [^{35}S]methionine (Translabel), $^{32}P_i$, and antiphosphotyrosine monoclonal antibody PY-20 were from ICN Biochemicals.

Oocyte maturation and extract preparation. Cytosolic extracts of immature and mature sea star oocytes were prepared as previously described (64). Stage VI *Xenopus* oocytes were obtained from ovary by collagenase digestion and sorting by size. Oocytes were treated at 20°C for up to 8 h with progesterone (10 μg/ml) in OR-2 medium (73). The extent of maturation was determined by scoring the percentage of oocytes that displayed a distinct white spot in the animal hemisphere, indicative of GVBD, which typically occurred about 7 to 8 h after progesterone treatment. Immature or mature oocytes were allowed to settle, the OR-2 medium was removed, and the cell pellet was ruptured in homogenizing buffer A (20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid [HEPES; pH 7.5], 10 mM ethylene glycol-bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid [EGTA], 2.5 mM $MgCl_2$, 1 mM dithiothreitol [DTT], 1 mM phenylmethylsulfonyl fluoride, 2 mM Na_2VO_4, 40 mM β-glycerol phosphate, 3 μg of leupeptin per ml). The homogenate was centrifuged at 12,000 × *g*, and the resulting cytosol was frozen at −70°C (45). *Xenopus* egg crushate was similarly prepared from eggs dejellied with cysteine.

For in vivo ^{32}P labeling of immature and mature *Xenopus* oocytes, two groups of 100 manually dissected stage VI oocytes were placed in 24-well plates containing 5 mCi of $^{32}P_i$ per ml in MBS (modified Barth's saline). After 4 h at 25°C, oocytes were transferred to nonradioactive MBS containing 0 or 10 μg of progesterone per ml for 16 h, at which time extracts were prepared.

Electrophoresis. One-dimensional SDS-polyacrylamide gel electrophoresis (PAGE) was performed on 1- or 1.5-mm-thick gels, using the buffer system described by Laemmli (43) and a 12.5% acrylamide–0.103% bisacrylamide separating gel. Samples were diluted with fivefold-concentrated SDS sample buffer (125 mM Tris-HCl [pH 6.8], 4% SDS, 0.01% bromophenol blue, 10% mercaptoethanol, 20% glycerol), boiled for 5 min, and electrophoresed for 17 h at 10 mA. Samples for two-dimensional gel electrophoresis were diluted into 0.3% SDS–65 mM DTT–20 mM Tris (pH 8.0)–1 mM EDTA. RNase-DNase solution was added to the lysate to final concentrations of 0.04 mg of RNase A and 0.1 mg of DNase I per ml, and the lysate was incubated for 1 min before being placed on dry ice. Lysates were then lyophilized and resuspended in 2DSB (9 M urea, 4% Nonidet P-40 [NP-40], 2% ampholytes [pH range, 3.5 to 10], 100 mM DTT). Two-dimensional electrophoresis was performed according to the method of Garrels (26), with modifications (40).

Immunoblotting studies. Proteins were separated by one- or two-dimensional gel electrophoresis and transferred to an Immobilon membrane either in transfer buffer (25 mM Tris, 192 mM glycine, 20% methanol) at 300 mA for 3 h (Fig. 1 and 4) or by semidry transfer (in 50 mM aminocaproic acid–300 mM Tris–25% isopropanol) (Fig. 2, 3, 7, and 8) at 0.8 mA/cm^2 for 2 h. Membranes were blocked with 3% gelatin (Fig. 1 and 4) or 5% bovine serum albumin plus 1% ovalbumin (Fig. 2, 3, 7, and 8). Blots were probed overnight with affinity-purified $p44^{mpk}$ antibody at a 1:1,000 dilution or monoclonal antibody PY-20 at 2 μg/ml or for 1 h with a 1:2,000 dilution of rabbit antiserum 7774. Following removal of the primary antibody, alkaline phosphatase-coupled immunoglobulin G was added to the blots, which were incubated for 2 to 3 h at room temperature. Alkaline phosphatase substrate (5-bromo-4-chloro-3-indolyl phosphate–Nitro Blue Tetrazolium) was added to the membrane, and color was developed for 5 to 15 min.

Column chromatography. Oocyte cytosol (ca. 2 mg of protein) was loaded onto a Mono Q column (1-ml bed volume) equilibrated in buffer B (12.5 mM β-glycerol phosphate, 12.5 mM morpholinepropanesulfonic acid [MOPS; pH 7.2], 0.5 mM EGTA, 7.5 mM $MgCl_2$, 2 mM DTT). The column was developed with a 15-ml linear 0 to 0.8 M NaCl gradient in buffer C (12.5 mM β-glycerol phosphate, 12.5 mM MOPS [pH 7.2], 1 mM DTT) in a Pharmacia fast protein liquid chromatography system, and 250-μl fractions were collected.

In some experiments, oocyte cytosol (ca. 1 mg of protein) was passed over a 1-ml Cellex-P column that was directly

linked to a 1-ml phenyl-Sepharose column. MBP phosphorylating activity and $p44^{mpk}$ did not bind to Cellex-P and were eluted from phenyl-Sepharose with buffer C containing 3% Brij 35.

Kinase and protein assays. $p44^{mpk}$ MBP kinase activity was assayed as described previously (63). Protein was estimated by the method of Bradford (9), with bovine serum albumin as a standard.

Cloning of *Xenopus* p42. Total cellular RNA was extracted from *Xenopus* ovary by the method of Chirgwin (11) and used to prepare cDNA by reverse transcription with avian myeloblastosis virus reverse transcriptase. Degenerate oligonucleotide primers based on the peptide sequences YFLYQI, CILAEM, and HPYLEQ in the rat ERK1 sequence (8) were synthesized as follows: primer 1A, CGCGA ATTCTAYTTYYTNTAYCARAT; primer 1B, CGCGGAT CCCATYTCNGCNARXATRCA; and primer 1C, CGCGG ATCCYTGYTCNARRTANGGRG (Y = C + T, R = A + G, N = A + C + G + T, and X = A + G + T). These primers were used pairwise in the polymerase chain reaction (PCR). Primer 1A had an added *Eco*RI site, and primers 1B and 1C had added *Bam*HI sites. The pair 1A and 1B was expected to give a 300-bp fragment, while the pair 1A and 1C was expected to give a 600-bp fragment based on the ERK1 sequence. PCR products of the expected sizes were detected with ovary RNA but not with liver RNA. Digestion of the 600-bp PCR product created a 300-bp fragment that was cloned into pBluescript (Stratagene) and sequenced. This clone (PCR clone 4) was ^{32}P labeled by nick translation and used to screen a baby *Xenopus* ovary cDNA library, inserted in the *Eco*RI site of λZAP (Stratagene Cloning Systems, San Diego, Calif.). Thirty-six positive clones were identified, at a rate of approximately 4 per 10^4 phage.

