Developmentally Regulated Expression of a Mitogen-activated Protein Kinase in *Xenopus laevis*

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**Abstract**

Mitogen-activated protein (MAP) kinases are activated in somatic cells in response to many extracellular stimuli and in oocytes during meiotic maturation. We have examined the tissue specificity of expression of a MAP kinase (Xp42) in adult and larval *Xenopus laevis*. MAP kinase RNA and protein were abundant in the nervous system and lymphoid tissues and were readily detected in most other organs. A remarkably high level of RNA was detected in ovary. Fractionation of oocytes showed that MAP kinase RNA is expressed at the highest level in small oocytes, suggesting that it is a maternal RNA that is stored for early embryogenesis. The levels of MAP kinase RNA and protein did not change from the time of fertilization through to late blastula. The results are consistent with functions for MAP kinases in signal transduction in embryonic as well as adult cells.

**Introduction**

MAP kinases are a small group of serine/threonine-specific protein kinases that are activated by phosphorylation on tyrosine and threonine residues in response to different extracellular signals (reviewed in Refs. 1–5). In mammals, two MAP kinases, of approximate sizes 42 and 45 kDa, have been characterized (6, 7). A third protein kinase, of 54 kDa, is activated in response to some stimuli apparently by tyrosine and threonine phosphorylation (8–9). The structure of this kinase has not been reported. The 42 kDa and 45 kDa MAP kinases, also known as ERK2 and ERK1, are closely related in sequence and share 83% sequence identity (6). They are more distantly related to other protein kinases, such as CDC2 and ERK3.

MAP kinase activities have been detected, using microtubule-associated protein 2 or myelin basic protein as substrates, in extracts derived from many cell types, including fibroblasts, adipocytes, adrenal chromaffin, lymphocytes, epithelial cells, pheochromocytoma, epidermoid carcinoma, and hepatocytes (4). Activity is detected only if the cells are stimulated with agents that induce cell proliferation, differentiation, or acute responses, such as secretion. Active MAP kinases have also been detected by virtue of their phosphotyrosine content in various cultured cells, in hippocampal slices, and in mature oocytes (4). Following the cDNA cloning of rat ERK1 and ERK2, specific antibodies to these proteins were generated, and analysis of various mouse tissues showed variable expression of the RNA and protein for both ERK1 and ERK2 (7). In adults, expression of both proteins was highest in the nervous system. Different regions of the brain showed different ratios of ERK1 to ERK2, perhaps indicating differences in function. However, in cell types where activation of the 42 and 45 kDa MAP kinases has been studied, both kinases appear to be activated equally by the stimuli tested.

Two laboratories have cloned cDNAs for MAP kinases from the African clawed toad, *Xenopus laevis* (10, 11). The two cDNAs predict proteins that are 98.5% identical in sequence and equal in size (361 residues). Both sequences are 96% identical to ERK2, the mammalian 42 kDa MAP kinase, and 83% identical to ERK1 (45 kDa). The differences between the two *Xenopus* cDNAs could represent allelic variation or could be due to the apparent duplication of the *X. laevis* genome that is believed to have occurred during speciation. It seems unlikely that the few protein sequence differences between the two proteins would permit differences in function. Additional MAP kinase genes in *Xenopus* have not been detected.

The widespread expression of MAP kinases in mammals, and their activation by diverse stimuli, have suggested a fundamental role in the response of cells to extracellular signals. However, there is to date no genetic evidence that MAP kinases are required for any cellular response in multicellular organisms. Proteins with specialized functions often show unique patterns of tissue distribution. In order to gain more insight into the possible functions of MAP kinase, we have examined MAP kinase expression in developing *Xenopus* oocytes, embryos, and whole animals.

