Developmental regulation of transcription factor AP-2 during *Xenopus laevis* embryogenesis

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ABSTRACT

We have isolated a cDNA clone encoding the *Xenopus* homologue of the transcription factor AP-2 (XAP-2). The predicted amino acid sequence derived from the *Xenopus* cDNA shows very strong conservation with the amino acid sequence of human AP-2, suggesting that this protein is evolutionarily conserved, at least among vertebrates. This is further substantiated by the demonstration that an *in vitro* translation product of XAP-2 cDNA bound specifically to an AP-2 binding site from the human MT-II_A gene. Northern blot analysis of *Xenopus* embryo RNA revealed the existence of three major XAP-2 mRNA species that were only detectable after the midblastula transition (when embryonic transcription is activated), with peak accumulation of the transcripts occurring during gastrulation. Therefore, in contrast to other *Xenopus* transcription factors, XAP-2 is not maternally derived but arises exclusively from zygotic transcription. Unlike the situation in cultured human teratocarcinoma (NT2) cells, retinoic acid treatment did not induce XAP-2 mRNA in *Xenopus* embryos, even though the treatment had a pronounced morphogenetic effect on the embryos. Our results suggest that XAP-2 may play a distinctive role during *Xenopus* embryogenesis.

INTRODUCTION

One useful approach for analyzing regulation of gene expression in higher eukaryotes is the study of *trans*-acting protein factors that modulate transcription rate through binding to a gene's *cis*-acting DNA elements. Certain of such transcription factors have been shown to have their expression or activity regulated in a cell-type-specific, tissue-specific, or cell-cycle-dependent fashion (1–4; for review, see 5). Few transcription factors, however, have been shown to be regulated during embryonic development. In *Xenopus*, most transcription factors responsible for gene expression at the onset of embryonic transcription, a stage called the midblastula transition (MBT; 6–8), are believed to be maternal in origin. Proteins binding to CCAAT and ATF/AP-1-like sequences have been found in nuclear extracts from early blastula embryos (9), a binding activity specific for

the cardiac actin CARG promoter element is present in unfertilized eggs (2), and heat-activatable heat shock transcription factor (HSF) activity has been demonstrated in unfertilized eggs and early embryos (10). All of these factors, since they are present before embryonic transcription is active, must be produced from maternal mRNA synthesized in the oocyte.

One transcription factor that may play a role in development is AP-2. This protein was first identified and purified from HeLa cells as a 50–52 kDa binding activity that recognized sequences common to the enhancer elements of SV40 and the human metallothionein-II_A (hMT-II_A) gene (11–13) and stimulated transcription *in vitro* of gene constructions containing the hMT-II_A promoter (12, 13). Binding sites for AP-2 have been found in a number of other human protein-coding genes, including proenkephalin (14), collagenase (15), growth hormone (12), and *c-myc* (12). From comparison of twelve such binding sites, a consensus sequence of 5'-CCCCAGGC-3' has been determined (13), although individual sites may vary substantially from the consensus. More recently, a cDNA encoding human AP-2 was cloned from a HeLa cDNA library (16).

AP-2 expression has been shown to be stimulated transiently during retinoic acid-induced differentiation of NT2 human teratocarcinoma cells (16, 17). Retinoic acid is a morphogen involved in vertebrate limb bud pattern formation (18–20), so response of AP-2 expression to this morphogen has led to the proposal that AP-2 may have an important role in regulating gene expression in early development (16). In *Xenopus*, retinoic acid has also been shown to affect embryonic primary axis formation (21) and anteroposterior patterning of the central nervous system (22).

In this paper, we report the cloning of a *Xenopus* cDNA that is homologous to human AP-2 cDNA. Developmental profiles of *Xenopus* AP-2 mRNA accumulation have revealed that expression is stage-dependent, suggesting that AP-2 may be a developmentally significant transcription factor in *Xenopus* embryogenesis.

