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PIPPin, a Putative RNA-Binding Protein Specifically Expressed in the Rat Brain[1]

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In looking for genes encoding RNA-binding factors, we prepared an expression library in λgt11, by cloning cDNAs corresponding to the polyadenylated fraction of RNA from rat brain at the embryonal day 18. The library was then screened by binding to a radioactive RNA, transcribed in vitro from a cDNA encoding the rat histone variant H3.3. Here we report some findings concerning a cDNA for a protein which contains two putative double stranded RNA-binding domains (dsBD). The corresponding message is specifically expressed in the brain. Moreover, Southern blot analysis showed that the gene is highly conserved from Drosophila melanogaster to man.

In the last few years an ever growing number of evidences has pointed to the importance of regulation of mRNA localization, stability and translation, in the control of gene expression, both in development (1–6) and in the maintenance of a differentiated cell phenotype (7–14). Posttranscriptional control processes are mediated by several RNA-binding proteins (RBPs) (5,9,15–20) and much progress has been made in the identification and characterization of the functions of many of these factors. The cloning of the corresponding cDNAs and the analysis of the amino acid sequences have led to the discovery of different conserved motifs in RBPs (for review, see: 17); in particular, several RBPs have in common one or more copies of a domain able to bind double-stranded RNA (dsRNA) (15,17–18). Here we describe a cDNA which encodes a protein that contains two putative dsRNA-binding domains and is expressed specifically in the rat brain.

MATERIALS AND METHODS

Cloning of putative RNA-binding proteins. Polyadenylated mDNA from developing rat brain was used as a template for cDNA synthesis. cDNA was then cloned in the λgt11 Sfi-Not vector (Promega, USA), according to the manufacturer's instructions. Screening of the library was done by probing the ability of the proteins (produced by the clones) to bind a [^{32}P]-radiolabeled H3.3 mRNA, transcribed in vitro from the T3 RNA polymerase promoter of a recombinant pGEM^{EX} (Promega, USA) plasmid which contains an H3.3 insert (acc.n. X73683: ref.21). We obtained two positive clones, the inserts of which were subsequently subcloned into the bluescript plasmid vector. One of these clones (called PIPPin cDNA) has been sequenced on both strands by the dideoxynucleotide chain termination method (22), using the Sequenase DNA-sequencing kit (United States Biochemicals, Cleveland, OH). Sequence alignments were done using MacMolly Tetra Program by Gene Soft.

Northern analysis. Total RNA was isolated from different rat adult tissues and from rat brains at different ages of development, according to Chomczynsky and Sacchi (23). Electrophoretic separation and Northern analysis of RNA were performed as described elsewhere (21). For standardization, the membranes were also probed with a 1.4 Kb Bam H1 fragment derived from the human ribosomal gene cluster, which specifically hybridizes to the 28S rRNA (24).

Southern analysis. 10μg of genomic DNA from different sources was digested with restriction enzymes, separated by 0.8% agarose gel electrophoresis and blotted onto a Hybond N$^+$ membrane (Amersham Int.). The blot was then hybridized with the [^{32}P]-labeled PIPPin cDNA. The membrane was washed for 20 min in 2XSSC/0.1% SDS at room temperature and for 20 min in 0.5XSSC/0.1% SDS at 55°C.

[1] The sequence of PIPPin cDNA has been deposited in the EMBL/GenBank/DDBJ databases (accession no. X89962).

