Supporting Information

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SI Methods

Plasmid Construction. ***Expression plasmids.*** Upstream ATGs in pGemT-easy (Promega) were mutated to increase translation efficiency by insertion of annealed oligonucleotides into the AatII–SacII sites, creating ATG-pGem. The sequences of oligonucleotides used were CGCTTGCTCCCGGCCGCCTTGGCGGCCGC and GGCCGCCAAGGCGGCCGGGAGCAAGCGACGT.

The poly(A)-binding protein (PABP) 1 rescue construct, PABP1Res, was generated by PCR using primers CAGTCACAATTGATGAATCCCAGTGCTCCC and CAGTCACTCGAGTTAAGCAGTTGGCACTCC and ligated into ATG-pGem. ePABPwobble was constructed to introduce silent mutations in the embryonic PABP (ePABP) coding sequence to make it resistant to morpholino antisense oligonucleotides. ePABP was amplified with two sequential PCR reactions: first with primers GGGGAATTCGATTATGAATGCAACCGGTGCTGGATATCCGC (sense) and GGGGAATTCTCAGATCAAAGATGGTTGGGCAC (antisense) and then with GGGGAATTCGATTATGAATGCTACTGGTGCTGGATATCCGC (sense) and same antisense primer. The EcoRI-digested product was ligated into ATG-pGem.

FLAG-tagged PABP1 (PABP1-FLAG) and PABP4 (PABP4-FLAG) were generated by PCR using primers CAGTCAGAGCTCATATGAATCCCAGTGCTCCCAGCTACCCAATGGCTTCC and CAGTCAAAGCTTTTATTTGTCATCGTCGTCCTTGTAGTCCATAGCAGTT GGCACTCCAGTTGC or CAGTCAGAGCTCATATGCACACAGCTACTAGCAGTTATCC and CAGTCAAAGCTTTCATTTGTCATCGTCGTCCTTGTAGTCCATAGAGGTAGCTGTGACAATTCCCAC, respectively. SacI–HindIII–digested products were inserted into pET28c (Novagen). A construct in which the C terminus of PABP1 was appended to the RNA recognition motifs (RRM) region of ePABP (eRRM/1Ct) was created by replacing an NheI–SalI fragment of ePABPwobble with the corresponding C-terminal region of PABP1, which was generated by PCR from PABP1Res with primers CCCGCTAGCCACTGTTCGTGTACCAAATCC and CATATGGTCGACCTGCAGGCGGCCGC and cut with NheI and SalI before ligation. To create a construct in which the ePABP C terminus was appended to the RRMs of PABP1 (1RRM/eCt), silent mutations were introduced into PABP1Res by site-directed mutagenesis to create an NheI site. The resulting plasmid (pGEM-PABP1mNheI) was digested with NheI–SalI to liberate the C terminus, which was replaced with the corresponding region of ePABP by ligation of a PCR product generated from ePABP-wobble using primers CCCGCTAGCATGAGGGCGATGCCTGGTCCTCTC and CATATGGTCGACCTGCAGGCGGCCGC and digested with NheI and SalI.

Glutathione S-transferase (GST)-PABP1 and GST-ePABP were generated by PCR using primers CCCCAATTGATGAATCCCAGTGCTCCCAGCTAC and CCCGCGGCCGCTTAAGCAGTTGGCACTCCAGTTGC or CCCCAATTGATGAATGCAACCGGAGCCGGATATCC and CCCGCGGCCGCTCAGAT CAAAGATGGTTGGGCAC, respectively. MfeI–NotI–digested products were inserted into pGEX-5×-1 (Pharmacia) digested with EcoRI–NotI. GST-PABP4 was generated by PCR using primers CCCGGATCCCCATGCACACAGC TACTAGCAGTTATCC and CCCGCGGCCGC TCAAGAGGTAGCTGTGACAATTCCC, and the product and pGEX-5×-1 were digested with BamHI–NotI before ligation.

Tethered-function assay plasmids. pMS2-PABP1 (previously known as "MS2-PAB"), pLG-MS2 (Luc-MS2), pLGENB1 (Luc-ΔMS2), pJK350 (β-galactosidase) (1), pMSPN, and pMS2-ePABP (2) have been described previously. pMS2-PABP4 was generated by PCR with primers CAGTCACTCGAGATGCACACAGCTACTAGC and TCTGAGACTAGTTCAAGAGGTAGCTGTGAC. The XhoI–SpeI–digested product was ligated into pMSPN.