MATERIALS AND METHODS

Library Screening, Subcloning, and Sequencing

The library used for screening was a *Xenopus laevis* stage 13 embryo cDNA library constructed in λgt11 (23). Human AP-2

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cDNA (clone AP2-9, a gift from Dr. T. Williams) was labeled with [α - 32 P] dCTP (Amersham) by nick translation using reagents from Bethesda Research Laboratories. Lambda plaques were transferred to BA85 nitrocellulose (Schleicher and Schuell) and screened with the probe according to the procedure outlined by Ausubel *et al.* (24). The screening was done at moderate stringency with two posthybridization washes in $1\times$ SSPE (0.15 M NaCl, 0.01 M NaH₂PO₄, 1 mM EDTA, pH 7.4), 0.1% SDS at 55°C. Positive plaques were picked, grown, and rescreened twice more to achieve greater than 90% homogeneity. The cDNA inserts in these clones were then subcloned into the Eco RI site of pBS+ (Stratagene) and sequenced at their 5' and 3' termini. One clone, XAP2-6a, was sequenced completely on both strands. Sequencing was carried out by the dideoxy method using Sequenase reagents (U.S. Biochemical Corp.).

In vitro Transcription and Translation

To transfer clone XAP2-6a intact into an expression vector, the entire open reading frame plus 31 bp upstream and 109 bp downstream was amplified from the phage DNA by polymerase chain reaction (PCR). The sense primer consisted of nucleotides 11 to 31 of clone XAP2-6a wherein is found a Bam HI site. The antisense primer consisted of nucleotides 1408 to 1428 of clone XAP2-6a with a Bam HI site added onto the 5' end. Amplification of 1 ng of clone XAP2-6a DNA was carried out in a reaction mixture containing 2.5 mM MgCl₂, 50 mM KCl, 10 mM Tris, pH 8.3, 0.01% (w/v) gelatin, 0.2 mM of each dNTP, 1 μ M of each primer, and 5 units of Taq polymerase (U.S. Biochemical Corp.) for 26 cycles in a Perkin-Elmer temperature cyclor. PCR reaction products were extracted with phenol/chloroform and chloroform, then precipitated with 2.5 volumes of absolute ethanol. Amplified DNA was digested with Bam HI and inserted into the Bam HI site of pET-3c (25), which contains a T7 promoter and transcription terminator. Absence of mutation in this construction, pET-3cXAP-2, was confirmed by DNA sequencing.

Xenopus AP-2 RNA synthesis was carried out using a Promega transcription kit. One μ g of pET-3cXAP-2 (linearized by digestion with Hind III) was transcribed in a 100 μ l reaction for 60 min at 37°C with T7 polymerase. Template DNA was removed by digestion with 2 units of RQ1 DNase for 15 min at 37°C, and RNA was isolated by sequential extractions with phenol/chloroform and chloroform followed by ethanol precipitation.

For *in vitro* translation, the RNA was heated to 65°C, then cooled on ice and added to 35 μ l of rabbit reticulocyte lysate (Promega) along with 120 μ Ci of [35 S]-methionine (Amersham). A control reaction set up in parallel contained no added RNA. Reactions were carried out at 30°C for 60 min.

Reaction products were analyzed by SDS-PAGE on a Hoeffer minigel apparatus. 2.5 μ l of each reaction mix were added to 10 μ l of protein sample buffer (25) and electrophoresed on a 10% polyacrylamide gel. Gel staining, destaining, and fluorography were performed as previously described (26).

DNA Mobility Shift Assay

The probe used for the mobility shift assay was a double-stranded synthetic 40 bp oligonucleotide (sense strand sequence: 5'-TCGACAGAACTGACCGCCCGCGCCCGTGTGCAGAGTCTGA-3') representing the distal basal level element (BLE) of the hMT-II_A promoter (nucleotides -188 to -159) which contains an AP-2 binding site (16). Conditions for the mobility

shift were as in Snape *et al.* (27), except that 0.5 ng of labeled probe were used in each binding reaction, 7.5 μ g of poly(dI·dC)·poly(dI·dC) were added to each binding reaction instead of 5 μ g, and 2 μ l of *in vitro* translation reaction mix were used in place of nuclear extract. Competitor DNA, consisting of 50 ng of unlabeled probe oligonucleotide or an unlabeled oligonucleotide containing a CArG box from the *Xenopus* cardiac actin gene (nucleotides -76 to -99; 2), was added where appropriate. Two sheets of paper were placed between the dried gel and the X-ray film during exposure to block signal from the [35 S]-methionine-labeled protein.