**Results**

**Xp42 RNA in Adult Xenopus Tissues and Oocytes.** To study tissue-specific expression of the Xp42 gene in adult *Xenopus*, we used a technique known as histoblotting (12). This method allows the detection of specific transcripts on whole animal cryostat sections fixed on nitrocellulose. Sagittal sections of adult decapitated female *X. laevis* were subjected to histoblotting analysis by hybridization at high stringency with random primer-labeled Xp42 cDNA probe. Relatively weak hybridization was detected in lung, kidney, skin, intestine, and muscles, and an even weaker signal in liver (Fig. 1, A and B). Xp42 RNA was also detected in brain by histoblotting sections made from a *Xenopus* head (data not shown). The strongest signal came from ovary, readily identified even in unstained sections by the presence of pigmented oocytes (Fig. 1, A and C). As a control, a histoblot was probed

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3 The abbreviations used are: MAP, mitogen-activated protein; kDa, kilodalton(s); cDNA, complementary DNA; kb, kilobase(s); SDS, sodium dodecyl sulfate; SSC, standard saline citrate; TPA, 12-O-tetradecanoylphorbol-13-acetate; MBS, modified Barth's saline.
Fig. 1. Expression of Xp42 and mos RNAs detected by histoblotting in adult Xenopus. Sagittal sections of normal (A–C, E, and G) or ovulated (D, F, H, and I) adult female Xenopus laevis were hybridized with an Xp42 cDNA probe (A–D and F), a Xenopus mos cDNA probe (G–I), or a nonspecific DNA probe (E), as described in “Materials and Methods.” Autoradiographs of the sections are shown in panels B–I; a hematoxylin-stained section corresponding to the autoradiographs in panels B and C is shown in panel A. The autoradiograph in panel B was overexposed (24 h) to show weak hybridization to various tissues and intense hybridization to ovary. A shorter exposure (3 h) at higher magnification is shown in panel C, to demonstrate the nonhomogeneous staining of the ovary. Other autoradiographs show 6-h exposures. All sections are shown with anterior at left. ooc, oocytes; ovr, ovary; ovid, oviduct; liv, liver; kid, kidney; mus, muscle.
with labeled vector, and no hybridization was observed (Fig. 1E). *Xenopus* MyoD and ErbA-specific probes were used as positive controls to check for specificity of the histoblot hybridization. The pattern of the signal was distinct for each gene, and in neither case was a strong signal observed in ovary (data not shown).

To confirm the high level of Xp42 RNA in ovary, total RNA from *Xenopus* adult tissues was analyzed by Northern blotting (Fig. 2A). In all tissues tested, three transcripts of approximately 4.9, 3.4, and 2.7 kb were found. All transcripts were much more abundant in ovary than in kidney, intestine (Fig. 2A), and lung (data not shown). Less hybridization was detected in liver, which was consistent with the histoblot hybridization data. A *Xenopus* kidney cell line (XTC) had similar levels of Xp42 RNA in liver. The relative ratios of the major 3.4- and 4.9-kb mRNAs appeared to be similar in different tissues. The 2.7-kb transcript was readily detected in ovary but hardly seen in somatic tissue RNA, probably because of its much lower abundance compared to the 4.9- and 3.4-kb mRNAs. The sizes of Xp42 transcripts were similar to those for transcripts from ERK2, but different from those of ERK1, in rat tissues (6). Testis expressed the 4.9-, 3.4-, and 2.7-kb RNAs at similar levels to kidney and intestine and contained increased amounts of a <1-kb RNA that was weakly detected in other tissues (Fig. 2C).