Yeast two-hybrid plasmids. pLexA-MS2, pACT-IRP, pACT-4GNt, pBTM-PABP1 1–2, pBTM-PABP1Ct, pACT-PAIP1 (1), pBTM-ePABP1-2, pBTM-ePABPCt (2), and pACT-xDazl (3) have been described previously. pBTM-PABP4Nt was created by PCR using primers CTAGCAGCTAGCAATGCACACAGCTACTAGCAGTTATCC and CTAGCAGGATCCCTAACGACACCCATATTTGCTAAACGTCTCCTTTAATCGCTC. The NheI–BamHI product was ligated into pBTMKnDB (gift from David Bernstein, University of Wisconsin-Madison, Madison, WI) digested with AvrII–BamHI. pBTM-PABP4Ct and pGAD-PABP4Ct were created by PCR using primers CAGTCAGAATTCCTTATCAACCAGTTCCAGCCTGCACC and CAGTCAGGATCCTCAAGAGGTAGCTGTGACAATTCC. The PCR product and pBTMknDB and pGADT7 (Clontech) were digested with EcoRI–BamHI before ligation. pGAD-PABP1Ct and pGAD-ePABPCt were created by PCR using primers GTCAGTGAATTCATCAACCCATACCAGCCACCAC and GTCAGTGGATCCTTAAGCAGTTGGCACTCCAGTTG or GTCAGTGAATTCCTCCTTGGGTCCTTCCAG and GTCAGTGGATCCTCAGATCAAAGATGGTTG, respectively. EcoRI–BamHI products were ligated into pGADT7. pACT-PAIP2 was created by PCR using primers CAGTCACCATGGCTATGAAAGATCCAAGTCGCAGC and CAGTCAGGATCCTCAAATATTTCCGTACTTCACCCC. NcoI–BamHI products were ligated into pACT2. pGBT9-eRF3 (4) was a kind gift of Bertrand Cosson (University of Rennes, Rennes, France).

In Vitro Translation. PABP1, ePABP, and PABP4 proteins were expressed from EST clones (IMAGE IDs 5542430, 4032449, and 5516025, respectively) using the TNT/SP6 system (Promega) supplemented with 10 μCi [^{35}S]methionine and 4 μM morpholino.

Bioinformatics. Analyses were performed using the following sequences:

Xenopus laevis: Poly(A)-binding protein, cytoplasmic (Pabpc) 1: NP_001080204.1; ePabp (Pabpc1l): NP_001082094.1; Pabpc4 (MGC80927): NP_001085857.1.
Xenopus tropicalis: Pabpc1: NP_001005051.1; ePabp (Pabpc1l): NP_001005062.1; Pabpc4: own prediction made from genomic sequence from *Xenopus* genome assembly version 4.1 (August 2005) and NM_001015753.1 (mRNA).
Gallus gallus: Pabpc1: NP_001026768.1; ePabp (Pabpc1l): XP_417367.2; Pabpc4: XP_417821.2.
Mus musculus sequences: Pabpc1: NP_032800.2; ePabp (Pabpc1l): NP_001107551.1; Pabpc2: NP_035163.1; Pabpc4: NP_570951.2; Pabpc5: NP_444344.1.
Homo sapiens sequences: Pabpc1: NP_002559.2; ePabp (Pabpc1l): NP_001118228.1; Pabpc3: NP_001118228.1; Pabpc4: NP_001129125.1; Pabpc5: NP_543022.1.
Drosophila sequence: NP_476667.1.

The protein multiple alignments were done using ClustalW (version 1.83) (5) and visualized using Genedoc (6). The phylogenetic tree was constructed by the neighbor-joining method. The reliability of each interior branch of a given topology was assessed using the bootstrap interior branch test with 1,000 replicates. Phylogenetic trees were constructed using MEGA (version 4.0.2; http://www.megasoftware.net/) (7).

RT-PCR Analysis. RT-PCR on *X. laevis* total RNA was performed as described previously (2) using the following primer sets:

ePABP: ATGCAGAGGCTAGCCACTATGAGGGCGATG; GCATTTTCCTTGGCTTGGTGGGCTTGCAGG
PABP1: GTCTCAGCGTGACCCTTCTC; AAGCTCAGTTTGCCTTTCCA
PABP4: GCAAATATGGGAAGACTCTG; GAGGAAAAGCAGACAAATCC
β-actin: GATAATGGATCTGGTATGTG; TGGTGACAATGCCATGTTC

Tethered-Function Analysis. Tethered-function assays were performed in stage VI oocytes as described (1) with three pools of five oocytes per experimental point. For the mRNA stability assay, total RNA from injected oocytes was extracted and analyzed by quantitative RT-PCR in a 7900HT Fast Real-Time PCR System (Applied Biosystems) as described (2).

Protein Purification and RNA-Binding Assays. GST and GST-fusion proteins were expressed in *Escherichia coli* BL21 (DE3) pLysS (Novagen) by induction with 1 mM isopropyl-β-D-thio-galactoside (IPTG) for 3 h at 30 °C. The 500-mL cultures were lysed in 10 mL BugBuster (Novagen) diluted 1× in PBS and supplemented with 0.5 mM DTT, 5 mg/mL lysozyme, 50 U/mL Benzonase (Novagen), and protease inhibitor mixture (Roche). GST proteins were purified by affinity chromatography using glutathione 4B Sepharose beads (GE Healthcare) according to manufacturer's instructions. Proteins were dialyzed to remove glutathione using MF-Millipore membranes against binding buffer [50 mM Tris HCl (pH 7.5), 150 mM NaCl, 1.5 mM $MgCl_2$, 0.1% Nonidet P-40, 1 mM DTT, protease inhibitor mixture] supplemented with 20% glycerol. RNA-binding assays were performed by GST chromatography. Indicated concentrations of dialyzed GST fusion proteins were incubated with glutathione 4B Sepharose beads in 1 mL binding buffer for 1 h at room temperature. After washing with 1 mL binding buffer, 1 nM A_{25} or C_{25} RNA oligonucleotides labeled at the 3′ end with Cy5 (Eurogentech) were added and incubated for a further 1 h at room temperature. Beads were washed five times in 1 mL wash buffer [50 mM Tris HCl (pH 7.5), 250 mM NaCl, 1.5 mM $MgCl_2$, 0.1% Nonidet P-40, 1 mM DTT, and protease inhibitor mixture], resuspended in loading buffer [125 mM Tris HCl (pH 6.8), 2% SDS, 10% glycerol, and 100 mM DTT] and boiled for 10 min to elute bound RNA. RNA was resolved on a polyacrylamide gel and detected by fluorescence on a phosphoimager. An aliquot of eluate was analyzed by Western blotting using an Odyssey Infrared Imager (LI-COR Biosciences): Immobilon-FL membranes (Millipore) were probed with a goat anti-GST antibody (1:5,000; Abcam) and a secondary anti-goat Alexa Fluor 680 nm (1:10,000; Invitrogen) in Odyssey blocking buffer (LI-COR Biosciences) according to the manufacturer's instructions.