Egg, Embryo, and Oocyte Sample Preparation

Xenopus laevis eggs were obtained, fertilized, dejellied, and maintained in Steinberg's solution as described by Heikkilä *et al.* (28). Embryos were staged according to Nieuwkoop and Faber (29). At each stage sampled, approximately 50 eggs or embryos were placed in sterile 1.5 ml microcentrifuge tubes, frozen on dry ice, and stored at -80°C. Defolliculated oocytes were prepared by surgically removing ovary and treating with 0.2% collagenase (Type IA, Sigma) in OR-2 solution (30).

Retinoic Acid Treatment

Embryos were treated with retinoic acid as follows: At stage 9, embryos were divided into two Petri dishes containing Steinberg's solution. All subsequent steps were done under illumination by only a red safelight to prevent breakdown of the drug. Retinoic acid was added to one dish to a final concentration of 1 μ M from a 1 mM stock in methanol. An equivalent volume of methanol was added to the control dish. Both dishes were stored in the dark. Sampling times are described in terms of time after addition of retinoic acid, and the embryonic stage reached by control embryos at the time of sampling is shown in parentheses. After 30 min (stage 9), 60 min (stage 9), and 2 h (stage 10), embryos were sampled from both dishes. After two h of exposure, half of the retinoic acid-treated embryos were transferred to a third dish containing only Steinberg's solution. At 6 h (stage 11), 16 h (stage 21-22), 24 h (stage 27), and 40 h (stage 34-35) after the initial addition of retinoic acid, embryos were sampled from all three dishes.

RNA Isolation and Northern Blot Analysis

RNA was isolated from embryos, eggs, and oocytes as outlined previously (31) except for the following modifications: DNA was not removed by LiCl precipitation; rather, DNA was digested by 1 unit of RQ1 DNase (Promega) at room temperature for 30 min in a buffer containing $1\times$ T7 transcription buffer, 40 units of RNasin (Promega), and 10 μ M DTT. The RNA was then extracted with phenol/chloroform and precipitated by the addition of 0.1 volumes of 3M sodium acetate and an equal volume of isopropanol.

RNA was denatured with glyoxal using the method of Thomas (32) and separated on 1.2% agarose gels in 10 mM sodium phosphate buffer, pH 6.8. RNA was blotted to Nytran (Schleicher and Schuell) as described elsewhere (33).

DNA probes were labeled with [α - 32 P] dCTP as described above. Hybridization was carried out according to the procedure of Church and Gilbert (34) except that the hybridizations were done in glass tubes in a Robbins hybridization incubator instead of in sealed bags. Blots were hybridized overnight at 65°C then washed twice at 65°C for 15 min in $2\times$ SSPE, 0.1% SDS and twice at 65°C for 15 min in $0.2\times$ SSPE, 0.1% SDS.

Southern Blot Analysis

For Southern blotting, 7.5 µg per sample of homozygous *Xenopus laevis* DNA (HD-1; kindly provided by Dr. R. Tompkins) were digested overnight at 37°C with a variety of restriction enzymes. The digested DNA was electrophoresed on a 1% agarose gel and transferred to Nytran as described by Maniatis *et al.* (35). Hybridization was carried out as for RNA blots.

