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Expression of the Poly(A)-Binding Protein during Development of *Xenopus laevis*

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We have isolated and sequenced cDNA clones encoding the poly(A)-binding protein of *Xenopus laevis* oocytes. Polyclonal antiserum was raised against a fusion protein encoding 185 amino acids of the *Xenopus* poly(A)-binding protein. This antiserum localizes the poly(A)-binding protein to subcellular sites associated with protein synthesis; in the retina, immunoreactive protein is detected in the synthetically active inner segment of the photoreceptor but not in the transductive outer segment. Transcripts encoding the poly(A)-binding protein are present in oocytes, although no protein is detected on protein blots. In contrast, the levels of both transcripts and protein increase in development, which correlates with the observed increase in total poly(A) during *Xenopus* embryogenesis (N. Sagata, K. Shiokawa, and K. Yamana, Dev. Biol. 77:431–448, 1980).

Most eucaryotic mRNAs are polyadenylated posttranscriptionally at their 3′ ends. The presence of a poly(A) tail has been shown to have a positive effect on the stability of globin mRNA injected into HeLa cells (18), on synthetic mRNAs injected into *Xenopus* oocytes (6), and on newly synthesized mRNA of HeLa cells in which polyadenylation has been blocked with cordycepin (34). In contrast, studies have found no correlation between the relative length of the poly(A) tail and message stability in *Dictyostelium* species (30). In separate experiments, the presence of a poly(A) tail appears to increase the translational efficiency of adenylated mRNA relative to deadenylated mRNA in reticulocyte lysates (5, 20) or when the mRNAs are injected into *Xenopus* oocytes (6, 9). These studies have left the physiological role of the poly(A) tail unresolved. An alternative avenue of investigation is the study of the protein(s) bound to the poly(A) tail. In vivo, mRNAs are associated with specific proteins to form RNA-protein complexes known as messenger ribonucleoprotein particles (13, 21). It is likely that these RNA-binding proteins modulate aspects of mRNA stability or translation and that an investigation of the protein(s) bound to the poly(A) tail will contribute to our understanding of the functional significance of the poly(A) tail itself. The 78-kilodalton (kDa) poly(A)-binding protein (A^+BP) was first described by Blobel (3) and is one of the most highly conserved and well characterized of the eucaryotic messenger ribonucleoprotein particle proteins. That the poly(A)-binding protein does indeed bind to the poly(A) tail of adenylated mRNAs in vivo has been demonstrated by using the techniques of UV cross-linking (1, 29) and nuclease protection (2). Its functional role in mRNA metabolism is unclear, although recent studies have indicated that it is a positive regulator of translation, as demonstrated by its ability to rescue translation in rabbit reticulocyte lysates which had been "poisoned" by poly(A) (14).

In the present study, a rabbit antiserum generated against the yeast poly(A)-binding protein was used to screen a λgt11 chicken erythroid library (23) and the resulting cDNA clone was ^{32}P labeled and used to screen a *Xenopus laevis* oocyte λgt10 library (25), yielding several overlapping cDNAs. Sequence analysis of the *Xenopus* oocyte cDNAs reveals an open reading frame of 1,899 nucleotides, encoding a 633-amino-acid polypeptide with a predicted molecular mass of 68.5 kDa (Fig. 1). The predicted amino acid sequence is 96% identical to that of the human poly(A)-binding protein (12), and while it is only 42% identical to the deduced yeast sequence (1, 26), the RNA-binding region (32) is highly conserved.

A portion of the *Xenopus* poly(A)-binding protein clone (underlined in Fig. 1) was ligated in frame into the vector pRIT2T (Pharmacia, Piscataway, N.J.), which was used to produce a fusion protein consisting of the immunoglobulin G binding domain of protein A and 185 amino acids from the poly(A)-binding protein. This fusion protein was used to generate the rabbit antiserum designated A^+BP-Pst/Ab. To demonstrate that the antiserum was monospecific for the poly(A)-binding protein in *Xenopus* embryos, Freon-extracted (15) protein from 40 tadpoles was separated by two-dimensional nonequilibrium gel electrophoresis (24), transferred to nitrocellulose, and probed with the A^+BP-Pst/Ab antiserum (Fig. 2B) or preimmune serum (Fig. 2C). The A^+BP-Pst/Ab antiserum specifically recognizes a single polypeptide, and no immunoreactive polypeptides were detected by using the preimmune serum. The immunoreactive protein is a relatively minor cellular constituent, as evidenced by the low intensity of the comigrating spot on the silver-stained gel (Fig. 2A). Significantly, the immunoreactive protein of Fig. 2B comigrates with the [^{35}S]methionine-labeled poly(A)-binding protein produced by in vitro translation of pSP64T-ABP mRNA in a rabbit reticulocyte lysate (Promega Biotec, Madison, Wis.) (Fig. 2D). These data demonstrate that A^+BP-Pst/Ab monospecifically recognizes the poly(A)-binding protein in crude preparations of tadpole protein.