The histoblot hybridization showed that Xp42 mRNA was not homogeneous throughout the ovary. There were “holes” corresponding to the position of large oocytes (Fig. 1, A and C). A normal *Xenopus* adult female ovary contains oocytes of different stages of development: stages 1 (oocytes <0.3 mm in diameter) through 6 (oocytes 1.2–1.4 mm in diameter) according to Dumont (13). Each oocyte is tightly sheathed in follicular cells and is surrounded by theca. To explain the differences in signal intensities from different parts of the ovary, we separated oocytes from supporting ovary tissues and sorted them according to size. This separation does not remove the follicular cells from the oocytes (14). Oocytes of different stages were used for extraction of RNA and Northern blotting experiments (Fig. 2A). Equal amounts of total oocyte RNA were analyzed with the Xp42 cDNA probe. The Xp42 transcripts were found to be most abundant in mRNA from the smallest oocytes analyzed (stage 2, 0.3–0.45 mm in diameter). The quantity of Xp42 mRNA, relative to total RNA, decreased progressively during oocyte development, and it was particularly low in stage 6 (>1.2 mm in diameter) oocytes. The abundance of Xp42 RNA in stage 6 oocytes was similar to that in eggs.
This situation resembles that described for the mos and ras protooncogenes. In those cases, the quantity of specific mRNA per oocyte is constant, and the apparent decrease in specific mRNA relative to total RNA is due to accumulation of rRNA and other maternal RNAs in growing oocytes (15, 16). Using histoblot and Northern blot hybridization, we examined expression of mos RNA in Xenopus tissues and oocytes. Both methods detected high levels of hybridization of mos cDNA probe with ovary and none in other Xenopus tissues (Fig. 1, G and I, and Fig. 2B). The level of mos RNA declined during oogenesis (Fig. 2B), approximately in parallel with the decline in Xp42 RNA. Because Sagata et al. (15) showed that in reality oocytes of each stage contain very similar amounts of mos RNA, we conclude that the number of Xp42 transcripts per oocyte is also constant. Presumably, Xp42 RNA is accumulated prior to stage 2 of oocyte development, and its quantity does not change between stages 2 and 6. In this respect, Xp42 RNA would resemble other polyadenylate-containing RNAs (17).

To determine whether there were any changes in Xp42 RNA during oogenesis, we examined gonadotropin-treated female Xenopus by histoblotting. Injection of gonadotropin into adult Xenopus female induces maturation of stage 6 oocytes. Maturation is the progress of meiosis from prophase I to metaphase II, at which point the mature oocytes arrest again, leave the ovary, and migrate to the oviduct. As shown in Fig. 1, D and F, the overall shape and morphology of the ovary are changed by gonadotropin treatment, but the level of hybridization of the Xp42 cDNA probes is still high. The ovary still contains oocytes in stages 1 through 5, and hybridization of the small oocytes is very strong. The stage 6, mature oocytes have entered the oviduct and hybridize no more strongly than the surrounding tissues (Fig. 1F). We hybridized a parallel section from the ovolated frog with a mos probe. Interestingly, the distribution of hybridization signals of Xp42 and mos cDNA probes was almost identical (Fig. 1, H and I). These results lend support to the conclusion that the density of Xp42 RNA in mature oocytes is lower than in the remainder of the ovary.

Xp42 RNA Expression during Development. We collected eggs, fertilized them, and followed the levels of Xp42 RNA by Northern blotting during early development (Fig. 2C). The amount of Xp42 mRNA was identical for eggs and embryos of all stages tested, from the fertilized egg (stage 1) to late blastula (stage 19). The signal did not change at the midblastula transition (stage 8.5), the point at which most maternal RNA is degraded and zygotic transcription starts. Because Xp42 mRNA is probably also degraded at this time, zygotic transcription must rapidly restore the level.

We also examined Xp42 RNA levels by histoblotting at several later times during development (Figs. 3 and 4). Hybridization of an Xp42 cDNA probe to stage 56 tadpoles (Fig. 3A) showed high levels of Xp42 RNA in the nervous system—brain, spinal cord, cranial nerves, and olfactory organs (indicated by 1, 2, 3, and 4, respectively, in Fig. 3), in contrast to low level expression in muscle tissue (6). Hybridization to neural structures continued through stage 59, when hind limbs are developing (Fig. 3, B and C). Strong hybridization to kidney (13), liver (10), and intestine (11) was also observed. Expression in the liver declined during tadpole development. Expression in muscles (6) was generally low, but the developing limb buds (9) of stage 59 tadpoles showed high expression (Fig. 3B, center and right, and 3C, right). The tissues in the developing limb buds could not be identified. Thymus (12) was detected in stage 59 embryos (Fig. 3B, center, and 3C, left). Coronal sections of an immature frog, stage 66 (Fig. 4), showed weaker hybridization to the kidney (10), liver (6), and intestine (8), and hybridization to muscle (4) was low, as in the adult (Fig. 1B). Strongest hybridization at this stage was detected in the thymus (9) and developing ovary (7). Strong hybridization was also detected in the spleen (14), which was practically enveloped by the anterior part of the ovary. Hybridization in the thymus was most conspicuous around the periphery, corresponding to the cortex, which contains the highest density of lymphocytes. High expression in the ovary of a stage 66 frog was confirmed by Northern blotting (Fig. 2C).