Fifteen positive plaques were isolated and plaque purified. Phagemid DNA was prepared from the 15 clones and digested with *Eco*RI. Eight of the clones had common 650- and 530-bp *Eco*RI fragments. Clone 3 was selected for further analysis. Single-stranded DNA was prepared from clone 3 or selected subclones and sequenced on both strands, using the dideoxy method and synthetic primers (62). Sequences were analyzed by using GenePro (Riverside Scientific, Seattle, Wash.) and compared with other protein sequences in the Protein Identification Resource data base, release 26.0.

The sequence shows three *Eco*RI sites within the clone 3 cDNA, creating fragments of 649 (5′), 126, 532, and 156 (3′) bp (the small fragments would not have been detected by restriction analysis). The 532-bp fragment contains the entire sequence of PCR clone 4 (see Fig. 6). Restriction analysis of the other seven clones showed that four also contained a 530-bp *Eco*RI fragment, together with another fragment of variable size, and the other three contained single, larger fragments. The data are consistent with 12 of 15 clones containing the same, or closely related, cDNAs, with different 3′ or 5′ endpoints. The high frequency of clones containing the same 5′ endpoint (creating the 649-bp fragment) suggests that this *Eco*RI site, like the next three (defining the 126- and 532-bp fragments), is encoded in the cDNA. However, the isolation of cDNA clones lacking the 532-bp *Eco*RI fragment suggests that the two *Eco*RI sites near the 3′ end of clone 3 (which lie in the 3′ untranslated region) may not be present in all cDNAs. This suggests that there may be other cross-hybridizing mRNAs containing different sequences. Northern (RNA) analysis of *Xenopus* adult ovary RNA showed two hybridizing species: a major band of about 5 kb and a minor band of about 3.5 kb (56).

Protein sequence analysis of $p44^{mpk}$. Tryptic peptides derived from purified sea star $p44^{mpk}$ were separated by narrow-bore reverse-phase high-performance liquid chromatography, using a Waters peptide analyzer equipped with a Vydac C4 column (2.1 by 150 mm) as described previously (1). Prior to application to the column, 10 μl of trifluoracetic acid (TFA) in H_2O (10:90, vol/vol) and 5 μl of 1 M EDTA were added to the digest. Chromatography solvents were solvent A (0.1% TFA in H_2O) and solvent B (0.08 to 0.095% TFA in H_2O-CH_3CN [30:70, vol/vol]). UV absorption values of the solvents were matched by titrating the TFA concentration in solvent B. Peptides were eluted with a 0 to 70% acetonitrile gradient, monitored at 215 nm, and collected manually. Isolated peptides were sequenced in a model 477A gas-liquid-phase sequenator (Applied Biosystems), and the resulting phenylthiohydantoin amino acid derivatives were analyzed in a model 120A (Applied Biosystems) chromatography system according to standard procedures.

Preparation of anti-p42 polyclonal antisera. PCR clone 4 was subcloned into a pATH bacterial expression vector (25). Expression of the PCR clone 4-TrpE fusion protein was induced with 3β-indoleacrylic acid. Total bacterial lysates were run on preparative SDS gels. The fusion protein was electroeluted from gel slices and used as an immunogen with complete Freund adjuvant to inject rabbits. Immunization with incomplete Freund adjuvant was repeated every 3 weeks. Antiserum 7774 was collected 1 week subsequent to the first boost.

Immunoprecipitation of *Xenopus* p42. Oocyte or egg extracts were solubilized in 2 volumes of 1.5% SDS–14 mM 2-mercaptoethanol with heating at 98°C for 3 min. The solubilized extract was further diluted into 10 volumes of NP-40 IPB (1% NP-40, 10 mM HEPES [pH 7.5], 2 mM EDTA, 50 mM NaF, 0.2 mM Na_3VO_4, 0.1% 2-mercaptoethanol, 40 μg of aprotinin per ml). The solution was incubated with 5 μl of antiserum 7774 at 0°C for 60 min. Formaldehyde-fixed *Staphylococcus aureus* was added to recover the immune complexes and incubated at 0°C for 30 min. The immune complexes were washed once through NP-40 IPB containing 10% sucrose and twice through NP-40 IPB. The resulting pellet was resuspended in SDS sample buffer.

In vitro transcription and translation of λ clone 3 cDNA. Ten micrograms of *Not*I-linearized pBluescript DNA, derived from λ clone 3, was incubated in transcription buffer containing 50 mM Tris-HCl (pH 7.5), 6 mM $MgCl_2$, 4 mM spermidine, 5 U of Inhibit-Ace, 500 μM ribonucleoside triphosphates, 500 μM $m^7G(5')ppp(5')G$, 10 mM DTT, and 100 U of T3 RNA polymerase in a total volume of 100 μl. The solution was incubated for 2 h at 37°C. The resulting RNA transcripts were extracted twice with phenol-chloroform (1:1, vol/vol) and once with chloroform and were ethanol precipitated. RNA was dissolved in H_2O. For in vitro translation, 3 μl of the in vitro transcript was incubated in a translation system consisting of rabbit reticulocyte lysate (Promega), 2 U of Inhibit-Ace, 20 μM amino acids (all but methionine), and 80 μCi of [^{35}S]methionine. The in vitro translation products were prepared for two-dimensional gel analysis as described above. For oocyte injections, 0.8 μCi of [^{35}S]methionine per oocyte was dried, dissolved in 50 nl of H_2O or RNA, and injected into the cytosol of manually dissected Dumont stage VI oocytes. Oocytes were incubated at 25°C for 24 h and then incubated with or without progesterone.

Nucleotide sequence accession number. Nucleotide sequence accession number M60977 has been assigned by GenBank for Xp42.

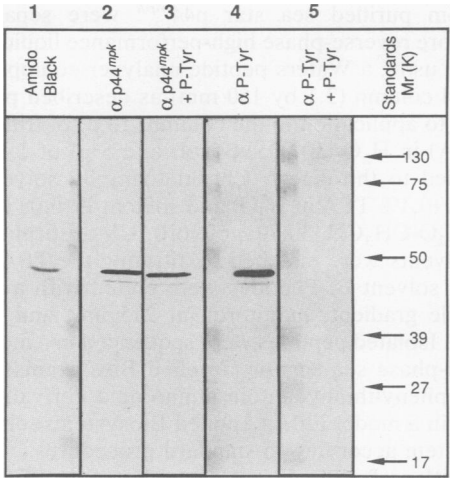

FIG. 1. Immunoblotting of purified sea star oocyte $p44^{mpk}$ with anti-$p44^{mpk}$ and antiphosphotyrosine antibodies. Purified $p44^{mpk}$ (ca. 2 μg) from GVBD-positive sea star oocytes was subjected to SDS-PAGE, transferred to a nitrocellulose membrane, and immunoblotted (Materials and Methods). Lanes: 1, amido black-stained blot of purified $p44^{mpk}$; 2, Western blot of purified $p44^{mpk}$ probed with anti-$p44^{mpk}$ antibodies (α); 3, assay in which anti-$p44^{mpk}$ antibodies were incubated with 2 mM phosphotyrosine before probing of the $p44^{mpk}$ blot; 4, Western blot probed with antiphosphotyrosine monoclonal antibody PY-20; 5, assay in which antibody PY-20 was incubated with 2 mM phosphotyrosine before probing of the blot. M_r values for standards are given in thousands (K).