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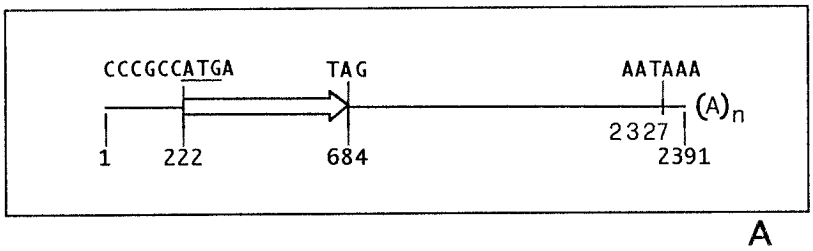
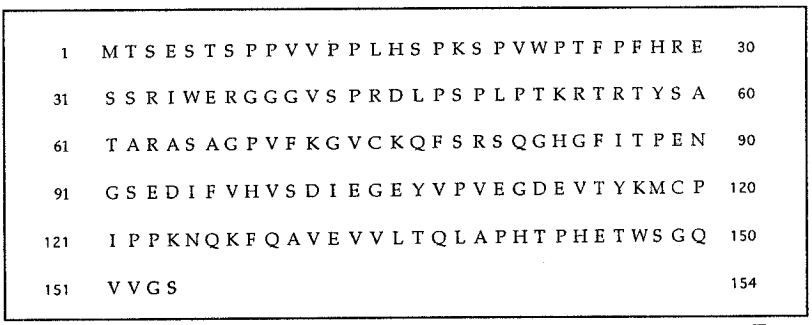
RESULTS AND DISCUSSION

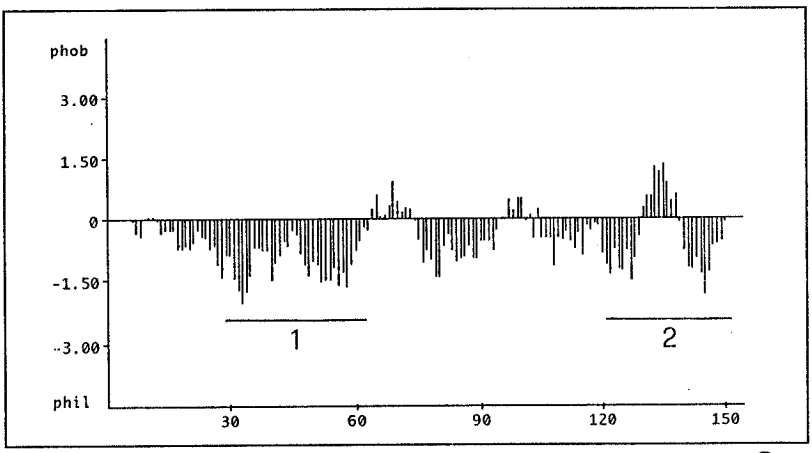
Analysis of the PIPPin cDNA. A schematic drawing of PIPPin cDNA is shown in Fig. 1A. This cDNA corresponds probably to the full length mRNA, since this insert (2391 bp long) hybridizes to a 2.5 Kb message (see below). The sequence context surrounding the putative initiation codon (indicated in the figure) is, according to Kozak (25), a highly favorable one, thus suggesting that this ATG (nt 222–224) is in fact the first translated triplet. The open reading frame ends up at nt 683, after which a stop codon is present. The complete amino acid sequence of the putative PIPPin protein is reported in Fig. 1B. This protein is expected to have a molecular weight of about 17 KDa



A



B



C

FIG. 1. Features of PIPPin cDNA and protein. (A) Schematic drawing of the cDNA clone. The initiation and stop codons which define the most probable open reading frame are indicated, together with the polyadenylation signal and the poly (A) tail. (B) Complete sequence and (C) hydropathy plot of the putative PIPPin protein. The regions which show chemical homology to dsRNA-binding proteins (see Fig. 2) are indicated as 1 and 2.