Yeast Two-Hybrid Analysis. Yeast two-hybrid analyses were performed with strains Mav99 (8) and L40ura$^-$ as described (9). Interactions were detected by activation of the lacZ reporter, revealed by β-galactosidase assay.

1. Gray NK, Coller JM, Dickson KS, Wickens M (2000) Multiple portions of poly(A)-binding protein stimulate translation in vivo. *EMBO J* 19:4723–4733.
2. Wilkie GS, Gautier P, Lawson D, Gray NK (2005) Embryonic poly(A)-binding protein stimulates translation in germ cells. *Mol Cell Biol* 25:2060–2071.
3. Collier B, Gorgoni B, Loveridge C, Cooke HJ, Gray NK (2005) The DAZL family proteins are PABP-binding proteins that regulate translation in germ cells. *EMBO J* 24: 2656–2666.
4. Cosson B, et al. (2002) Characterization of the poly(A) binding proteins expressed during oogenesis and early development of *Xenopus laevis*. *Biol Cell* 94:217–231.
5. Higgins DG, Thompson JD, Gibson TJ (1996) Using CLUSTAL for multiple sequence alignments. *Methods Enzymol* 266:383–402.
6. Nicholas KB, Nicholas HB, Jr. (1997) *GeneDoc: A Tool for Editing and Annotating Multiple Sequence Alignments* (Distributed by the authors).
7. Kumar S, Tamura K, Jakobsen IB, Nei M (2001) MEGA2: Molecular evolutionary genetics analysis software. *Bioinformatics* 17:1244–1245.
8. Vidal M, Brachmann RK, Fattaey A, Harlow E, Boeke JD (1996) Reverse two-hybrid and one-hybrid systems to detect dissociation of protein-protein and DNA-protein interactions. *Proc Natl Acad Sci USA* 93:10315–10320.
9. Zhang B, Kraemer B, SenGupta D, Fields S, Wickens M (1999) Yeast three-hybrid system to detect and analyze interactions between RNA and protein. *Methods Enzymol* 306: 93–113.

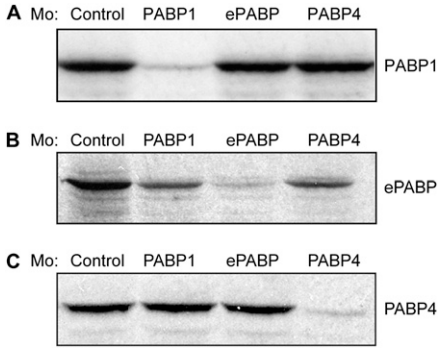


Fig. S1. Translation of PABPs is inhibited efficiently by specific morpholinos. (*A*) PABP1, (*B*) ePABP, or (*C*) PABP4 mRNA was translated in vitro in the presence of [^{35}S]methionine and morpholino antisense oligos (Mo): Control, PABP1-A (PABP1), ePABP-A (ePABP), or PABP4.