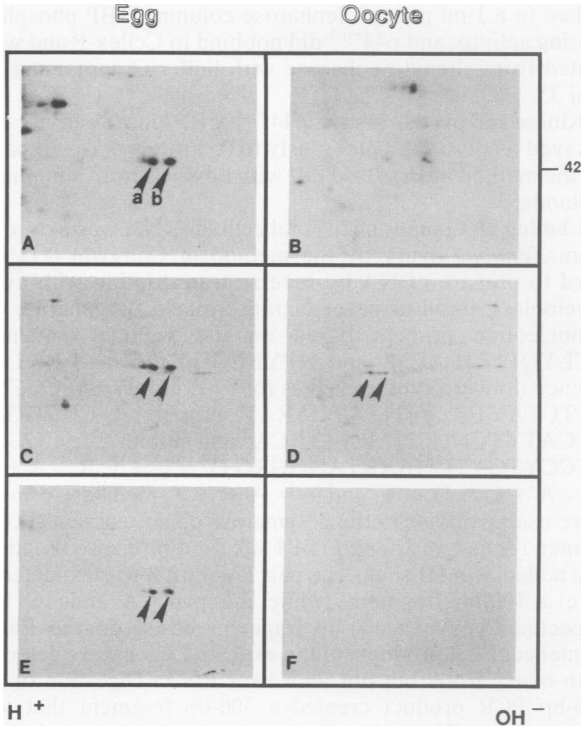

FIG. 2. Immunoblot analysis of phosphoproteins in *Xenopus* oocytes and eggs with anti-$p44^{mpk}$ antibodies and antiphosphotyrosine antibodies. Samples (100 μg) of *Xenopus* oocyte and egg crushates (Materials and Methods) were labeled by incubation with 1 mCi of $^{32}P_i$ in the presence of sodium pyruvate, NAD^+, and fructose 1,6-bisphosphate (14). Phosphoproteins were separated in the first dimension by isoelectric focusing at pH 3.5 to 10 and in the second dimension by 15% SDS-PAGE. Proteins were transferred to an Immobilon membrane and probed with affinity-purified anti-$p44^{mpk}$ antibodies (C and D) or antiphosphotyrosine monoclonal antibody PY-20 (E and F). The blots were autoradiographed to locate ^{32}P-proteins (A and B). The ^{32}P spots (arrowheads) overlie the immunoreactive spots shown below.

RESULTS

Tyrosine phosphorylation of $p44^{mpk}$ during sea star oocyte maturation. $p44^{mpk}$ was purified from mature sea star oocytes (63) and used to prepare a rabbit polyclonal antiserum, which was affinity purified over a $p44^{mpk}$-agarose column (64). Western immunoblots of purified $p44^{mpk}$ probed with the anti-$p44^{mpk}$ antibodies (Fig. 1, lane 2) revealed a strong immunoreactive band which comigrated with the silver-stained protein following electrophoresis on SDS-polyacrylamide gels (lane 1). The ability of a protein-tyrosine phosphatase inhibitor, sodium orthovanadate, to stabilize the kinase activity of $p44^{mpk}$ during its purification from GVBD-positive sea star oocytes hinted that the kinase might be subject to tyrosine phosphorylation (65). This was substantiated by the successful immunoblotting of purified $p44^{mpk}$ with monoclonal antibody PY-20, which recognizes tyrosine-phosphorylated proteins (lane 4). Binding of monoclonal antibody PY-20 to $p44^{mpk}$ was dependent on the presence of a phosphorylated tyrosine residue, since a parallel incubation of the antiphosphotyrosine antibodies in the presence of 2 mM phosphotyrosine completely eliminated binding of PY-20 (lane 5) but not of anti-$p44^{mpk}$ (lane 4). Tyrosine phosphorylation of $p44^{mpk}$ coincided with stimulation of its MBP phosphorylating activity near the time of GVBD, as shown by immunoblotting of partially purified $p44^{mpk}$ with antibody PY-20 (65).

***Xenopus* p42 reacts with anti-$p44^{mpk}$ and has properties of an MBP kinase.** In view of the tyrosine phosphorylation of $p44^{mpk}$ during sea star oocyte maturation, we were intrigued by the prospect that the tyrosine-phosphorylated 42-kDa proteins in frog eggs (named $pp42^{Xa}$ and $pp42^{Xb}$ in reference 14) might represent amphibian homologs. Extracts prepared from crushed *Xenopus* eggs or oocytes were incubated with ^{32}P, and the radiolabeled proteins were resolved by two-dimensional electrophoresis and transferred to an Immobilon membrane (Fig. 2). The two phosphorylated forms of Xp42 were identified by their positions on the gel relative to internal landmarks (Fig. 2A, marked a and b). These spots reacted strongly with antiphosphotyrosine monoclonal antibody PY-20 in metaphase-arrested eggs but not stage VI oocytes (Fig. 2E and F). In addition, these spots were also specifically recognized by affinity purified anti-$p44^{mpk}$ antibodies in *Xenopus* eggs (Fig. 2C). In the oocyte, the anti-$p44^{mpk}$ antibodies bound proportionately less well to the lower amounts of phosphorylated Xp42 present. A protein that might correspond to the nonphosphorylated form of Xp42 was not detected in two-dimensional gel blots of oocytes. This appears to be due to a higher affinity of the anti-$p44^{mpk}$ antibodies against phosphorylated Xp42 than against unphosphorylated Xp42, suggesting that the phosphorylation sites were involved in epitope recognition. The antiserum is not simply recognizing phosphotyrosine, however, because a number of tyrosine-phosphorylated proteins, including the PDGF receptor and pp41 of PDGF-stimulated 3T3 cells, are not detected with this serum (56). Also, recognition of phosphorylated Xp42 was not reduced by competition with phosphotyrosine (data not shown).

A putative unphosphorylated form of Xp42 was detected

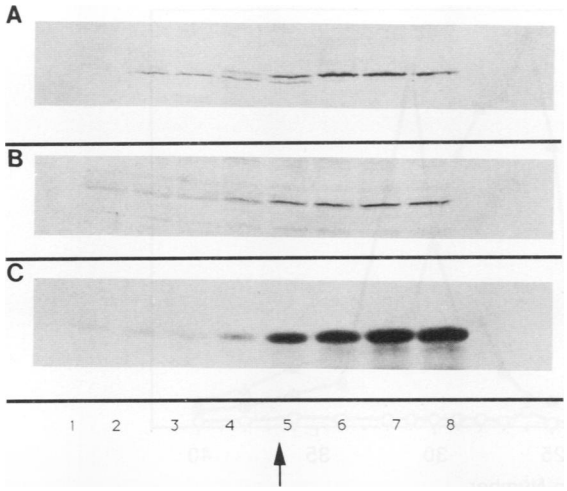

FIG. 3. Time course of p42 activation during progesterone-induced *Xenopus* oocyte maturation. Stage VI oocytes were treated with progesterone (10 μg/ml) for the times (in hours) indicated at the bottom. At each time point, oocyte extract was prepared (see Materials and Methods) and used for Western blotting and kinase assays. (A) Western blot of crushate probed with anti-p44^{mpk} antibodies (the decreased immunoreactivity at zero time was not reproducible); (B) Western blot of crushate probed with antiphosphotyrosine antibody PY-20; (C) MBP phosphorylating activity of crushate. The arrow indicates the time of 50% GVBD.

following one-dimensional SDS-PAGE. An approximately 40-kDa protein reacting with anti-p44^{mpk} was detected in stage VI oocytes (56) and in the early stages of progesterone-induced maturation (Fig. 3A), but at 4 h after stimulation it was replaced by a more strongly reactive 42-kDa protein that also reacted with antiphosphotyrosine (Fig. 3B). An apparent molecular weight increase upon phosphorylation has been reported previously for mouse p42 (16).