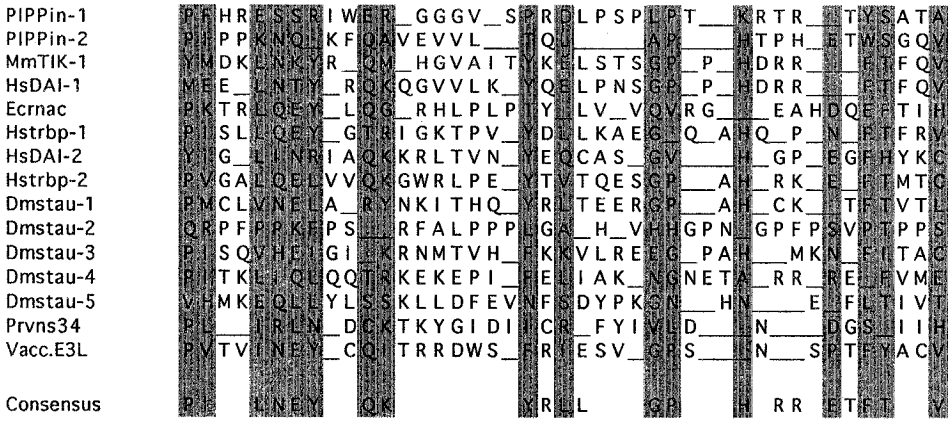


FIG. 2. Multiple alignment of regions 1 and 2 (see Fig. 1) of PIPPin with motifs found in dsRNA-binding proteins. The consensus sequence is shown below. The sequences are as follows: MmTIK-1, mouse TIK (9–45); HsDAI, human dsRNA-dependent kinase: 1 (11–46), 2 (101–136); Ecrnac, *E. coli* RNase III (156–192); Hstrbp, human TAR-binding protein: 1(10–46), 2 (139–176); Dmstau, D. melanogaster staufen gene product: 1 (312–348), 2 (485–523), 3 (579–615), 4 (712–750), 5 (950–986); Prvns34, porcine group C rotavirus ns34 protein (336–368); Vacc.E3L, vaccinia virus E3L (118–153).

and a statistical pI of 7.7. Moreover, the hydropathy plot of PIPPin (Fig. 1C) shows that it is a very hydrophilic protein.

The comparison of the putative sequence of PIPPin with other protein sequences deposited in the Gene data banks revealed two regions (residues 26–63 and 120–152, respectively, indicated as 1 and 2 in Fig. 1C) with chemical homology to dsRNA-binding proteins. The alignment of the two regions with the motifs found in these proteins (Fig. 2) was done by MacMolly Complign Module and further edited manually to match the alignment reported by others for the same motifs (15,17). Conserved positions, which include both basic and hydrophobic amino acid, are scattered in the PIPPin 1 and 2 putative dsRNA-binding domains. In addition, the hydropathy plots of the two regions are very similar to those obtained (data not shown) for the other motifs reported in Fig. 2.

Northern analysis. Fig. 3A shows a representative example of Northern blot analysis of RNA samples from rat brains of different ages of development, hybridized to the PIPPin insert. A single species of about 2.5 Kb was evidenced at high stringency. The intensity of this band increases

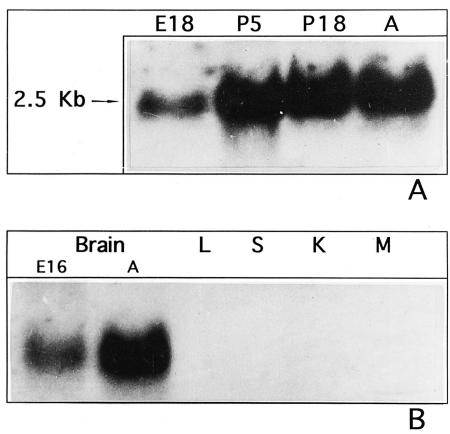


FIG. 3. Northern blot analysis of total cellular RNA from rat brains of different ages of development (A) or from different rat tissues (B), hybridized to the PIPPin insert. E, embryonal, P, postnatal day of development; A, adult brain. L, liver; S, spleen; K, kidney; M, muscle.

FIG. 4. Southern blot analysis of rat, human and drosophila genomic DNA, hybridized to the PIPPin insert. The restriction enzymes used are indicated. H, human; Dr., D. melanogaster.

between the embryonal day 18 (E18) and the postnatal day 5 (P5), keeping thereafter an almost constant level, up to adulthood. This finding suggests that the corresponding protein is specifically required during brain maturation but also for the maintenance of some differentiated function. We asked then whether this hypothetical function was typical or not of the brain. As shown in Fig. 3B, when total RNAs from different adult rat tissues were hybridized to the PIPPin insert, the 2.5 Kb message was clearly visible only in the brain samples. So, whatever its function, PIPPin seems to be a brain-specific protein.