Xp42 Protein in Xenopus Adult Tissues. To test whether variations in Xp42 RNA content in different adult tissues paralleled variations in Xp42 protein content, we analyzed protein extracts from different Xenopus tissues by Western blotting using 1913 antibody. This antibody was raised to a synthetic peptide (KELIFEETARFQPGY), corresponding to residues 347–361 at the COOH terminus of both cloned forms of Xp42 (10, 18). In mammalian cells (Swiss mouse 3T3 cells, monkey kidney CV-1 cells, and dog epithelial TRMP cells), antisemum 1913 detects two MAP kinases (p42 and p45) whose mobility changes when phosphorylation is induced by mitogenic stimulation (data not shown). Mammalian p42 and p45 (rat ERK2 and ERK1) show 15 of 15 and 13 of 15 amino acid sequence identity with Xp42 in the region recognized by the antibody, although both have additional residues at the COOH terminus.

In all Xenopus adult tissues tested, the antibody detected an approximately 40 kDa protein of approximately the same mobility as Xp42, detected in Xenopus oocytes (used as a control) (Fig. 5). Slight variations in mobility could be due to differential expression of the two cloned forms of Xp42, which migrate slightly differently on SDS gels despite being identical in molecular size. Besides this 40 kDa band, other weak bands of much lower molecular mass were detected in some tissues. These could be cross-reacting bands or breakdown products. Most were too small to encode functional protein kinases.

The highest level of Xp42 was observed in brain, spinal cord, and spleen, and less was observed in skin, intestine, testis, liver, and ovary (Fig. 5). The content of Xp42 was relatively lower in kidney, adrenal, heart muscle, and whole blood. Xp42 was not detected in plasma. If the Xp42 in blood is restricted to the blood cells, their content of Xp42 must be high. Xp42 was not detected in skeletal muscle, despite detectable Xp42 RNA hybridization.

The levels of Xp42 protein in adult tissues were thus in general agreement with the levels of Xp42 RNA, i.e., high in brain and spleen, and lower in skin, intestine, and testis. Apparent discrepancies were observed for liver, kidney, and muscle: kidney and muscle contained more RNA than liver, but Xp42 protein abundance progressively decreased, comparing liver to kidney to muscle. The most marked difference was that we did not detect an increased level of Xp42 in ovary; it was comparable to the level in other tissues (Fig. 5). This fact supported...
the idea that the high level of mRNA in ovary is mostly inactive in translation and is probably stored in the oocytes for translation in embryogenesis.

Phosphorylation of Xp42 at either tyrosine or threonine or both can be detected due to decreased mobility on a SDS gel relative to the nonphosphorylated form (18). In eggs, all Xp42 is phosphorylated (Fig. 5, pp42; Refs. 10, 19, and 20). In most Xenopus adult tissues, the protein was present in nonphosphorylated form. Small quantities (extremely faint bands) of what may be phospho-Xp42 were detected in adrenal, heart muscle, brain, kidney, and skin. In Xenopus tadpole tissues, in contrast...
Fig. 4. Distribution of Xp42 RNA in a young frog (stage 66). Histoblot hybridization of 6 coronal sections taken from various levels of the same frog. Upper panels, autoradiographs; lower panels, hematoxylin staining. Tissues are labeled: 1, brain; 2, skin; 3, cranial nerve; 4, muscle; 5, eye; 6, liver; 7, ovary; 8, intestine; 9, thymus; 10, kidney; 11, stomach; 12, esophagus; 13, olfactory organs; 14, spleen.

Fig. 5. Expression of Xp42 protein detected by Western blotting of tissues. Protein was extracted from various tissues and analyzed by SDS-polyacrylamide gel electrophoresis and Western blotting with antibody to a COOH-terminal peptide of Xp42. Ovary samples were obtained from an ovulated adult (post ov.), a normal adult, and an immature female (imm.). Other samples were from adult Xenopus. pp42, the phosphorylated form of Xp42 found in eggs; p42, the nonphosphorylated form of Xp42 found in oocytes.
to adult tissues, we always detected a significant amount of the putative phosphorylated form of Xp42, although nonphosphorylated Xp42 was predominant (data not shown).