RESULTS

In order to isolate *Xenopus* AP-2 cDNA sequences, we screened a stage 13 *Xenopus* embryo cDNA library (23) at moderate stringency using human AP-2 cDNA (16) as a probe. Preliminary sequence data from the eight positive clones suggested that one clone, XAP2-6a, most closely corresponded to the human AP-2 cDNA and included a complete open reading frame, so this clone was chosen for subsequent study (Fig. 1A). The 1573 bp nucleic acid sequence of clone XAP2-6a is not shown, but is available for examination through the EMBL Data Library under accession no. M59455. The open reading frame (represented by an open box in Fig. 1A) identified in the *Xenopus* sequence could encode

Fig. 1. A. Diagrammatic representation of clone XAP2-6a. The open reading frame is depicted by an open box. The Eco RI sites used in subcloning XAP2-6a and generating the hybridization probe are denoted by E. The hatched box represents the part of the clone used as a probe in hybridization studies. B. Comparison of the amino acid sequence deduced from the DNA sequence of the open reading frame of clone XAP2-6a to the deduced amino acid sequence of human AP-2. The entire *Xenopus* sequence is presented, but the human sequence is shown only where it differs from the *Xenopus* sequence. In two places (after position 10 and position 412) where the *Xenopus* sequence lacked an amino acid that was found in the human sequence, dashes have been inserted into the *Xenopus* sequence to preserve the sequence overlap. Translation stop codons are represented by asterisks(*).

a polypeptide of 425 amino acids with a mol wt of 49,659, very similar to the size determined for human AP-2 (12, 16). Comparison of the predicted amino acid sequence from the *Xenopus* open reading frame to the amino acid sequence for human AP-2 (16) revealed striking homology between the two sequences (Fig. 1B). The two sequences differ at the amino terminus, but overall show 88.1% identity at the amino acid level. If conservative amino acid substitutions are allowed, the degree of similarity between the two sequences is 92.7%. Because of this high degree of conservation, we believe that the cDNA we isolated encodes *Xenopus* AP-2 (XAP-2).

To further substantiate our claim that our cDNA encodes XAP-2, we subcloned a PCR-amplified segment (containing the entire open reading frame) of clone XAP2-6a in frame into the T7 expression vector pET-3c (25). This construction was transcribed *in vitro* using T7 polymerase and the resulting RNA was translated *in vitro* in a rabbit reticulocyte lysate. Translation products were analyzed by SDS-PAGE, shown in Fig. 2A. Compared to a control translation reaction in which no exogenous RNA was added (lane 1), the translation reaction containing the *in vitro* transcribed RNA (lane 2) produced a single polypeptide of approximately 48 kDa, which closely agrees with the predicted mol wt of 49.6 kDa.

The *in vitro* translation reaction product was tested for DNA binding properties in a DNA mobility shift assay using a radiolabeled oligonucleotide containing the AP-2 binding site from the distal BLE of the hMT-II_A gene (16) as a probe. As is evident in Fig. 2B, the translation product bound to the probe (lane 3). This binding was abolished by the addition a 100-fold excess of unlabeled probe oligonucleotide (lane 5), but was not affected by the addition of a 100-fold excess of a non-specific

Fig. 2. The polypeptide encoded by clone XAP2-6a binds specifically to an oligonucleotide containing an AP-2 site. A. Fluorograph of an SDS-polyacrylamide gel of *in vitro* translation reaction products. RNA transcribed from an expression vector containing the open reading frame of clone XAP2-6a was added to a rabbit reticulocyte lysate mixture and the translation products were labeled with [³⁵S]-methionine. Lane 1: No RNA added. Lane 2: *In vitro* transcribed RNA added. Molecular weight markers (in kDa) are shown on left. An arrow indicates the position of the 48 kDa translation product. B. DNA mobility shift assay of the translation reaction mixtures in Fig. 2A. The probe used was a [³²P]-labeled oligonucleotide containing an AP-2 site from the hMT-II_A gene. The positions of bound and unbound probe are indicated. Lane 1: No protein added. Lane 2: Protein from lane 1 in Fig. 2A added. Lane 3: Protein from lane 2 in Fig. 2A added. Lane 4: Same as lane 3 except that 100-fold excess of unlabeled CARG box competitor has been added. Lane 5: Same as lane 3 except that 100-fold excess of unlabeled AP-2 binding site competitor has been added.

competitor, an oligonucleotide containing a CArG box from the *Xenopus* cardiac actin gene (2; lane 4). Clone XAP2-6a therefore encodes a polypeptide that binds specifically to DNA containing an AP-2 binding site.