Further evidence for a relationship between p44^{mpk} and Xp42 was provided by MBP kinase assays. During *Xenopus* oocyte maturation there was a tight correlation between the time course of stimulation of MBP phosphorylating activity in extracts from *Xenopus* oocytes (Fig. 3C) and phosphorylation of Xp42. Although the antibody reacts more strongly with activated p42, the detection of unphosphorylated p42 in immature oocytes suggests activation by phosphorylation rather than de novo synthesis.

In addition to correlating Xp42 phosphorylation with MBP kinase activation, we tested whether Xp42 and MBP kinase activity copurified on an ion-exchange column. Extracts of GVBD-positive and immature *Xenopus* oocytes were analyzed by Mono Q fractionation. In this system, p44^{mpk} from maturing sea star oocytes elutes at 330 to 430 mM NaCl (63, 64) (Fig. 4A, ▲). The major maturation-activated MBP kinase from *Xenopus* oocytes was detected in fractions 28 to 32 (390 to 470 mM NaCl) (Fig. 4A, ●); this precisely correlated with the elution position of the 42-kDa protein band that was immunoreactive with anti-p44^{mpk} antibody (Fig. 4C). The inactive form of the *Xenopus* MBP kinase eluted in fractions 28 and 29 (Fig. 4B). The tighter binding of the stimulated form of the MBP kinase with the positively charged resin was consistent with its increased phosphorylation. Thus, Xp42 has many properties expected for a maturation-activated MBP kinase.

A cDNA clone for *Xenopus* p42. Following the publication of the sequence of ERK1, a MAP kinase (8), and aware of the apparent similarity between Xp42 and mouse p42/MAP kinase (16), we set out to clone Xp42. We used the ERK1 protein sequence to design oligonucleotides for PCR, using segments of protein sequence that (i) were conserved between ERK1 and its yeast relatives FUS3 and KSS1, (ii) were not conserved in other protein kinases, and (iii) had low codon degeneracy (see Materials and Methods). One 5′ and two different 3′ primers were made. Following PCR with cDNA derived from *Xenopus* ovary mRNA, we obtained products of the expected molecular sizes with both pairs of primers (56). After cleavage of restriction enzyme sites present in the oligonucleotides used for PCR and subcloning, a DNA fragment was obtained whose sequence was related to that of *ERK1*. This fragment was used to probe a λZAP cDNA library prepared from *Xenopus* baby ovary mRNA, using *Eco*RI linkers.

Phagemid DNA was generated from 15 positive λ clones and characterized by *Eco*RI restriction mapping. Eight clones were apparently identical, containing approximately 650- and 530-bp fragments (see Materials and Methods). One of these clones was sequenced completely (λ clone 3; Fig. 5). The sequence of clone 3 reveals a single open reading frame of 361 residues (Fig. 5B). Two tandem termination codons are followed 9 bases downstream by an in-frame methionine codon. This potential initiation site is followed 15 codons later by another methionine codon. Neither codon is in a perfect consensus for initiation of translation. If the first methionine is used for initiation, the amino terminus of the resultant protein would contain the curious sequence $MetAla_5Ser_2AsnProGly_3ProGlu$ before the next methionine is reached. Since our data do not indicate which initiation site is used (although the same site appears to be used in oocytes and in rabbit reticulocyte lysate; see below), we have numbered residues from the first methionine. The open reading frame contains all of the residues that are conserved in all protein kinases (Fig. 6, *) and has the sequence motifs characteristic of serine/threonine kinases rather than tyrosine kinases (32).

Alignment of the sequence with that of rat ERK1 reveals 83% sequence identity and a higher percentage of sequence similarity (Fig. 6). Comparison with FUS3 and KSS1 shows 49% identity with each and lower identity with other protein kinases present in the data base. We have also determined the amino acid sequences of four peptides obtained from purified p44^{mpk}. Alignment of these partial sequences with those of clone 3 and ERK1 strongly suggests that p44^{mpk} is a close relative (Fig. 6).

Evidence that λ clone 3 encodes Xp42. Since there is more than one ERK1-related cDNA in a rat brain cDNA library, there may be mRNA for more than one ERK1-related protein kinase in *Xenopus* ovary. Therefore, it was important to test whether our cDNA clone encoded Xp42. Antiserum 7774 was raised to a bacterial fusion protein containing TrpE joined to the PCR clone 4 sequence, representing residues 246 to 318 of the open reading frame. This antiserum was tested as a probe on immunoblots of *Xenopus* proteins and used for immunoprecipitation. Antiserum 7774 reacts on immunoblots with two proteins in *Xenopus* oocyte extracts, one of 40 kDa and one of 42 kDa (Fig. 7A, lanes 1 and 2). Following progesterone-induced maturation, the lower band disappears and the upper band becomes more intense. The upper band aligns with Xp42, detected by antiphosphotyrosine antibodies in mature but not immature *Xenopus* oocytes (Fig. 7A, lanes 3 and 4), and the disappearance of the lower band suggests a mobility shift similar to that detected with anti-p44^{mpk}. Unfortunately, the 7774