Expression of Xp42 Protein during Oogenesis and Early Embryogenesis. In order to determine whether Xp42 protein is expressed in early oocytes and during early embryogenesis, we examined protein extracts by Western blotting. In oocytes of all stages tested (stages 1–6), 1913 antibody detected the nonphosphorylated form of Xp42 (Fig. 6A). When proteins from the equivalent of one oocyte were loaded on the gel, the level of Xp42 was approximately constant during oogenesis, despite a marked increase in total oocyte protein (Fig. 6A). This means that Xp42 was either not synthesized or was turned over at constant rate during oogenesis so the quantity of Xp42 per cell remained constant.

To test whether Xp42, present in developing oocytes, might play a role in signal transduction, we treated stage 3/4 (0.6–1.0 mm in diameter) oocytes with the phorbol ester TPA (Fig. 6C). Treatment with TPA for 6–60 min stimulated a mobility shift of an estimated 20% of Xp42 molecules in stage 3/4 oocytes (Fig. 6C). Since these oocytes were not dejelliculated, we cannot determine whether the putative phosphorylated Xp42 was present in the oocyte or follicle cells. There are approximately 6000 follicle cells/oocyte (14), but they are very small compared with the oocyte, and some follicle cells may have been removed by collagenase. Addition of TPA to stage 6 oocytes did not induce the phosphorylation of Xp42 seen in stage 3/4 oocytes, but after 16 h, there was a shift to about 50% phosphorylated form (Fig. 6C). TPA has been reported to accelerate, but not induce, maturation of stage 6 oocytes (21).

After progesterone induction of stage 6 oocytes, there appears to be little, if any, synthesis of Xp42, because the quantities of Xp42 in stage 6 oocytes and eggs are equal. In eggs, however, all of the Xp42 has the mobility characteristic of the phosphorylated form. Incubation of stage 6 oocytes for 48 h with cycloheximide did not cause the content of Xp42 to drop detectably, suggesting that the protein does not turn over in cycloheximide-treated oocytes. Phosphorylated Xp42 also appears to be stable in eggs until fertilization. At this time, Xp42 is dephosphorylated and inactivated (20, 22), and the dephosphorylated protein is stable.

We have followed the expression of Xp42 in embryos, from fertilization to the blastula stage (Fig. 6B). The content of Xp42 per embryo did not change. Also, there was no sign of Xp42 phosphorylation at the times studied.

*J. Posada and J. A. Cooper, unpublished results.

1J. Posada, unpublished data.

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Fig. 6. Amount and phosphorylation state of Xp42 protein in developing oocytes and early embryos. A and B, Western blot detection of Xp42 protein in extracts from oocytes of stages 1 through 6 (A) and embryos stages 1 through 9.5 (B). Each lane was loaded with protein derived from equal numbers of oocytes or embryos. C, kinetics of phosphorylation of Xp42 in TPA-treated stage 6 and stage 3/4 oocytes. TPA was added to 1 μg/ml concentration. pp42, the phosphorylated form of Xp42 found in eggs; p42, the nonphosphorylated form of Xp42 found in oocytes.
The constant level of Xp42 suggests that either Xp42 protein is stable, or it turns over and is resynthesized from stored maternal RNA. The quantity of total protein is approximately constant during this period of development, so the concentration of Xp42 relative to total protein is also constant, but the large increase in cell number means that the average quantity of Xp42 per cell decreases by at least 3 orders of magnitude.

Discussion

In the adult, we found the highest expression of Xp42 mRNA in the ovary, specifically in stage 2-4 oocytes, where it appears to be stored, possibly to allow Xp42 protein synthesis through oogenesis and early embryogenesis. During development of Xenopus from larval stages to the adult, Xp42 mRNA is expressed at high levels in the developing nervous system, lymphoid organs (thymus and spleen), developing limb buds, and the developing ovary—all regions of active cell proliferation, differentiation, and signal transduction. Lower levels of RNA were detected in other tissues. The Xp42 protein was detected in all tissues studied and was most abundant in the nervous system and lymphoid organs. The results are consistent with an essential role for MAP kinases in all cell types, as expected from the wide range of extracellular stimuli that provoke MAP kinase activation.