The availability of cDNA clones and a specific antiserum allowed us to investigate the levels of poly(A)-binding protein mRNA and protein during *Xenopus* development. Total RNA isolated from oocytes and developing embryos was resolved by formaldehyde-agarose gel electrophoresis, transferred onto nitrocellulose, and hybridized (11) with a

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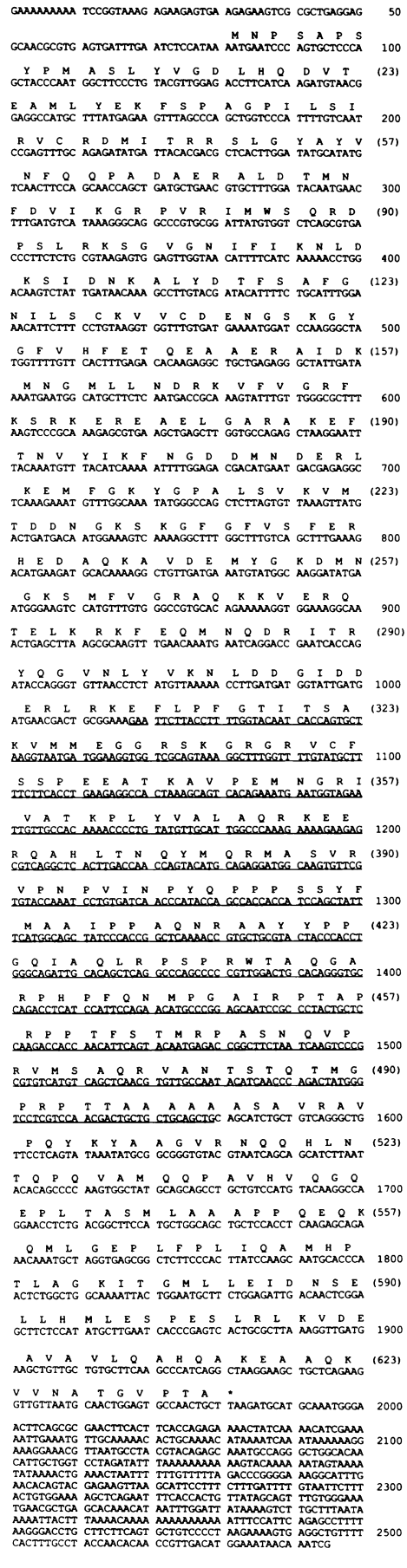

FIG. 1. The 2.55-kilobase *Xenopus* poly(A)-binding protein cDNA. Xen ABP-EF. Three overlapping cDNA clones were isolated from an oocyte λgt10 library (25) and sequenced by using the dideoxy-chain termination method (28). The nucleotides are numbered on the right, and the amino acid position is given in parentheses. The sequence and corresponding amino acids used in the construction of the poly(A)-binding protein–protein A fusion construct are underlined. The entire coding region (nucleotides 1 through 2053) was ligated into the *Bgl*II site of pSP64T (22), allowing for the efficient in vitro synthesis of poly(A)-binding protein mRNA.

^{32}P-labeled cDNA probe specific for the poly(A)-binding protein mRNA, revealing a single hybridizing band of approximately 3.7 kilobases (Fig. 3A). Stage VI oocytes contain detectable levels of poly(A)-binding protein transcripts (Fig. 3A, lane 1), which decrease shortly after fertilization (lane 2). Transcript levels rise during the blastula (lane 4), gastrula (lane 5), neurula (lane 6), tailbud (lane 7), and tadpole (lane 8) stages. Northern (RNA) blot analysis of poly(A)$^+$ RNA demonstrates that the same pattern of post-fertilization transcript decrease and postblastula increase is observed (data not shown). To demonstrate that equivalent amounts of RNA were loaded into each lane, the Northern blots were rehybridized with a ^{32}P-labeled DNA probe specific for *Xenopus* 27S ribosomal RNA (data not shown).