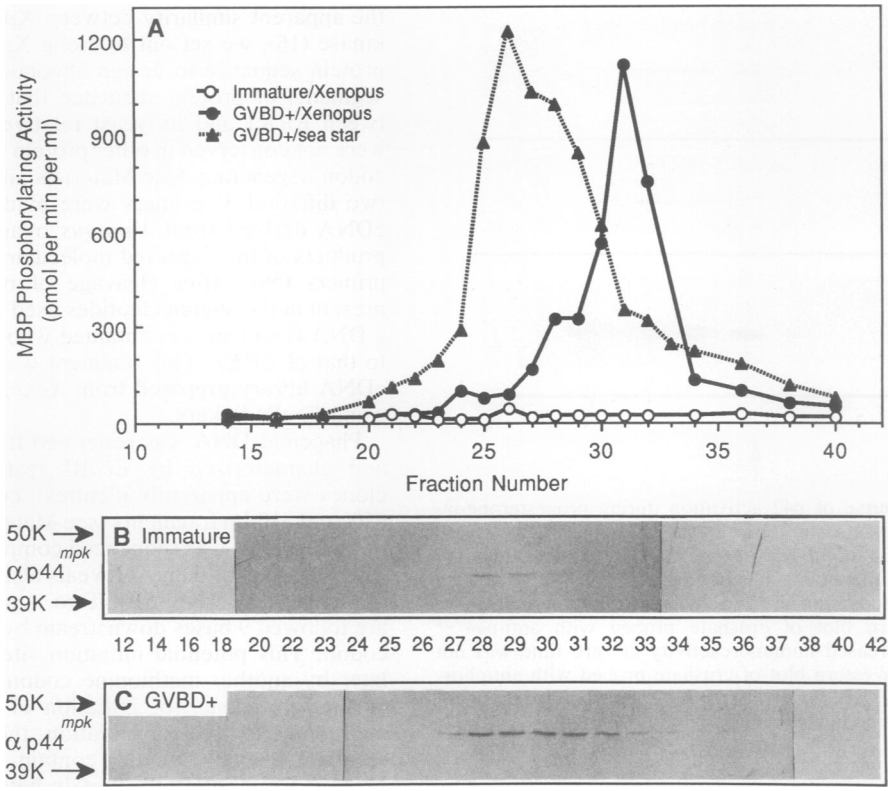

FIG. 4. Mono Q fractionation of $p44^{mpk}$ from sea star and *Xenopus* oocytes. Cytosolic extracts (ca. 12 mg of protein) from immature *Xenopus* oocytes (○) or from mature *Xenopus* (●) or sea star (▲) oocytes harvested at the time of GVBD were loaded onto a Mono Q column that was developed with a linear gradient of 15 ml of 0 to 0.8 M NaCl. Fractions (250 μl) were collected. Following SDS-PAGE of the Mono Q fractions and transfer to nitrocellulose, the 35- to 50-kDa region was probed with affinity-purified rabbit anti-$p44^{mpk}$ antibody (αp44). (A) MBP phosphorylating activity in the Mono Q fractions; (B) immunoblot of Mono Q fractions of immature *Xenopus* oocytes; (C) immunoblot of Mono Q fractions of GVBD-positive *Xenopus* oocytes.

antibodies recognize only SDS-denatured Xp42, precluding any assay for kinase activity in the immunoprecipitates.

Two-dimensional gel analysis and immunoblotting with antiserum 7774 indicates that both oocytes and eggs contain a common set of 42-kDa proteins (Fig. 8Aa and b). Eggs differ from oocytes, however, in the disappearance of a 40-kDa spot representing nonphosphorylated Xp42 (Fig. 8Aa, arrowhead) and the appearance of a pair of 42-kDa spots that line up precisely with the two ^{32}P-labeled phosphorylated forms of Xp42 (Fig. 8Ab, arrow, and data not shown). The unchanging 42-kDa proteins most likely correspond to the 42-kDa band seen on SDS gels of oocytes (Fig. 7A, lane 1). Perhaps these 42-kDa proteins are ERK-related proteins whose phosphorylation does not change during oocyte maturation. They are unlikely to represent alternate usage of the different initiation codons present within the cDNA cloned, because the larger protein is more basic, rather than more acidic as predicted from the sequence.

Further evidence that antiserum 7774 detects Xp42 was provided by immunoprecipitation. Immunoprecipitation of [^{35}S]methionine-labeled *Xenopus* oocytes and eggs revealed single bands of 40 and 42 kDa, respectively (56). Probing of immunoprecipitates with antiphosphotyrosine antibody revealed a tyrosine-phosphorylated 42-kDa protein in immunoprecipitates prepared from eggs but not oocytes (Fig. 7A, lanes 5 and 6). When oocytes were labeled with $^{32}P_i$ and then allowed to mature and immunoprecipitated, much more labeled 42-kDa protein was detected in mature than immature oocytes (Fig. 7A, lanes 7 and 8). Phosphoamino acid analysis showed that the 42-kDa protein phosphorylated in egg extract contained predominantly phosphotyrosine, as found previously for Xp42 (16), and had the same tryptic phosphopeptide map (56). Small amounts of phosphothreonine were also detected. Our previous detection of phosphoserine in Xp42 labeled in egg extracts and purified from two-dimensional gels (16) may have represented cross-contamination with other phosphoproteins or differential labeling of Xp42 in vitro versus in intact oocytes.

These results confirm that antiserum 7774, raised to a sequence contained within the open reading frame of λ clone 3, recognizes the nonphosphorylated and phosphorylated forms of Xp42. It may, however, be capable of recognizing other related protein kinases, so recognition does not constitute proof that Xp42 is encoded by λ clone 3. Further evidence addressing this possibility was obtained by synthesis of the product of λ clone 3 in vitro, by transcription with phage polymerase and translation in reticulocyte lysate. The [^{35}S]methionine-labeled product formed a single, 40-kDa spot on two-dimensional gel electrophoresis (Fig. 8Ac). When this product was mixed with oocyte and egg extracts, analyzed by two-dimensional gel electrophoresis, and immunoblotted with antiserum 7774, the [^{35}S]methionine-labeled in vitro translation product aligned precisely with the putative nonphosphorylated form of Xp42, detected in oocyte but not egg extracts (Fig. 8Aa and b, arrowheads). This finding demonstrates that the product of the cDNA clone