The expression pattern of XAP-2 during *Xenopus* embryogenesis was examined by Northern blot analysis of RNA isolated from various embryonic stages (Fig. 3A). The probe used in these studies was a 1 kbp Eco RI fragment from clone XAP2-6a (represented by a hatched box in Fig. 1A). Interestingly, the XAP-2 probe hybridized to three major bands with sizes of approximately 2.6 kb, 2.2 kb, and 1.8 kb. Based on these sizes, it would appear that the cDNA clone we isolated is not full length, but lacks at least 200 bp. The sizes given are approximate because, like human AP-2 mRNA (16), the XAP-2 mRNA bands were quite diffuse compared to RNA bands which appeared when the same blot was rehybridized with other cDNA probes (data not shown). None of the XAP-2 bands was detected in egg, stage 6, or stage 8 RNA samples (lanes 1–3), but all were first detectable at stage 9 (lane 4), shortly after the midblastula transition. Although their accumulation began at the same stage, the three RNA species did not show identical expression patterns. The 1.8 kb band appeared to be somewhat transient, showing maximal accumulation at stage 11 (midgastrula; lane 7), then decreasing to low levels by stage 27 (tailbud; lane 11). The 2.2 kb band also peaked at stage 11, but was still strongly detectable as late as stage 45 (swimming tadpole; lane 13). The 2.6 kb RNA was not strongly detected in late blastula/early gastrula stages, but showed increased accumulation as development progressed, up to at least stage 45. This transcript, and the 2.2 kb RNA, have been detected in some tissues (skin, kidney, and brain), but not others (liver, lung), from adult frogs, demonstrating that these transcripts are not limited to embryos and are tissue-specific. The 1.8 kb RNA is detected in skin, kidney, and brain only at very low levels (A. M. Snape, unpublished observations).

Fig. 3. Pattern of XAP-2 mRNA accumulation during embryonic development. A. Total RNA was isolated from *Xenopus* eggs and from embryos at various stages, electrophoresed, blotted to Nytran, and hybridized to an XAP-2 cDNA probe. Lane 1: Egg. Lane 2: Stage 6. Lane 3: Stage 8. Lane 4: Stage 9. Lane 5: Stage 10. Lane 6: Stage 10½. Lane 7: Stage 11. Lane 8: Stage 12. Lane 9: Stage 15. Lane 10: Stage 20. Lane 11: Stage 27. Lane 12: Stage 36. Lane 13: Stage 45. Transcript sizes: a=2.6 kb; b=2.2 kb; c=1.8 kb. B. Overexposure of a Northern blot of oocyte, egg, and embryo RNA. Lane 1: Oocyte. Lane 2: Egg. Lane 3: Stage 8. Lane 4: Stage 11. Lane 5: Stage 20.

To determine if XAP-2 transcripts were present at any point during oogenesis, RNA from defolliculated oocytes of mixed stages was blotted along with RNA from egg and three embryonic stages (Fig. 3B). Even after overexposing the blot, no XAP-2 mRNA was observed in ovary, egg, or stage 8 samples (lanes 1–3). To ensure even RNA loading and the integrity of the RNA samples, both blots in Fig. 3 were stripped of XAP-2 probe and rehybridized with a probe from the cDNA clone r5, which represents an unidentified ubiquitous RNA (31). From this reprobing, intact RNA was confirmed in all lanes (data not shown). It appears, therefore, that XAP-2 gene expression does not occur during oogenesis, but commences only after the midblastula transition.