We next assayed the levels of poly(A)-binding protein present during development to determine whether the pattern of protein expression paralleled that observed for the mRNA. Protein isolated from oocytes, fertilized eggs, and gastrula, neurula, and tadpole stage embryos was used in a Western blot (immunoblot) analysis by using the A$^+$BP-Pst/Ab antiserum (Fig. 3B). Immunodetectable poly(A)-binding protein (approximate molecular mass, 70 kDa) is only seen in neurula (lane 5) and tadpole (lane 6) stage embryos. The presence of immunodetectable α-fodrin (Fig. 3C) and total protein (Fig. 3D) indicates that comparable amounts of protein were loaded onto the gel for each developmental stage. While Western blot analyses did not detect the poly(A)-binding protein in oocytes or early embryos (Fig. 3B), both whole-mount immunocytochemistry of oocytes (R. Stambuk and R. T. Moon, unpublished data) and direct analysis of oocyte messenger ribonucleoprotein particles (33) reveal that this protein is present. Taken together, the data from the Northern and Western analyses indicate that the expression of the poly(A)-binding protein is not constitutive but is instead modulated in oocytes and the developing embryo. Significantly, the time course for the accumulation of poly(A)-binding protein transcripts and polypeptides correlates with the observed increase in the steady-state amount of poly(A) (27). In contrast, the overall rate of mRNA synthesis is declining at this time (31), indicating that there is no positive correlation between mRNA synthesis and the levels of the poly(A)-binding protein. This suggests that embryos may actively regulate the expression of the poly(A)-binding protein in response to changes in the level of its binding site [i.e., poly(A)].

The antiserum was next used in an attempt to determine the localization of the poly(A)-binding protein within a given cell or cell population. To this end, tadpoles were processed for immunocytochemistry as described previously (17, 19) and incubated with either affinity-purified (10) A$^+$BP-Pst/Ab or preimmune serum as the primary antiserum. Specific staining was visualized by the use of a biotinylated goat anti-rabbit secondary antiserum, followed by incubation with a streptavidin-horseradish peroxidase conjugate. Specific staining was observed in the cell layers of the retina, in the epithelial and fiber cells of the lens, and in the epithelial