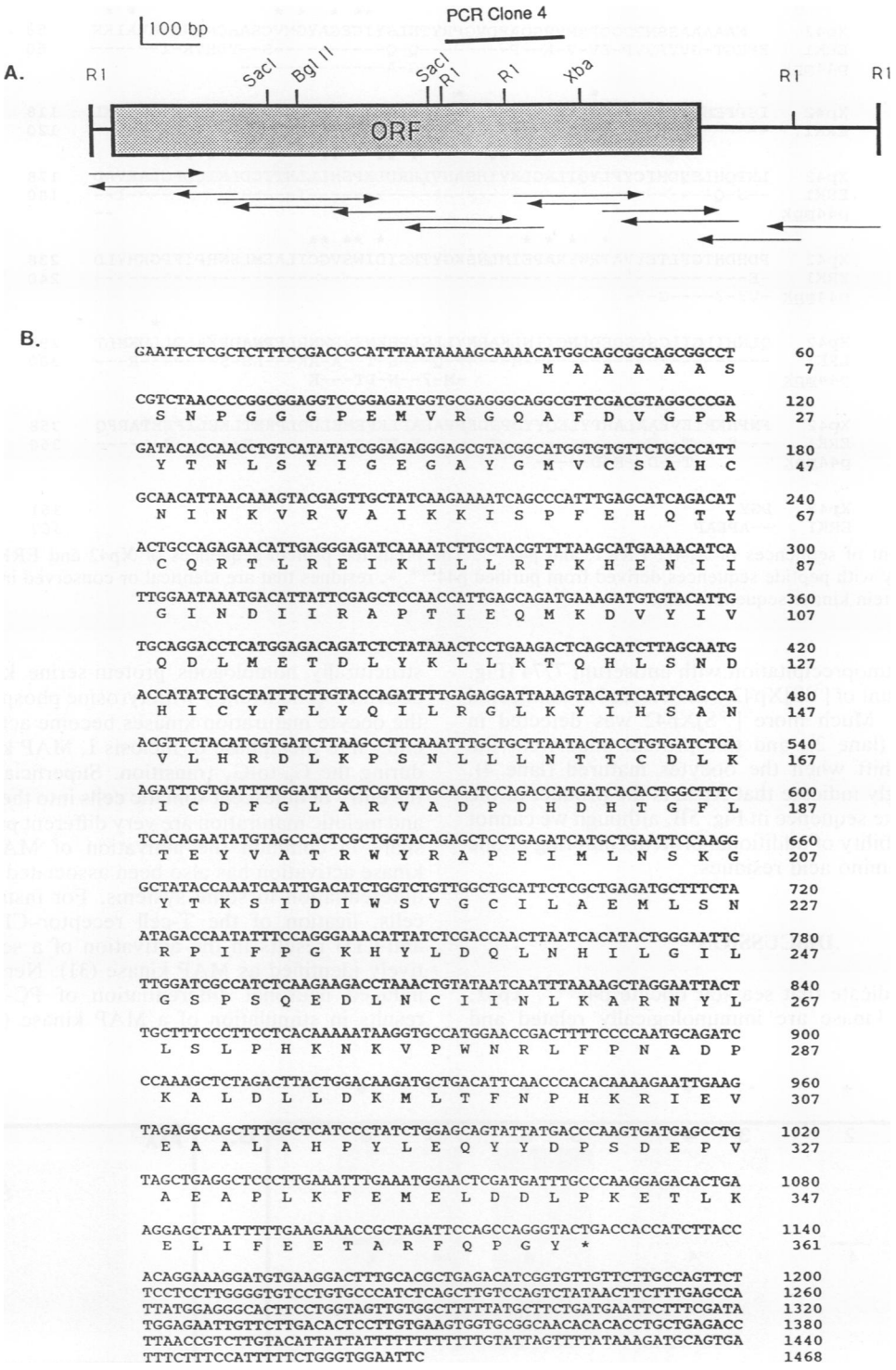

FIG. 5. Structure and sequence of an Xp42 cDNA clone. (A) Restriction map of the insert in λ clone 3. Selected restriction sites, the location of the open reading frame (ORF), and the location of the PCR clone used to screen the λ library are shown at the top. Arrows indicate sequencing runs. (B) Nucleotide sequence and predicted amino acid sequence of the insert in λ clone 3.

that was sequenced encodes a protein identical in molecular mass and isoelectric point to nonphosphorylated Xp42. Unfortunately, insufficient [^{35}S]methionine radioactivity has been obtained for two-dimensional peptide map comparison.

We have also examined the products of mRNA, synthesized in vitro from the cloned open reading frame, translated in *Xenopus* oocytes. mRNA was injected into oocytes that were incubated for 24 h in the presence of [^{35}S]methionine. Some oocytes were then matured with progesterone. Lysates of control and mRNA-injected oocytes and eggs were

```
                                                  ** *  * *             * *
Xp42     MAAAAASSNPGGGPEMVRGQAFDVGPRYTNLSYIGEGAYGMVCSAHCNINKVRVAIKK     58
ERK1   EPRGT-GVVPVVP-EV-V-K--P---------Q-Q----------S--YDHVR-T------     60
p44mpk                                -G-A----------?--

       *              *           *
Xp42   ISPFEHQTYCQRTLREIKILLRFKHENIIGINDIIRAPTIEQMKDVYIVQDLMETDLYKL   118
ERK1   -----------------Q---G-R---V---R--L----L-A-R----------------   120

                          *  **      * **   **          * ***
Xp42   LKTQHLSNDHICYFLYQILRGLKYIHSANVLHRDLKPSNLLLNTTCDLKICDFGLARVAD   178
ERK1   --S-Q-------------------------------------I---------------I--   180
p44mpk                                                             --

                     *  * * *            * ** **
Xp42   PDHDHTGFLTEYVATRWYRAPEIMLNSKGYTKSIDIWSVGCILAEMLSNRPIFPGKHYLD   238
ERK1   -E-----------------------------------------------------------   240
p44mpk -V?-?----G-?-

                                                                  *
Xp42   QLNHILGILGSPSQEDLNCIINLKARNYLLSLPHKNKVPWNRLFPNADPKALDLLDKMLT   298
ERK1   ----------------------M------Q---S-T--A-AK---KS-S-------R---   300
p44mpk                              -M-?--N-PT---K

       *
Xp42   FNPHKRIEVEAALAHPYLEQYYDPSDEPVAEAPLKFEMELDDLPKETLKELIFEETARFQ   358
ERK1   ---N---T--E-------------T------E-FT-D---------R------Q------   360
p44mpk        T--D--K-D-----

Xp42   PGY                                                            361
ERK1   --APEAP                                                        367
```

FIG. 6. Alignment of sequences of Xp42, ERK1, and p44^{mpk}. The predicted protein sequences of Xp42 and ERK1 were aligned for maximum homology with peptide sequences derived from purified p44^{mpk}. *, residues that are identical or conserved in at least 62 out of a collection of 65 protein kinase sequences (32).

analyzed by immunoprecipitation with antiserum 7774 (Fig. 8B). A small amount of [^{35}S]Xp42 was detected in uninjected oocytes (lane 6). Much more [^{35}S]Xp42 was detected in injected oocytes (lane 2), and this protein underwent the expected band shift when the oocytes matured (lane 4). These data strongly indicate that Xp42 is the product of the open reading frame sequence in Fig. 5B, although we cannot exclude the possibility of additional proteins differing at one or more neutral amino acid residues.

DISCUSSION

Our findings indicate that sea star oocyte p44^{mpk}, Xp42, and a rat MAP kinase are immunologically related and structurally homologous protein-serine kinases which are activated concurrently with tyrosine phosphorylation. While the oocyte maturation kinases become active at the time of entry into metaphase of meiosis I, MAP kinase is activated during the G_0-to-G_1 transition. Superficially, it seems that the entry of quiescent somatic cells into the mitotic cell cycle and meiotic maturation are very different processes, but they share in common the activation of MAP kinases. MAP kinase activation has also been associated with induction of differentiation in some systems. For instance, in Jurkat T cells, ligation of the T-cell receptor–CD3 complex with anti-CD3 results in the activation of a serine kinase putatively identified as MAP kinase (31). Nerve growth factor-induced neuronal differentiation of PC-12 cells likewise results in stimulation of a MAP kinase (49). Induction of