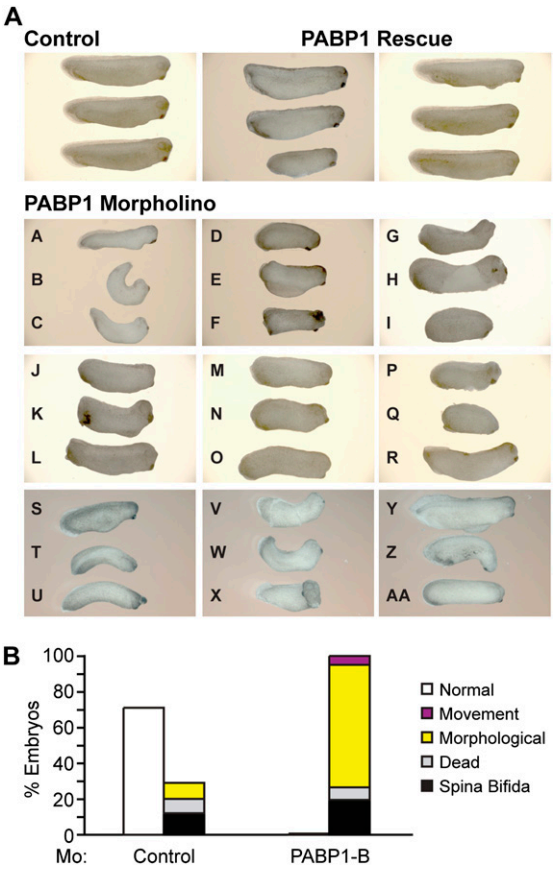


Fig. S2. PABP1 depletion causes multiple developmental defects. (*A*) Representative photographs of stage 29/30 embryos injected with control or PABP1-A (PABP1) morpholino ± 10 ng of PABP1 rescue mRNA. PABP1 morphants display the following defects: spinal curvature (embryos B, C, G, H, K, O, R, T, U, V, W, Z); abnormal development of tail and/or fin (embryos G, H, L, O, AA); absence of posterior elongation (embryos D, E, F, J, K, M, N, P, V); abnormal development of anterior structures (embryos J, V, Z, AA); ventral edema (embryos A, E, K, S, Y); and developmental arrest (embryos I, Q). Most PABP1-rescued embryos do not show morphological or movement defects. (*B*) An alternative PABP1 morpholino (PABP1-B) causes developmental defects and lethality similar to those caused by PABP1-A. Embryos were injected with control or PABP1-B morpholino; percentages of stage 26 embryos displaying the indicated phenotypes are shown. Data represent the average of three independent experiments with ~300 embryos per experimental point.

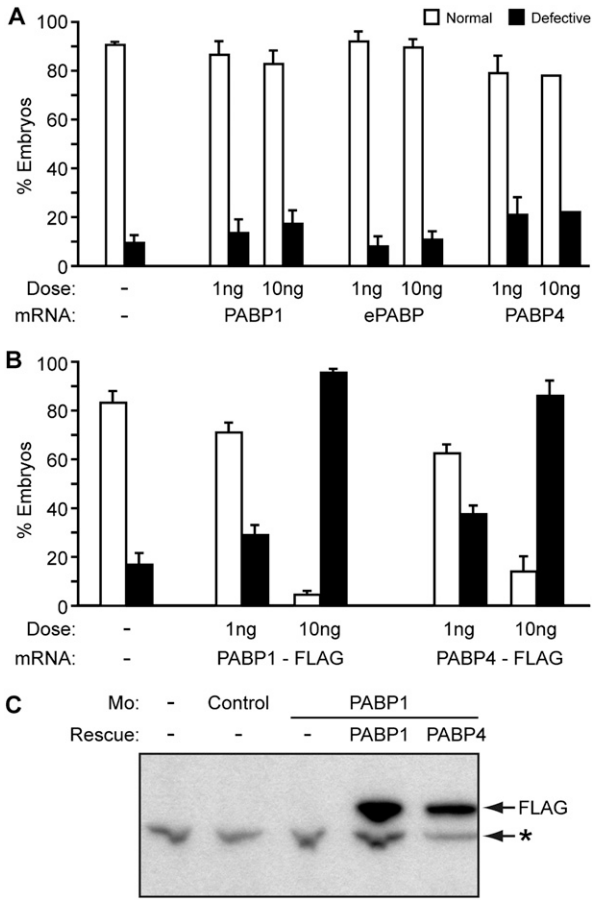


Fig. S3. PABP overexpression does not result in early developmental defects. Embryos were injected with (*A*) 1 ng or 10 ng of mRNA encoding PABP1, ePABP, or PABP4 or (*B*) 1 ng or 10 ng of mRNA encoding PABP1-FLAG or PABP4-FLAG. Embryos were allowed to develop until stage 47–50; the percentages of normal and defective embryos are shown. In *A* phenotypic defects were not observed at a significant level over control, permitting their use in rescue experiments. In *B* the 10-ng dose of PABP1-FLAG and PABP4-FLAG induced embryonic defects, possibly associated with the FLAG-tag. Therefore, the 1-ng dose was used in all FLAG rescue experiments. (*C*) PABP1-FLAG and PABP4-FLAG proteins are expressed at similar levels. Western blot analysis of whole-cell extracts from stage 16–18 embryos injected with control or PABP1-A (PABP1) morpholino ± 1 ng of PABP1-FLAG or PABP4-FLAG rescue mRNA, using an anti-FLAG antibody. Asterisk indicates a nonspecific band.