The cDNA probe and antibodies used should detect the products of both of the Xenopus MAP kinase genes that have been sequenced (10, 11). In most tissues, there is a general concordance between RNA and protein levels, suggesting that regulation of protein abundance probably does not occur at a translational or posttranslational level. The major exception is ovary, which has a disproportionately large quantity of MAP kinase RNA but an exceptional level of MAP kinase protein. Thus, much of the RNA in ovary may not be translated. Our analysis of the distribution of MAP kinase RNA and protein in oocytes at different stages of oogenesis is consistent with a model in which the RNA is produced in very large amounts for storage in the initial stages of oogenesis, and in which MAP kinase protein is also made at that time. During the subsequent growth of the oocyte, there is no further increase in the quantity of MAP kinase RNA, and MAP kinase protein quantity is also constant, implying balanced synthesis and degradation. This suggests that MAP kinase RNA resembles other polyadenylated RNAs that are stored in the oocyte in being accumulated early, before rRNA (17). The quantities of MAP kinase RNA and protein are also constant in early embryogenesis, from fertilization through to late blastula. The volume and protein content of the embryo change little over this time, but the number of cells increases by orders of magnitude. The MAP kinase protein concentration in mature oocytes and early embryos is similar to that in many adult tissues, and is presumably adequate for signaling.

A growing number of mRNAs are being found in stored form in Xenopus oocytes. Examples include RNAs for Mos, cyclins, Ras, Vg-1, and MAP kinase. Most of the stored maternal RNAs are believed to participate in development prior to the midblastula transition. For example, Mos is required for oocyte maturation, cyclins are needed for the embryonic cell cycles, Ras may function in oocyte maturation of signaling events in the early embryo, and Vg-1 is an RNA that is localized in the vegetal hemisphere of the egg and participates in early inductive events (15, 16, 23-25). Other RNAs, such as MyoD, are not stored in the egg (26). Because MAP kinase mRNA is stored in the oocyte, we anticipate that it is required for events occurring during oogenesis or early embryogenesis prior to the midblastula transition. In the stage 6 oocyte, MAP kinase is activated during meiotic maturation (10, 19, 20, 22, 27). A kinase that phosphorylates, and activates, MAP kinase has been purified from eggs (28). Activated MAP kinase may be involved in cytoskeletal changes at meiosis (11). During early embryonic development, prior to the midblastula transition, cell-cell interactions are required for inductive events, including ectoderm and mesoderm induction and definition of the dorsal-ventral and anterior-posterior axes (29-33). If MAP kinase is involved in intracellular transduction of the signals that are responsible for early inductive events, then our failure to detect phosphorylation of MAP kinase in early embryos implies that activation is temporally or spatially restricted.

The MAP kinase in undcveloped oocytes may function to transduce signals that regulate oogenesis. The MAP kinase of small oocytes can be phosphorylated in response to TPA, as occurs in mammalian (4) and Xenopus tissue culture cells (data not shown). This suggests that MAP kinase in small oocytes could participate in the response of these cells to signals from the stroma of the ovary. Later in oogenesis, fully developed oocytes respond to in vivo or in vitro progression into meiosis by stoichiometric phosphorylation and activation of MAP kinase.

In tadpoles and adult tissues, MAP kinase RNA and protein were most abundant in the nervous system (brain, spinal cord, cranial and spinal nerves, sensory olfactory epithelium, and retina) and lymphoid tissues (spleen and thymus), and abundant in liver, kidney, skin, and intestine. The abundance of MAP kinase RNA in brain and olfactory organs declined during development, being greater in stage 56 than stage 59 or 66 of development. On the other hand, MAP kinase RNA seemed to be less abundant in ovary from juvenile compared with fullgrown females. These observations suggest changes in expression during the development of specific organs. The status of MAP kinase in Xenopus skeletal muscle is uncertain, since we detected RNA but only traces of protein. Possibly, MAP kinase gene expression is present in Xenopus muscle but is hard to extract, or, alternatively, the preponderance of a few, specialized contractile proteins in muscle leads to a relative underrepresentation of “housekeeping” proteins such as MAP kinase.