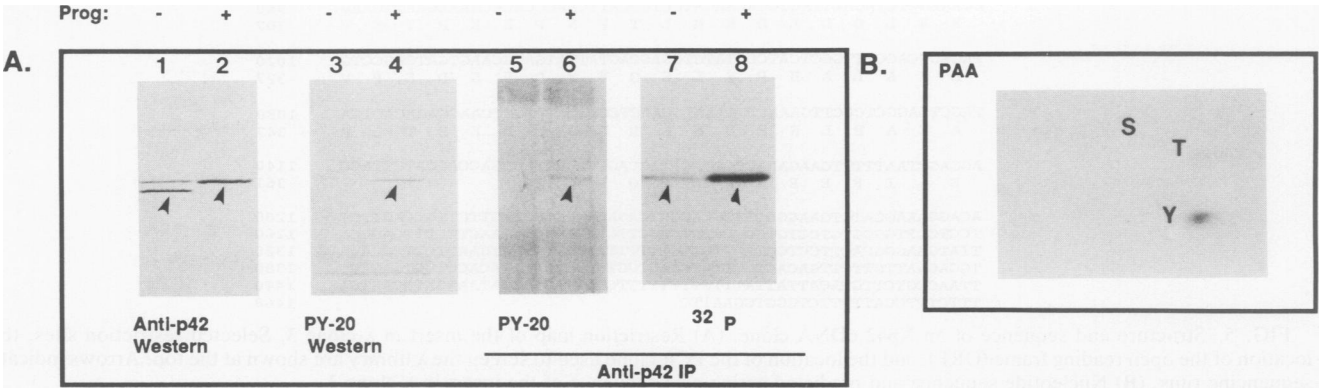

FIG. 7. Characteristics of anti-Xp42 antiserum 7774. (A) Extracts were prepared from either immature control or mature *Xenopus* oocytes (without [−] and with [+] progesterone [Prog], respectively). In lanes 1 through 4, extracts were analyzed on a 12.5% SDS-polyacrylamide gel and immunoblotted with either antiserum 7774 (lanes 1 and 2) or antiphosphotyrosine antibody PY-20 (lanes 3 and 4). Lanes 5 through 8 contain immunoprecipitates prepared with antiserum 7774. In lanes 5 and 6, nonradioactive immunoprecipitates were analyzed on a 12.5% SDS-polyacrylamide gel and immunoblotted with PY-20. In lanes 7 and 8, immunoprecipitates were prepared by using antiserum 7774 from immature or mature oocytes that had been labeled in vivo for 4 h with ^{32}P and then incubated without ^{32}P in the presence or absence of progesterone for 16 h. (B) Two-dimensional phosphoamino acid (PAA) analysis of [^{32}P]Xp42 immunoprecipitated with antiserum 7774 from mature oocytes (panel A, lane 8). Positions of nonradioactive markers: S, phosphoserine; T, phosphothreonine; Y, phosphotyrosine.

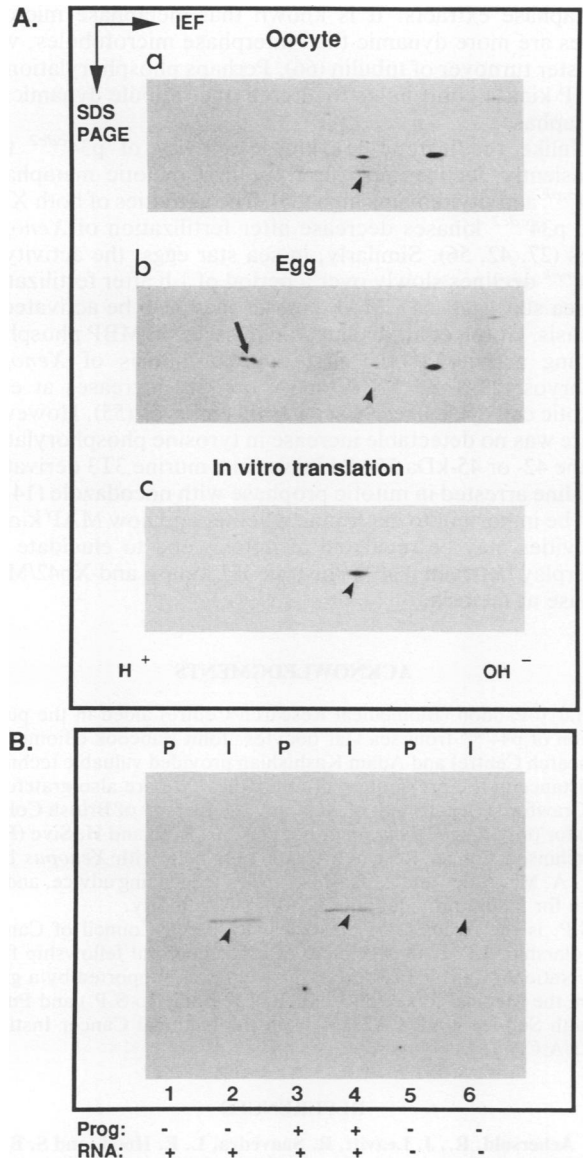

FIG. 8. Evidence that Xp42 is encoded by λ clone 3. (A) Two-dimensional gel electrophoresis of [^{35}S]methionine-labeled reticulocyte translation products of RNA synthesized from λ clone 3, mixed with nonradioactive *Xenopus* oocyte and egg proteins, showing that the protein encoded by the cDNA comigrates with an oocyte protein recognized by antiserum 7774. A total of five gels were run: oocyte proteins alone, egg proteins alone, oocyte proteins mixed with ^{35}S-proteins, egg proteins mixed with ^{35}S-proteins, and ^{35}S-proteins alone. All gels were immunoblotted and probed with antiserum 7774 and then exposed to film to locate the ^{35}S-labeled in vitro translation products. No immunoreactive spots were detected when the ^{35}S-labeled translation products were analyzed alone. (a) Oocyte proteins recognized by 7774; (b) egg proteins detected by 7774; (c) [^{35}S]methionine-labeled in vitro translation products detected by autoradiography. Arrowheads mark positions of [^{35}S]methionine-labeled protein encoded by λ clone 3; the arrow indicates immunoreactive spots that coincide with ^{32}P-labeled phosphorylated forms of Xp42. IEF, Isoelectric focusing. (B) Synthesis of Xp42 in oocytes injected with RNA synthesized in vitro from λ clone 3. Oocytes were microinjected with [^{35}S]methionine with (+) or without (−) RNA. After 24 h at 25°C, injected oocytes were incubated in the absence or presence of progesterone (Prog). Extracts were prepared and immunoprecipitated with preimmune (P) or immune (I) serum from rabbit 7774. Immunoprecipitates were analyzed on a 12.5% SDS-polyacrylamide gel and exposed to film. Arrowheads point to 40-kDa protein precipitated from RNA-injected, immature oocytes (lane 2), 42-kDa protein precipitated from RNA-injected, mature oocytes (lane 4), and 40-kDa protein precipitated from immature oocytes that received no RNA (lane 6).

adrenal chromaffin cells to secrete catecholamines features activation of a MAP kinase (21).

The interplay between protein tyrosine kinases and serine kinases in transducing mitogenic information from the cell surface to intracellular effector molecules is largely unknown. So far, there are few examples of direct activation of protein-serine kinases by tyrosine phosphorylation. The 76-kDa *raf* proto-oncogene-encoded protein-serine kinase is activated by the PDGF receptor, although whether by tyrosine phosphorylation, serine phosphorylation, physical association, or a combination is unclear (50). The protein-serine phosphorylating activity of murine MAP kinase is stimulated in parallel with its phosphorylation on tyrosine and threonine residues (57). Dephosphorylation studies indicate that phosphorylation of both tyrosine and serine/threonine residues is required for full activity (3, 5, 64) but in vitro rephosphorylation, and activation, by purified tyrosine and serine/threonine kinases has not been reported. Without these key experiments, it is not possible to determine whether the phosphorylations of MAP kinase at tyrosine and serine/threonine residues are dependent or independent events. Phosphorylation at serine or threonine could be autophosphorylation (in *cis* or *trans*) triggered by prior tyrosine phosphorylation. Alternatively, because some ostensibly serine/threonine-specific protein kinases are actually able to phosphorylate also at tyrosine (37), autophosphorylation at tyrosine could be triggered by serine/threonine phosphorylation by another protein kinase. This seems unlikely because sea star p44^{mpk} does not have detectable tyrosine kinase activity (13). Finally, the serine/threonine and tyrosine phosphorylation events may be catalyzed by two or more different protein kinases. In this case, MAP kinases could be at the confluence of multiple signaling pathways.