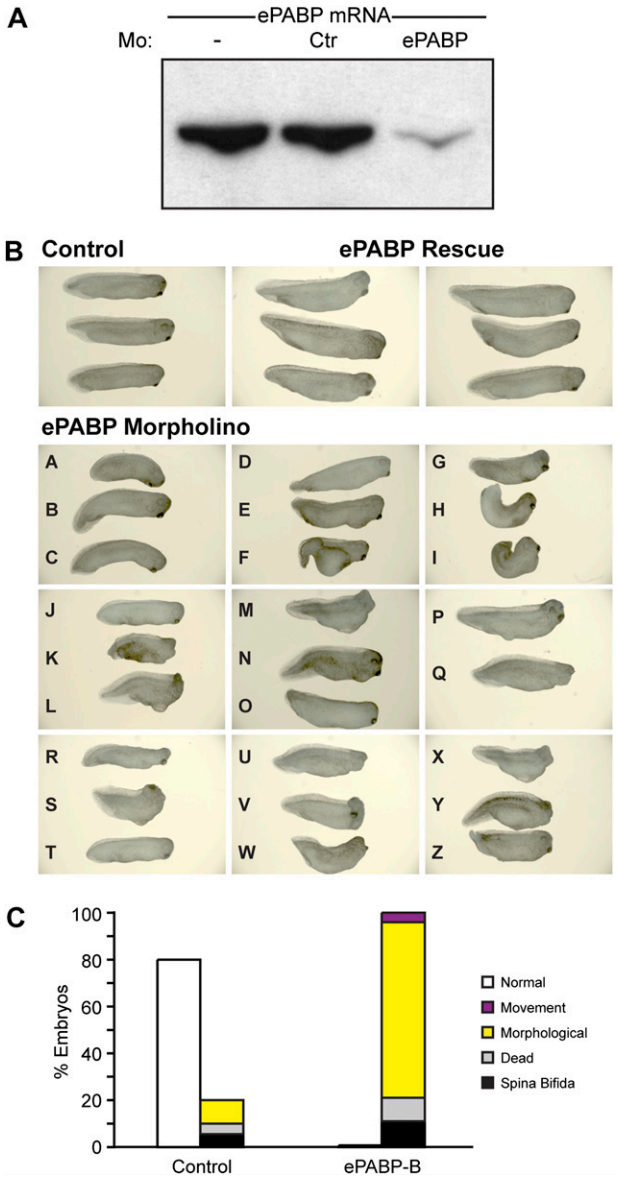
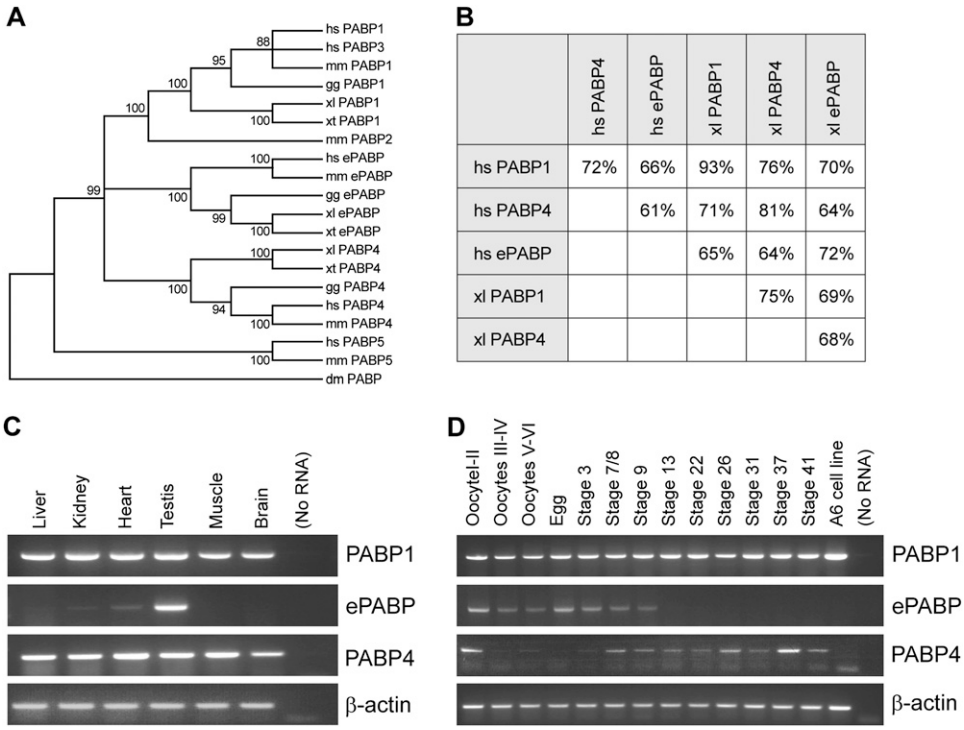


Fig. S4. ePABP depletion causes widespread developmental defects. (*A*) Western blot analysis of whole-cell extracts from stage VI oocytes coinjected with an ePABP-expressing mRNA ± control (Ctr) or ePABP-A (ePABP) morpholino, using an ePABP-specific antibody. This shows that ePABP expression is inhibited efficiently in vivo by the morpholino. (*B*) Representative photographs of stage 30–32 embryos injected with control or ePABP-A morpholino ± 10 ng of ePABP rescue mRNA. ePABP morphants display the following defects: spinal curvature (embryos A, B, C, H, V, W, Y); abnormal development of tail (embryos D, J); absence of posterior elongation (embryos A, F, G, K, S, Z); abnormal development of anterior structures (embryos K, L, M, Q, R, S, T, U, X, Y); and ventral edema (embryos E, F, I, K, L, M, N, S, Y). Embryo O appears morphologically normal. The majority of ePABP-rescued embryos do not show morphological defects. (*C*) An alternative ePABP morpholino (ePABP-B) causes the same developmental defects and lethality as ePABP-A. Embryos were injected with control or ePABP-B morpholino; percentages of stage 26–29 embryos displaying the indicated phenotypes are shown. Data represent the average of four independent experiments, with ~500 embryos per experimental point.



	hs PABP4	hs ePABP	xl PABP1	xl PABP4	xl ePABP
hs PABP1	72%	66%	93%	76%	70%
hs PABP4		61%	71%	81%	64%
hs ePABP			65%	64%	72%
xl PABP1				75%	69%
xl PABP4					68%