Our results may be compared with those obtained for mammalian MAP kinases. MAP kinase activities have been detected in cells of hematopoietic (monocyte, lymphocyte), neural crest (melanocyte, pheochromocytoma, adrenal chromaffin), mesenchymal (fibroblast, adipocyte), and endodermal (hepatocyte, epithelial) origins. By Northern and Western blotting, Boulton et al. (6, 7) have detected ERK1 or ERK2 in most cells and tissues tested. However, the ratio of the two species varies greatly. At one extreme, cultured astroglia express RNA for ERK1 but not ERK2, and at the other, neuronally induced P19 embryonal carcinoma cells express RNA for ERK2 but not ERK1. In tissues, less extreme situations were found,
but ERK1 RNA predominates in spinal cord, intestine, and lung and ERK2 RNA in hippocampus, muscle, and heart (6). By Western blotting, ERK1 protein outweighs ERK2 protein in testis, heart, intestine, and lung. More equal amounts of the two proteins were detected in spinal cord, total brain, thymus, spleen, and kidney (7). As in Xenopus, muscle contained less MAP kinase protein than expected from the amount of RNA. However, insulin-stimulated rabbit muscle contains a MAP kinase activity that can stimulate a Rsk-like protein kinase (34), so the apparent underabundance in muscle may be an artifact. The two Xenopus MAP kinases bear much closer similarity in RNA sizes and protein sequences to mammalian ERK2 than to ERK1, but they may fulfill the functions of both ERKs. Indeed, the divergence of the two ERKs in mammals may not necessarily denote differences in function: both are activated more or less equally by the same MAP kinase activators, purified from nerve growth factor-stimulated PC12 cells or epidural growth factor-stimulated A431 cells (35, 36). The two ERKs may show subtle differences in substrate specificity, however, and functional specialization could have occurred in mammals but not amphibians.

The expression of Xenopus MAP kinase in various embryonic and adult tissues speaks to possible roles for MAP kinases in signal-regulated cell proliferation, differentiation, and neuronal signaling. A requirement for MAP kinases for each of these processes may be demonstrated by future experiments using inhibitors, antisense, dominant-negative, or gene disruption approaches.

Materials and Methods

Animals. Xenopus laevis were obtained from Xenopus I (Ann Arbor, MI) and NASCO (Briarcliff Manor, NY). Tissues were identified by reference to standard texts (37–39).

Histoblotting. The histoblotting technique has been described previously (12). Adult, decapitated frogs, immature frogs, and tadpoles were placed in traps made from aluminum foil, surrounded with O.C.T. compound (embedding medium for frozen tissue specimens; Miles, Elkhart, IN), and frozen in liquid nitrogen. Whole body sections (15 mm thick) were made on a cryostat (Bright Instrument Co., Huntingdon, England), placed on nitrocellulose membrane (type BAB5; Schleicher and Schuell, Keene, NH), and baked at 80°C under vacuum for 2 h. Blots were prehybridized in 50% formamide, 5X SSC (SSC contains 0.15 m NaCl and 0.015 m sodium citrate), 0.1% SDS, 100 µg/ml denatured salmon sperm DNA, and 0.1% each of Ficoll, polyvinyl pyrrolidone, and bovine serum albumin, at 4°C for 4–12 h. Hybridization was carried out in the same solution, with the addition of 32P-labeled probe, at 42°C for 24 h. Blots were washed first in 2X SSC with 0.2% SDS at room temperature, then twice in 1X SSC at 66°C, and finally in 0.5X SSC at 66°C. After washing, blots were dried at room temperature and exposed to XAR-5 film (Kodak) without intensifying screens. After exposure, histoblots were stained with hematoxylin and made transparent by soaking in Permount mounting solution for examination by light microscopy.