The presence of multiple XAP-2 mRNA species in embryos raised the possibility of a family of AP-2 genes in *Xenopus*. To explore this possibility, we performed a Southern blot of homozygous *Xenopus* DNA digested with various enzymes and probed with the XAP-2 probe (Fig. 4). In most digested samples, the XAP-2 probe hybridized to several bands of large size (lane 1 in particular). The observed DNA band sizes suggest that at least two, but probably not more than four, XAP-2 genes exist. Isolation and characterization of genomic clones will be required to clarify the relationship between the multiple mRNAs and genomic XAP-2 DNA.

Because human AP-2 expression was reported to be stimulated by retinoic acid (RA; 16, 17), and because RA has been shown to affect *Xenopus* embryonic development (21, 22), we tested whether XAP-2 expression could be induced by RA in developing embryos. Embryos were exposed to 1 µM RA beginning at stage 9, the period during which *Xenopus* embryos are the most sensitive to the morphological effects of RA (21), and RNA was prepared from the embryos at various times thereafter. As demonstrated in Fig. 5, RA treatment of embryos under these conditions did not significantly increase accumulation of XAP-2 mRNA (lanes 1–12). We did observe a decrease in XAP-2 expression upon RA treatment for 2 h (lane 6 compared to the control in lane 5) and for 6 h (lane 8 compared to lane 7). In

Fig. 4. Southern blot of *Xenopus* genomic DNA digested with various restriction enzymes and hybridized to XAP-2 cDNA. Size markers (in kbp) are shown at left. Lane 1: Bam HI. Lane 2: Bgl II. Lane 3: Eco RI. Lane 4: Hind III. Lane 5: Pst I. Lane 6: Sac I.

these two cases control embryos at the time of sampling were at stage 10 and stage 11, respectively, both of which correspond to early gastrulation, when XAP-2 mRNA shows its highest accumulation. It has been shown by Sive *et al.* (21) that RA retards development of *Xenopus* embryos during gastrulation. Conceivably, development of the RA-treated embryos may have lagged behind the control embryos sufficiently that they had not yet experienced the major increase in XAP-2 mRNA accumulation. This would result in RA-treated embryos showing an apparent decrease in XAP-2 mRNA levels compared to controls. The same result was not observed for embryos sampled at later stages because by those stages, XAP-2 mRNA accumulation has decreased and does not show as pronounced a change in so short a time period.

In case a transient period of XAP-2 inducibility was being missed earlier in development, we also tried adding RA to embryos soon after fertilization (four-cell stage) and allowing exposure to continue for 24 h (lanes 13 and 14), but XAP-2 expression was still not affected. Several other treatment programs were tried, such as 40 h of exposure to RA beginning at stage 9, 2 h of exposure beginning at stage 9 followed by removal of RA and recovery of embryos for various periods up to 40 h, and 2 h of exposure beginning at four-cell stage followed by removal of RA and recovery of embryos for 24 h. None of these approaches had any reproducible effect on XAP-2 gene expression (data not shown). While our list of treatment regimens was not exhaustive, enough different conditions were tried that we feel confident concluding that general XAP-2 expression is not readily induced by RA in embryos. The lack of induction of XAP-2 could not be attributed to inaction of the RA because all but the shortest exposures to the drug resulted in major morphological abnormalities, with virtually all embryos having a dorsoanterior index (21) of 1 to 2 (data not shown). We cannot rule out that within embryos a subset of cells responded to RA treatment with increased XAP-2 expression; *in situ* hybridization with the XAP-2 probe will be required to investigate this possibility.

Fig. 5. Northern blot analysis of total RNA from embryos exposed to retinoic acid (RA). Embryos were treated with RA beginning at stage 9; times listed are from that point (except for lanes 13 and 14). Embryos not exposed to RA (control embryos) were grown in parallel with treated embryos and sampled at the same time points. Odd-numbered lanes show RNA from control embryos; even-numbered lanes show RNA from RA-treated embryos. Lanes 1 and 2: 30 min. Lanes 3 and 4: 60 min. Lanes 5 and 6: 2 h. Lanes 7 and 8: 6 h. Lanes 9 and 10: 16 h. Lanes 11 and 12: 24 h. Lanes 13 and 14: Embryos treated beginning at 4-cell stage for 24 h.