Fig. S5. PABP4 is conserved in vertebrates. (*A*) Neighbor-joining phylogenetic tree of vertebrate PABP family members rooted with *Drosophila melanogaster* (dm) PABP. Bootstrap (1,000 replicates) values are shown at the nodes. PABPs from *Homo sapiens* (hs), *Mus musculus* (mm), *Gallus gallus* (gg), *X. laevis* (xl), and *Xenopus tropicalis* (xt) are compared. (*B*) Table showing the percentage of amino acid identity between *X. laevis* and human PABP1, PABP4, and ePABP. (*C* and *D*) RT-PCR analysis of total RNA extracted from the indicated *X. laevis* adult tissues (*C*) or developmental stages (*D*) using primers specific for the indicated PABPs. β-actin serves as an internal control. In adults, ePABP mRNA is present only in the gonads, consistent with the protein expression (1, 2) whereas PABP1 and PABP4 mRNAs are widespread. During development, PABP4 mRNA is most abundant in early oocytes and after the midblastula transition (stage 7/8 embryos). Although ePABP protein levels begin to diminish after approximately stage 35 (2, 3), ePABP mRNA is absent after midblastula transition. Whilst PABP1 mRNA is present in all stages analyzed, PABP1 protein levels start to rise significantly only after stage 15, continuing to increase until approximately stage 41 (2, 3).

1. Wilkie GS, Gautier P, Lawson D, Gray NK (2005) Embryonic poly(A)-binding protein stimulates translation in germ cells. *Mol Cell Biol* 25:2060–2071.
2. Cosson B, et al. (2002) Characterization of the poly(A) binding proteins expressed during oogenesis and early development of *Xenopus laevis*. *Biol Cell* 94:217–231.
3. Voeltz GK, Ongkasuwan J, Standart N, Steitz JA (2001) A novel embryonic poly(A) binding protein, ePAB, regulates mRNA deadenylation in *Xenopus* egg extracts. *Genes Dev* 15: 774–788.

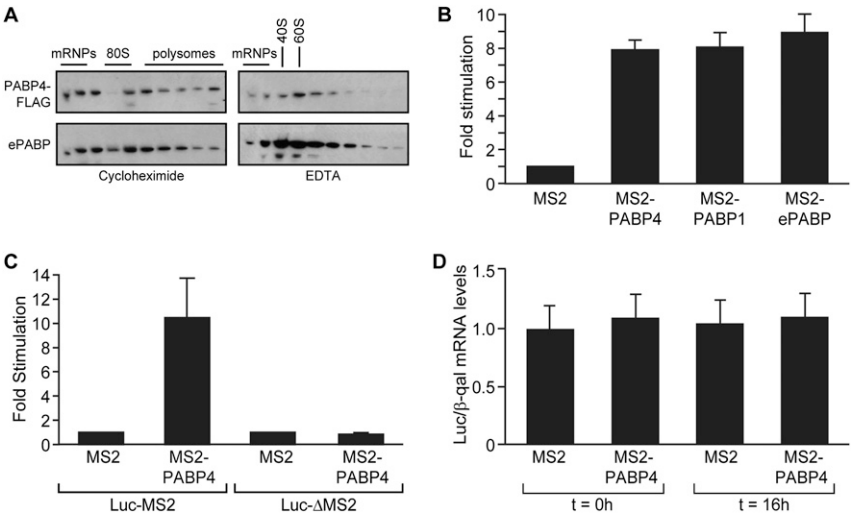


Fig. S6. *X. laevis* PABP4 stimulates translation. (*A*) A proportion of PABP4 associates with polysomes. Stage VI oocytes were injected with PABP4-FLAG–expressing mRNA, and the resulting whole-cell extracts were treated with cycloheximide or EDTA, fractionated on 10–50% sucrose gradients, and Western blotted with an anti-FLAG or anti-ePABP antibody. The positions of messenger ribonucleoprotein complexes (mRNPs), 80S (or 40S and 60S), and polysomes are indicated. Release of PABP4 and endogenous ePABP by EDTA is indicative of sedimentation into the heavier fractions resulting from association with actively translating polysomes. (*B*) Tethering of PABP4 activates reporter mRNA translation to a similar extent as PABP1 and ePABP. Stage VI oocytes expressing the indicated MS2-fusion protein were coinjected with an unadenylated luciferase reporter mRNA containing 3′ UTR MS2 binding sites (Luc-MS2) and a β-galactosidase control mRNA. Luciferase was normalized to β-galactosidase activity, and the fold stimulation relative to MS2 (set to 1) is shown. Changes in β-galactosidase activity normally are <15%. (*C*) PABP4 stimulates only the translation of reporter mRNAs to which it is tethered. Oocytes were injected as in *B*, with Luc-MS2 or with an identical reporter lacking the MS2 binding sites (Luc-ΔMS2) and β-galactosidase control mRNA. Data in *B* and *C* represent the average stimulations of at least three independent experiments; error bars indicate SEM. (*D*) Tethering of PABP4 does not affect mRNA stability. Oocytes were injected as in *B*, with Luc-MS2 and β-galactosidase mRNAs. Total RNA was extracted either immediately or after 16 h and analyzed by quantitative RT-PCR with primers specific for the reporter mRNAs. Normalized luciferase/β-galactosidase values are shown. Data represent the average of two independent experiments; error bars indicate SEM. Thus, the effects of PABP4 are the direct result of changes in mRNA translation and not changes in mRNA stability, as previously shown for PABP1 and ePABP (1, 2).