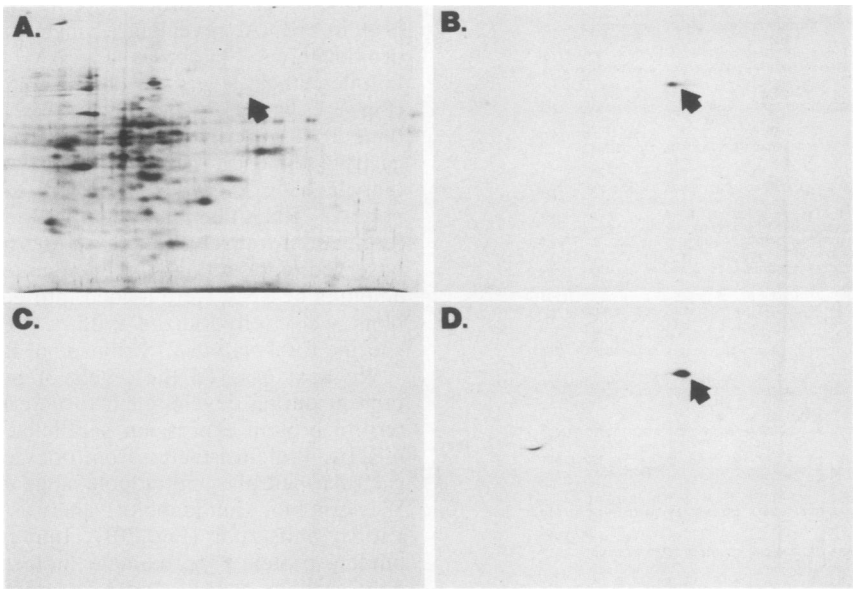

FIG. 2. Comigration in two dimensions of the in vitro translation product of pSP64T-ABP mRNA and a tadpole polypeptide recognized by the A^+BP-Pst/Ab antiserum. Freon-extracted proteins were separated by nonequilibrium pH gradient gel electrophoresis (24) in the first dimension and sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis in the second dimension. All gels were run in parallel, and all autoradiograms were exposed for 16 h at −80°C with an intensifying screen. The arrows in panels A, B, and D point to comigrating proteins. (A) Silver-stained (4) two-dimensional gel of protein isolated from three tadpoles. (B) Western blot of the protein from 40 tadpoles, probed with the A^+BP-Pst/Ab antiserum (1:1,000 dilution). (C) Western blot identical to that shown in panel B, except that it was probed with preimmune serum. (D) Autoradiogram of the [^{35}S]methionine-labeled in vitro translation product of the synthetic poly(A)-binding protein mRNA, separated on an identical two-dimensional gel.

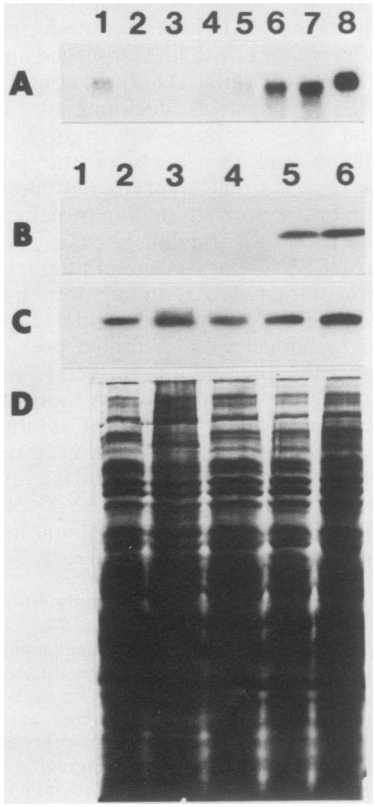

FIG. 3. Expression of the poly(A)-binding protein during *Xenopus* development. (A) Total RNA (15 μg) was resolved by using a formaldehyde-agarose gel, transferred to nitrocellulose, and hybridized with a ^{32}P-labeled (8) probe consisting of nucleotides 1 through 498 of the *Xenopus* cDNA clone, Xen ABP-EF. Only the relevant portion of the autoradiogram is shown; these are the only hybridizing bands observed and have an estimated size of 3.7 kilobases (exposed for 16 h). The total RNA was isolated from stage VI oocytes (lane 1), fertilized eggs (lane 2), cleavage stage embryos (lane 3), blastula stage embryos (lane 4), gastrula stage embryos (lane 5), neurula stage embryos (lane 6), tailbud stage embryos (lane 7), and tadpole stage embryos (lane 8). (B) Freon-extracted protein (equivalent to three oocytes or embryos) was resolved by sodium dodecyl sulfate–10% polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with preimmune serum (lane 1) or the A^+BP-Pst/Ab antiserum (lanes 2 to 6). Protein was isolated from stage VI oocytes (lanes 1 and 2), fertilized eggs (lane 3), gastrula stage embryos (lane 4), neurula stage embryos (lane 5), and tadpole stage embryos (lane 6). The estimated molecular mass is 70 kDa (exposed 16 hours). (C) An identical control blot probed with antiserum specific for *Xenopus* α-fodrin (molecular mass, 240 kDa) (11). Only the relevant portion of the autoradiogram is shown, and lane 1 is again the preimmune control. (D) Coomassie-stained gel identical to lanes 2 through 6 from panel A, demonstrating that equivalent amounts of protein were loaded in each lane.

and endothelial cells of the cornea (Fig. 4A). Within the retina, the inner and outer plexiform layers stained weakly and the outer segments of the photoreceptors did not stain. From the pattern of staining observed in the photoreceptor cells, we conclude that the poly(A)-binding protein is present in subcellular regions associated with protein synthesis (i.e., the inner segment) and not found in locations that are not active in protein synthesis (i.e., the outer segment) (7). It is interesting that the fiber cells of the lens interior stain less