Southern analysis. The function of PIPPin is not only brain-specific; it is also probably conserved in evolution. As shown in Fig. 4, indeed, a PIPPin-like gene seems to be present in organisms as distant as man and drosophila.

CONCLUSIONS

In this paper we describe a cDNA encoding a putative brain-specific protein that seems to have been highly conserved in evolution. Its primary sequence contains at least two regions which show chemical homology to proteins for which the ability to bind RNA has been already demonstrated. This finding, together with the fact that we isolated this cDNA by binding of an mRNA to the proteins produced by an expression library, strongly suggests that PIPPin is an RNA-binding protein.

However, functional tests are necessary (and in progress) to demonstrate definitively the functions of PIPPin and its role in brain maturation.

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REFERENCES

1. Berleth, T., Burri, M., Thoma, G., Bopp, D., Richstein, S., Frigerio, G., Noll, M., and Nusslein-Volhard, C. (1988) *EMBO J.* **7,** 1749–1756.
2. St Johnston, D., Beuchle, D., and Nusslein-Volhard, C. (1991) *Cell* **66,** 51–63.
3. Ferrandon, D., Elpick, L., Nusslein-Volhard, C., and St Johnston, D. (1994) *Cell* **79,** 1221–1232.
4. Sallés, F. J., Lieberfarb, M. E., Wreden, C., Gergen, J. P., and Strickland, S. (1994) *Science* **266,** 1996–1999.
5. Murata, Y., and Wharton, R. P. (1995) *Cell* **80,** 747–756.
6. Curtis, D., Lehman, R., and Zamore, P. D. (1995) *Cell* **81,** 171–178.
7. Wilhelm, J. E., and Vale, R. D. (1993) *J. Cell Biol.* **123,** 269–274.
8. Klausner, R. D., Rouault, T. A., and Harford, J. B. (1993) *Cell* **72,** 19–28.
9. Gray, N. K., and Nentze, M. W. (1994) *EMBO J.* **13,** 3882–3891.
10. Kislanskis, E. H., Zhu, X., and Singer, R. (1994) *J. Cell Biol.* **127,** 441–451.
11. Latham, V. M., Kislanskis, E. H., Singer, R., and Ross, A. F. (1994) *J. Cell Biol.* **126,** 1211–1219.
12. Steward, O. (1995) *Curr. Op. Neurobiol.* **5,** 55–61.
13. St Johnston, D. (1995) *Cell* **81,** 161–180.
14. Hentze, M. W. (1995) *Curr. Op. Cell Biol.* **7,** 393–398.
15. St Johnston, D., Brown, N. H., Gall, J. G., and Jantsch, M. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 10979–10983.
16. Mattaj, I. W. (1993) *Cell* **73,** 837–840.
17. Burd, C. G., and Dreyfuss, G. (1994) *Science* **265,** 615–620.
18. Bass, B. L., Hurst, S. R., and Singer, J. D. (1994) *Curr. Biol.* **4,** 301–313.
19. Taylor, S. J., and Shalloway, D. (1994) *Nature* **368,** 867–871.
20. McCarthy, J. E. G., and Kollmus, H. (1995) *Trends Biochem. Sci.* **20,** 191–197.
21. Castiglia, D., Cestelli, A., Scaturro, M., Nastasi, T., and Di Liegro, I. (1994) *Neurochem. Res.* **19,** 1531–1537.
22. Sanger, F., Nicklen, S., and Coulson, A. R. (1977) *Proc. Natl. Acad. Sci.USA* **74,** 5463–5467.
23. Chomczynsky, P., and Sacchi, N. (1987) *Anal. Biochem.* **162,** 156–159.
24. Erickson, J. M., Rushford, C. L., Dorney, D. J., and Wilson, G. N. (1981) *Gene* **16,** 1–9.
25. Kozak, M. (1991) *J. Cell Biol.* **115,** 887–903.