1. Gray NK, Coller JM, Dickson KS, Wickens M (2000) Multiple portions of poly(A)-binding protein stimulate translation in vivo. *EMBO J* 19:4723–4733.
2. Wilkie GS, Gautier P, Lawson D, Gray NK (2005) Embryonic poly(A)-binding protein stimulates translation in germ cells. *Mol Cell Biol* 25:2060–2071.

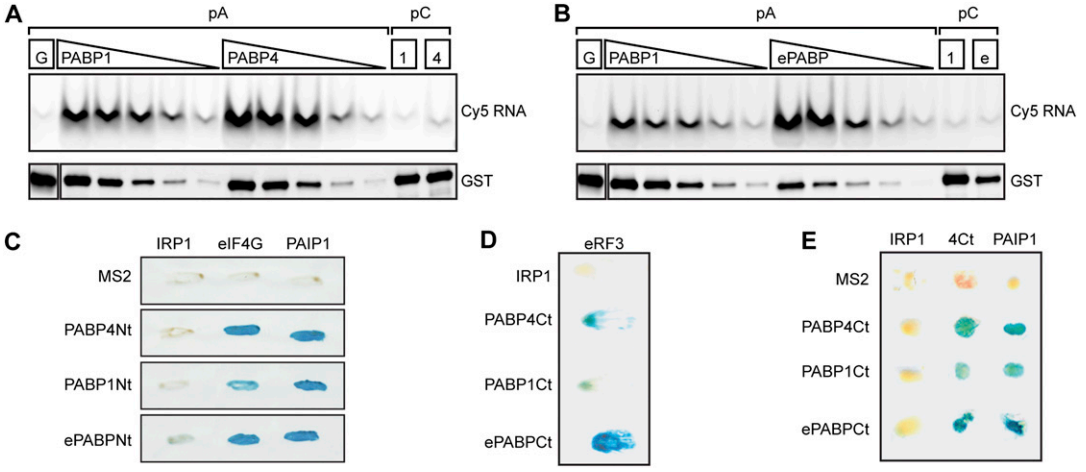


Fig. S7. PABP1, ePABP, and PABP4 activate translation through a similar mechanism. (*A* and *B*) PABP4, like PABP1 and ePABP, binds poly(A) but not poly(C) RNA efficiently. A range of concentrations (4, 2, 1, 0.5, and 0.25 nM) of GST-PABP1 and (*A*) GST-PABP4 or (*B*) GST-ePABP were immobilized on glutathione beads and incubated with 1 nM poly(A) RNA (pA). To control for specificity, 4 nM GST protein (G) was incubated with poly(A) RNA; and 4 nM PABP1 (1), PABP4 (4), and ePABP (e) were incubated with 1 nM poly(C) RNA (pC). After elution, RNA and proteins were detected by fluorescence (*Top*) and Western blotting with an anti-GST antibody (*Bottom*), respectively. (*C*) PABP4 interacts with initiation factors. Yeast two-hybrid analysis with the indicated PABP N termini (Nt) (LexA DNA-binding domain fusions) against eukaryotic initiation factor 4G (eIF4G) and poly(A)-binding protein interacting protein 1 (PAIP1) (Gal4 activation domain fusions) shows that PABP4 maintains these interactions. (*D*) The translation termination factor eukaryotic release factor 3 (eRF3) also is a PABP4 partner, as shown by yeast two-hybrid analysis with the indicated PABP C termini (Ct) (Gal4 activation domain fusions) against eRF3 (Gal4 DNA-binding domain fusion). (*E*) PABP4 can interact with itself and other PABP proteins. Yeast two-hybrid analysis with the indicated PABP C termini (LexA DNA-binding domain fusions) against the PABP4 C terminus (4Ct) or PAIP1 (Gal4 activation domain fusions) reveals PABP–PABP interactions. In *C*–*E*, iron regulatory protein-1 (IRP1) and MS2 are negative controls. Expression of β-galactosidase (blue) indicates protein–protein interaction.

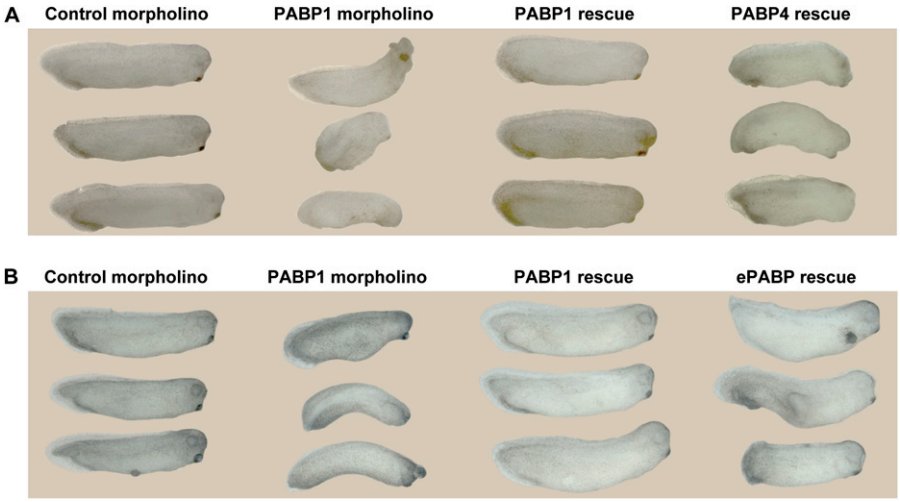


Fig. S8. PABP4 and ePABP cannot rescue PABP1-depleted embryos efficiently. (*A*) Representative photographs of stage 29/30 embryos injected with control or PABP1-A (PABP1) morpholino ± 1 ng of PABP1-FLAG or PABP4-FLAG rescue mRNA. (*B*) Representative photographs of stage 29/30 embryos injected with control or PABP1-A morpholino ± 10 ng of PABP1 or ePABP rescue mRNA.