Candidates for kinases involved in MAP kinase activation are now being described. Stimulatory factors have been identified in epidermal growth factor-stimulated 3T3 cells, although whether they are protein kinases is unknown (3). Earlier studies showed that tyrosine phosphorylation of p42/MAP kinase is dependent on protein kinase C (PKC), a protein-serine kinase activated indirectly by phospholipid degradation in mitogen-activated cells (40, 72). PKC activation during oocyte maturation has been inferred from the effects of agonists and inhibitors (69). PKC could trigger MAP kinase tyrosine phosphorylation by regulating a tyrosine kinase. One candidate tyrosine kinase known to be a substrate for PKC is p60$^{c\text{-}src}$ (30), which is present in *Xenopus* eggs (56, 67). Alternatively, direct phosphorylation of p42 by PKC may be a prerequisite for subsequent tyrosine phosphorylation and activation. However, p44^{mpk} does not appear to be a substrate for either p60$^{c\text{-}src}$ or PKC in vitro (13). Of course, it is also possible that an increased rate of tyrosine phosphorylation is caused by a change in the substrate (MAP kinase) or that increased phosphorylation results from a reduced rate of dephosphorylation, as a result of decreased activity of a phosphatase or a change in the substrate (phosphorylated MAP kinase).

The targets for MAP kinase may be the same or different

in oocytes and fibroblasts. In vitro, purified murine MAP kinase can phosphorylate, and activate, a *Xenopus* oocyte enzyme, S6K-II (70). S6K-II is normally activated during *Xenopus* oocyte maturation, prior to GVBD (23). It is not clear whether Xp42 is activated before or after the increase in S6K-II activity. In fibroblasts, homologs of S6K-II are activated rapidly when quiescent cells are stimulated with calf serum, 12-*O*-tetradecanoylphorbol-13-acetate, or expression of the protein-tyrosine kinase $p60^{v\text{-}src}$ (10, 71). The kinetics of MAP kinase activation and p42 tyrosine phosphorylation are sufficiently rapid to permit MAP kinase to activate S6K-II in stimulated cells (15, 57). Mixing experiments indicate that an active MAP kinase can activate an S6 peptide kinase, probably related to S6K-II, in 3T3 cell extracts (2). We are presently testing directly whether microinjection of $p44^{mpk}$ can activate S6 protein phosphorylation as well as other aspects of meiotic maturation.

Sea star oocyte $p44^{mpk}$ and the murine MAP kinase share many similarities with respect to size, physical properties, substrate specificity, and regulation (5, 36, 57, 58, 63, 64). In this report, we have demonstrated that sea star $p44^{mpk}$ and Xp42 are tyrosine phosphorylated in concert with their activation, as has been previously reported for murine MAP kinase (59). Moreover, polyclonal anti-$p44^{mpk}$ antibodies recognized mouse 3T3 cell p42/MAP kinase (56). Partial protein sequence data indicate that $p44^{mpk}$ is a bona fide member of the Xp42/ERK1 family. Whether $p44^{mpk}$ is more closely related to ERK1 or Xp42 requires more sequence information.

Despite the similarities between $p44^{mpk}$, Xp42, and ERK1, it seems possible that they are encoded by distinct genes and are close relatives rather than true phylogenetic homologs. This possibility is occasioned by the apparent complexity of MAP kinases in 3T3 cells, in which there are two inducible MBP kinases (4) and four phosphotyrosine-containing related proteins of 42 and 45 kDa are recognized by anti-$p44^{mpk}$ antibody (15, 41, 56). The nonphosphorylated form of the 42-kDa phosphoproteins was identified previously in unstimulated 3T3 cells and found to have an apparent molecular mass of 40 kDa (16), the same as that of Xp42. The sequence of ERK1 predicts a protein that is very similar to Xp42, both in the kinase domain and in the acidic carboxy-terminal domain, but ERK1 is longer at both the carboxy and amino termini (8) (the full amino-terminal sequence has not been reported). Therefore, ERK1 may correspond to the 45-kDa phosphoproteins, and Xp42 could be the *Xenopus* homolog of mammalian p42/MAP kinase. The larger size of $p44^{mpk}$ relative to MAP kinase suggests that it may be the sea star equivalent of the 45-kDa phosphoproteins. This might explain some different properties of purified $p44^{mpk}$ and MAP kinase. For example, sea star $p44^{mpk}$ undergoes autophosphorylation on serine (64), whereas MAP kinase does not autophosphorylate in vitro (59). The two enzymes also show differences in substrate specificity and inhibition by β-glycerolphosphate (54, 58, 63). On the other hand, $p44^{mpk}$ may be the phylogenetic homolog of Xp42, and the larger size of the sea star protein could be due to evolutionary divergence.

The dramatic and sustained activation of $p44^{mpk}$ and Xp42 during meiosis (12, 55) implicates these kinases in meiotic maturation. Recently, a MAP kinase activity that is probably identical to Xp42 has been purified from *Xenopus* eggs (29). Either this kinase or purified murine MAP kinase is capable of reorganizing microtubules in vitro (29). When MAP kinase is incubated with an interphase extract of *Xenopus* oocytes in the presence of purified centrosomes, the microtubule length is dramatically shortened, to mimic that present in metaphase extracts. It is known that metaphase microtubules are more dynamic than interphase microtubules, with a faster turnover of tubulin (66). Perhaps phosphorylation by MAP kinase contributes to altered microtubule dynamics at metaphase.

Unlike the histone H1 kinase activity of $p34^{cdc2}$ that transiently declines just after the first meiotic metaphase, $p44^{mpk}$ activity remains high (55). The activities of both Xp42 and $p34^{cdc2}$ kinases decrease after fertilization of *Xenopus* eggs (27, 42, 56). Similarly, in sea star eggs, the activity of $p44^{mpk}$ declines slowly over a period of 1 h after fertilization of sea star eggs (55). MAP kinases may also be activated at mitosis. Gotoh et al. detected an increase in MBP phosphorylating activity at the first zygotic mitosis of *Xenopus* embryos (29), and MBP kinase activity increases at each mitotic cell cycle in early sea urchin embryos (55). However, there was no detectable increase in tyrosine phosphorylation of the 42- or 45-kDa MAP kinases in a murine 3T3 derivative cell line arrested in mitotic prophase with nocodazole (14). It will be important to determine whether and how MAP kinase activities may be regulated at mitosis and to elucidate the interplay between $p34^{cdc2}$/histone H1 kinase and Xp42/MAP kinase at meiosis.

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