Preparation of Oocytes and Embryos. Oocytes were prepared from ovaries that had been digested with 0.5 mg/ml collagenase in MBS (88 mM NaCl-1 mM KCl-0.41 mM CaCl2-0.2 mM MgCl2-2.4 mM NaHCO3-10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4). Oocytes were staged according to size using nylon mesh (Nitex, Tetko, Fort Atkinson, WI). Oocyte sizes were measured using a graticule (13). Stage 6 oocytes were induced to mature with 10 µM progesterone (Sigma, St. Louis, MO).

Eggs were obtained from adult Xenopus females after injection with 500–800 units of human chorionic gonadotrophin (Steris Laboratories, Phoenix, AZ) and fertilized with minced testis, in 1X MBS containing gentamycin. Embryos were grown in 0.1X MBS at room temperature. Early embryos were dejellied in 2% cysteine (pH 7.5) and extensively washed in 0.1X MBS before extracting RNA or proteins.

Northern Blot Hybridization. Total RNA was isolated from tissues, oocytes, or dejellied embryos by using the acid guanidinium thiocyanate-phenol-chloroform procedure (40). RNA was denatured with 5 M sodium hydroxide, precipitated with ethanol, and analyzed by electrophoresis on 1.5% agarose gels in 0.4X M 3-(N-morpholino)propanesulfonic acid, pH 7.0-10 mM NaOOCCH3-0.2 mM EDTA-2.2 M formaldehyde (41). Gels were blotted to nitrocellulose membrane and probed with nick-translated probes as described for the histoblots.

Western Blots. Xenopus tissues, oocytes, or dejellied embryos were quickly homogenized using a Dounce homogenizer in an ice-cold solution containing 20 mM Tris-HCl, 1 mM dithiothreitol, 1 mM EDTA, and 20 µg/ml aprotinin, using 5 ml/1 g of tissue. Following centrifugation at 4000 × g for 10 min, the supernatants were diluted with an equal volume of 2X concentrated SDS gel sample buffer. Protein concentrations were measured using the Bradford protein assay (Bio-Rad, Richmond, CA). Approximately 80 µg of protein were loaded on each lane of an SDS-polyacrylamide gel (15% acrylamide-0.193% bisacrylamide). Following electrophoresis, the gel was transferred to Immobilon (Millipore, Bedford, MA) using a semidyde electroblotter. The blot was blocked with a solution containing 1% bovine serum albumin, 1% ovalbumin, and 0.05% Tween-20 in 0.15 M NaCl-10 mM Tris-HCl, pH 7.5, and probed with affinity-purified 1913 antibody, raised to a synthetic peptide, corresponding to residues 347–361 at the COOH terminus of Xp42 (18). The antisera was purified by passing over an Affi-Gel 15 column containing the peptide antigen and eluted with 0.1 M glycine HCl, pH 1.8. Blots were washed with 0.15 M NaCl-10 mM Tris-HCl, pH 7.5, probed with a 2000-fold dilution of alkaline phosphatase-conjugated antisera raised to rabbit immunoglobulin, washed again, and developed with 0.33 mg/ml p-nitro blue tetrazolium chloride-0.16 mg/ml 5-bromo-4-chloro-3-indolyolphosphate toluidine salt in 0.1 M Tris-HCl, pH 9.5-0.1 M NaCl-0.05 M MgCl2.

Probes. The Xp42 probe used was a restriction fragment of Xp42 cDNA containing 1306 base pairs, cloned into the EcoRI site of pBluescript KS+ (Stratagene, La Jolla, CA). This clone contains the entire open reading frame of Xp42, 41 base pairs of 5’ untranslated sequence and 182 base pairs of 3’ untranslated sequence. The Xenopus Mor probe was an approximately 1.5-kb fragment derived from the plasmid pTZXA+. (42). This contains the Xenopus mos cDNA cloned into the expression plasmid pTZ18R (Pharmacia LKB, Piscataway, NJ). For a nonspecific probe, pBR322 DNA was used. Restriction
fragments were purified by agarose gel electrophoresis and labeled using a random primed labeling kit (Boehringer Mannheim).

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References
26. Rupp, A. W., and Weintraub, H. Ubiquitous MyoD transcription at the midblastula transition precedes induction-dependent MyoD expres-

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