DISCUSSION

We have isolated a cDNA clone encoding the *Xenopus* homologue of the transcription factor AP-2. The predicted amino acid sequence from a long open reading frame in the cDNA was found to be very similar to that of its human counterpart, suggesting conservation of AP-2 function between distantly related species. This is supported by the observation that an *in vitro* translation product encoded by the *Xenopus* cDNA bound specifically to an oligonucleotide containing a human AP-2 binding site. In contrast to the sequence similarity, however, are differences in other characteristics of the AP-2 mRNAs of human and *Xenopus*. The human mRNA was reported to be 3.3 to 3.6 kb in size (16), whereas the largest major transcript observed in *Xenopus* embryos was 2.6 kb. Since the coding regions of both mRNAs are similar in size (about 1.3 kb), the incongruous transcript sizes are likely due to a difference in the size of the untranslated regions. Why this might be is unknown, but it may reflect differences in post-transcriptional regulation of AP-2 gene expression between human and *Xenopus*.

Also interesting was the detection of three distinct XAP-2 mRNA species whereas only one AP-2 band was observed in HeLa cells (16). We cannot conclude as yet whether the three transcripts are the products of separate XAP-2 genes or the result of alternate processing of the same initial transcript. (Results from the Southern blotting experiment in Fig. 4 are consistent with the existence of two or possibly more XAP-2 genes.) Numerous genes have been found to exist in multiple copies in *Xenopus* due to the tetraploid nature of the species (36). Currently, other positive clones isolated from the cDNA library are being characterized to determine if any correspond to the different mRNA species.

Regardless of the nature of the origin of the three transcripts, their existence is intriguing. Their size differences may be due to additional coding sequence present in the larger transcripts, which would suggest differences in function between the translation products, or to transcripts having different amounts of untranslated RNA, which, as discussed above, would raise the possibility of differential post-transcriptional regulation of XAP-2 gene expression. The latter possibility is consistent with the observation that the three XAP-2 mRNA species differ slightly in their patterns of developmental expression. No matter which explanation is correct (they are not mutually exclusive), the presence of multiple XAP-2 mRNAs that are regulated differently suggest that this factor plays more than a simple role during *Xenopus* embryogenesis.

What that role might be is still far from certain, but inferences can be drawn from our data. Unlike other *Xenopus* transcription factors, XAP-2 does not appear to be synthesized maternally; the earliest point in development that any of the XAP-2 transcripts was detected was stage 9, after the midblastula transition. Because XAP-2 mRNA is not detected in the embryo until after the MBT, this factor is unlikely to be directly involved in the general transcriptional activation that occurs at the MBT. Peak accumulation of two of the XAP-2 mRNA species (the 1.8 kb and 2.2 kb transcripts) occurred during gastrulation, implying that an important aspect of the factor's function occurs at this stage. XAP-2 might therefore regulate genes activated not at the MBT but sometime thereafter. Thus XAP-2 may constitute part of a second wave of transcriptional regulators that have more specialized roles in the embryo.

As stated in the Introduction, AP-2 mRNA accumulation has

been shown to increase in retinoic acid-induced human teratocarcinoma cells (16, 17). Furthermore, a recent publication by Mitchell et al. (37) proposed that AP-2 has a role in transcriptional regulation during retinoid-affected morphogenetic processes, because AP-2 mRNA was localized to mouse embryonic tissues that are developmentally sensitive to retinoic acid. However, treatment of *Xenopus* embryos with retinoic acid did not result in increased XAP-2 mRNA accumulation, even though the treated embryos showed substantial morphological abnormalities. While XAP-2 may be involved in regulating some morphogenetic processes affected by RA in *Xenopus* embryos, our results clearly indicate that RA-induced developmental aberrations in frog embryos do not depend on activation of the XAP-2 gene.

The availability of the XAP-2 clone will enable us to further explore the developmental regulation of this factor, and how this regulation contributes to the proper effectuation of the complex program of gene expression that directs embryonic development.

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