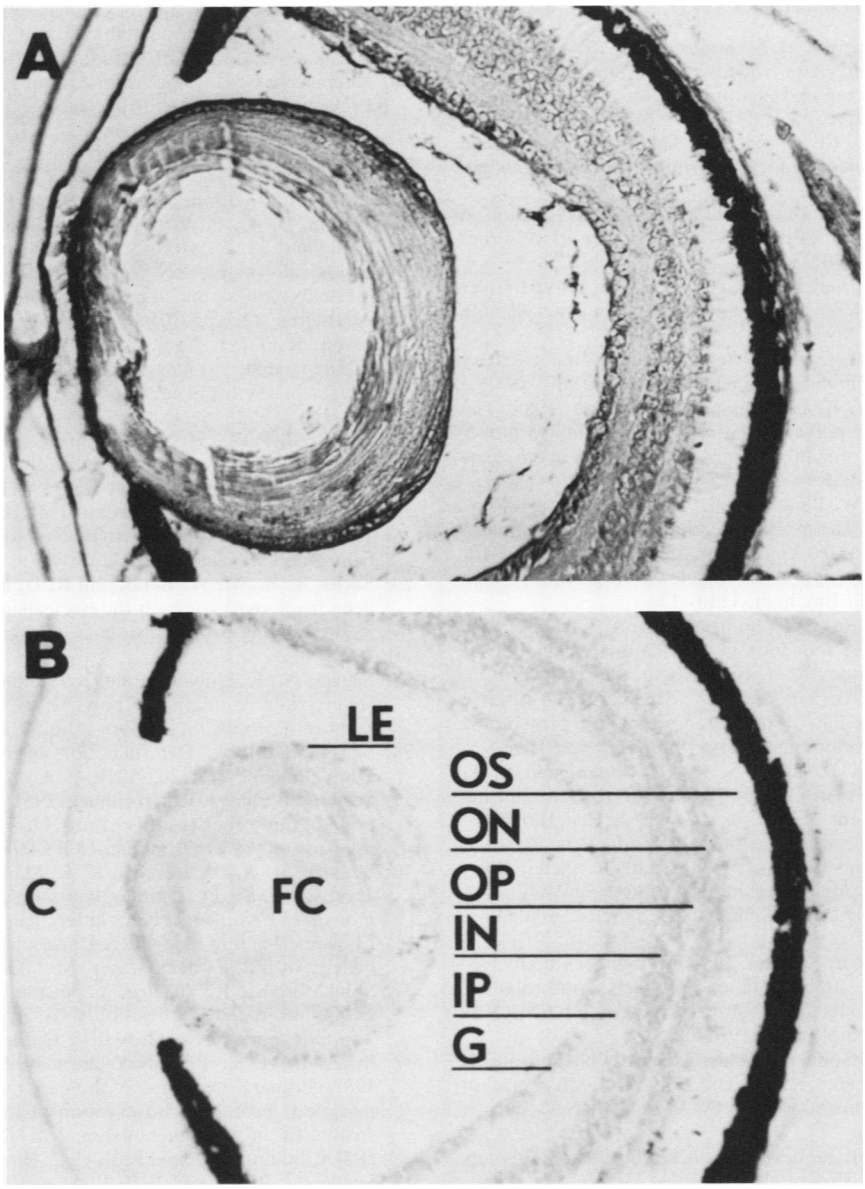

FIG. 4. Immunocytochemical localization of the poly(A)-binding protein in the eye of a *Xenopus laevis* tadpole. The primary antiserum was either affinity-purified A^+BP-Pst/Ab (A) or preimmune serum (B). FC, Lens fiber cells; LE, lens epithelia; OS, photoreceptor outer segments; ON, outer nuclear layer (photoreceptor inner segments); OP, outer plexiform layer; IN, inner nuclear layer; IP, inner plexiform layer; G, ganglion layer. Magnification, ×250. Please note that in panel A, the central portion of the lens was lost during processing.

intensely than the lens epithelia, indicating that these cells express different levels of the poly(A)-binding protein. As these fiber cells have lower levels of protein synthesis than the lens epithelia (16), the level of poly(A)-binding protein expression correlates with the level of protein synthesis in a cell. Examination of the pattern of staining in the remainder of the tadpole embryos reveals roughly equivalent staining throughout non-yolk-filled tissue layers (data not shown).

These data demonstrate that levels of the poly(A)-binding protein and its transcripts increase during development of *X. laevis* and that this increase correlates with developmental increases in levels of its binding site, poly(A) (27). Taken together with our immunocytochemical data showing the poly(A)-binding protein to be localized to cell types and subcellular domains most active in protein synthesis, these studies provide initial evidence that eucaryotes modulate the steady-state level and spatial distribution of this conserved RNA-binding protein relative to the abundance of poly(A) and to levels of protein synthesis.

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