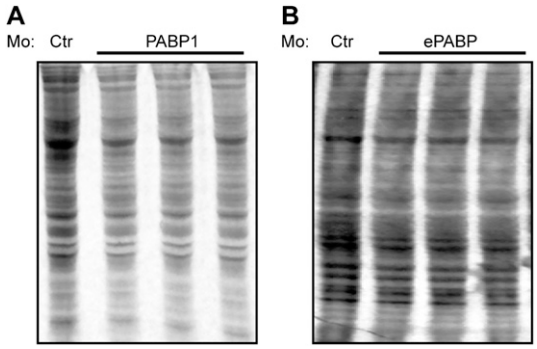


Fig. S9. Global protein synthesis is affected in PABP morphants. Protein extracts from triplicate samples of [^{35}S]methionine-labeled (*A*) PABP1 and (*B*) ePABP morphants (from Fig. 2 *C* and *D*) were resolved on 10% SDS/PAGE gels, showing that synthesis of a wide range of proteins is decreased compared with control (Ctr). Thus, the loss of newly synthesized proteins in Fig. 2 *C* and *D* represents a largely global loss of translation.

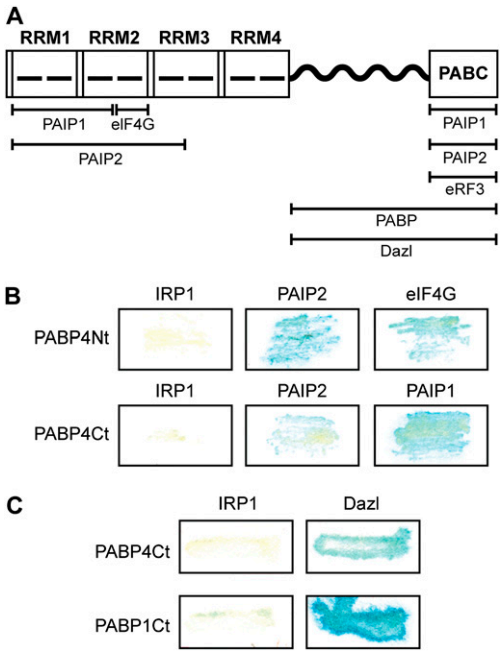


Fig. S10. Characterized protein interactions in *X. laevis* are not PABP specific. (*A*) Domain organization and mapped interactions of *X. laevis* PABP1. RRMs1–4 are shown as boxes. Each RRM contains two RNP motifs (RNP1 and RNP2, indicated by lines) that directly contact the mRNA. RRMs 1 and 2 direct the majority of poly(A) binding. The RNA-binding specificity of RRMs 3 and 4 is unclear but includes AU-rich sequences. The wavy line represents the variable proline-rich region that links the RRMs to the PABP C-terminal (PABC) domain (box). Binding sites of characterized interactions with the indicated proteins are depicted below as lines. Most of these interacting proteins are translation factors (including PABP itself) and also interact with ePABP and PABP4 (Fig. S7). Poly(A)-binding protein interacting protein 2 (PAIP2) is a negative regulator of PABP function (1), and Deleted in Azoospermia-like (Dazl) is an mRNA-specific binding protein that directly recruits PABP to target mRNAs (2). (*B*) PAIP2 interacts with *X. laevis* PABP1 and ePABP (3), and yeast two-hybrid analysis reveals that PAIP2 interacts similarly with both the N- and C-terminal domains of PABP4, suggesting that it does not underlie PABP specificity. (*C*) Yeast two-hybrid analysis shows that *X. laevis* Dazl interacts with the C terminus of PABP4 in addition to its published interactions with PABP1 and ePABP (2). Thus, this C terminal-interacting protein, which mediates mRNA-specific translation, cannot explain the requirement for individual PABPs. In keeping with this notion, Dazl knockdown in *X. laevis* results only in a loss of germ cells and not in lethality (4).

1. Khaleghpour K, et al. (2001) Translational repression by a novel partner of human poly(A) binding protein, Paip2. *Mol Cell* 7:205–216.
2. Collier B, Gorgoni B, Loveridge C, Cooke HJ, Gray NK (2005) The DAZL family proteins are PABP-binding proteins that regulate translation in germ cells. *EMBO J* 24:2656–2666.
3. Kim JH, Richter JD (2007) RINGO/cdk1 and CPEB mediate poly(A) tail stabilization and translational regulation by ePAB. *Genes Dev* 21:2571–2579.
4. Houston DW, King ML (2000) A critical role for Xdazl, a germ plasm-localized RNA, in the differentiation of primordial germ cells in Xenopus. *Development* 127:447–456.