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Zep: A Novel Zinc Finger Protein Containing a Large Proline-Rich Domain[1]

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Zep, a novel 49 kDa zinc finger protein, was found in the brain of day-13 mouse embryos and cloned. Zep contains two C2H2-type zinc finger motifs close to the N-terminal region. The majority of the molecule is composed of a proline-rich domain showing similarity to proline-rich domains in transcription factors and a salivary proline-rich protein. In addition to the proline-rich domain, Zep has an acidic domain and a serine/threonine-rich domain, all of which are frequently found in many transcription factors. The overall organization of Zep shows no similarity to any other proteins. There is a nuclear localization signal in Zep, and the Zep-GFP (green fluorescent protein) fusion protein is located predominantly in the nucleus. In the day-13 mouse embryo, Zep is strongly expressed in the nervous system, *i.e.* brain, spinal cord, and dorsal root ganglia, with strong to weak expression observed in other regions. Zep continues to be strongly expressed in the neonatal brain; however, its expression is weak in the brain and spinal cord of adult mice. *In situ* hybridization reveals strong signals for Zep mRNA in the cerebellum and olfactory bulb with moderate signals detected in the hippocampus and cortex. Strong Zep expression is observed in adult thymus, lung, spleen, testis, and ovary. Zep may be involved in the formation and remodeling of various tissues including nervous tissue, probably through transcriptional regulation.

Key words: embryos, nervous system, nuclear protein, proline-rich domain, zinc finger.

The construction of the central nervous system is a complex process requiring the cooperation of many bioregulatory factors. Various approaches have been taken to find novel bioregulatory factors, *i.e.* purification based on activity, cDNA cloning based on differential expression, and cDNA cloning based on common motifs. We analyzed heparin binding proteins in embryonic mouse brain to screen for new bioregulatory factors involved in the construction of the embryonic brain, since heparin binding proteins are often growth factors (*1, 2*), cell adhesion molecules (*3, 4*), or transcription factors (*5, 6*).

We found a novel protein with Cys_2His_2 (C2H2)-type zinc finger motifs and a large proline-rich domain. The C2H2-type zinc finger motif was first described in *Xenopus* transcription factor IIIA (*7*). This motif has since been found in many transcription factors (*8-10*), including those expressed in the central nervous system in adults or embryos. For example, Krox 20 is a transcription factor with binding sites in the promoter region of the HOX-1.4 gene; it is expressed in a segment-specific manner in the early stages of construction (*11*).

Here, we describe the structure and expression of the novel zinc finger protein Zep.

MATERIALS AND METHODS

Analysis of Heparin Binding Proteins—Brains from day-13 mouse embryos (300 brains, about 10 g) were homogenized in 80 ml of buffer A [10 mM Tris HCl buffer pH 7.5, containing 250 mM sucrose, 50 mM KCl, 5 mM $MgCl_2$, 1 mM PMSF, and protease inhibitors (Boehringer Mannheim; Complete™)]. The homogenates were centrifuged at 3,000 rpm for 10 min to remove the nuclear fraction and the supernatant was again centrifuged at 40,000 rpm for 1 h at 4°C. The supernatant was loaded onto a column of heparin-Sepharose (1.0×1.3 cm) equilibrated with 0.2 M NaCl in 20 mM sodium phosphate buffer, pH 6.8. Proteins were eluted successively with 5 ml of 0.5 M NaCl, 1.0 M NaCl, and 1.5 M NaCl in 20 mM sodium phosphate buffer, pH 6.8. The proteins were recovered by ethanol precipitation, and analyzed by two-dimensional gel electrophoresis performed according to the method of Benjamin *et al.* (*12*). The proteins were transferred electrophoretically from the gels onto PVDF membranes (Millipore; Immobilon-P^{SQ}) and stained with Coomassie Brilliant blue. The separated spots were excised, and analyzed using an automated

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Abbreviations: C2H2, Cys_2His_2; DAPI, 4′,6-diamidino-2-phenylindole; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFP, Green fluorescent protein; NLS, nuclear localization signal.

protein sequencer (PE Applied Biosystems, model 494A).

Isolation of Zep cDNA—A cDNA in the EST database (Genbank accession number W98748) whose predicted protein sequence showed homology to a heparin binding protein expressed in embryonic brain, was obtained by RT-PCR with the sense primer 5′-GGCGGTTGGTGTGG-AGAACT-3′ and the antisense primer 5′-AGACGGCGAC-TTATTGAACAG-3′. Approximately 1×10^7 plaques of a mouse day-13.5 embryonic brain cDNA library in λZap II (Stratagene) were screened using the cDNA fragment as the hybridization probe. Filters were hybridized overnight at 42°C in hybridization solution containing 50% formamide, 5×SSC, 50 mM sodium phosphate buffer, 5× Denhardt's solution, 1% SDS, and 100 mg/ml heat-denatured salmon sperm DNA. The filters were washed at 65°C in 2×SSC, 0.1% SDS, and finally in 0.2×SSC, 0.1% SDS at 65°C.

Subcloning and DNA Sequencing—Phage DNA from positive clones was digested with *Eco*RI, and the cDNA inserts were subcloned into the *Eco*RI site of the plasmid vector pUC 119. Nucleotide sequences of the fragments were determined by the dideoxy chain-termination method using an automated DNA sequencer (Licar model 4000).

Northern Blot Analysis—Total RNA (5 μg) was prepared from ICR mouse tissues by the acid guanidinium isothiocyanate-phenol chloroform method (*13*). The radioactive probe was the same as that used to screen the mouse day-13.5 embryonic cDNA library. The blots were washed at 65°C in 2×SSC, 0.1% SDS, and finally in 0.2×SSC, 0.1% SDS at 65°C. The membranes were exposed to a BAS-imaging plate and the radioactivity on the membrane was determined with a BAS 2000 Radioimage Analyzer (Fuji Film). As a control, a GAPDH probe was used (*14*).

In Situ Hybridization—Specimens from C57BL/6J mice were subjected to Nissl staining or *in situ* hybridization as described previously (*15*). As the Zep probe, an 0.8 kb *Eco*RI fragment of the cDNA (nucleotide numbers 1-801 in Fig. 2) was subcloned into pBluescript II SK(−). Sense and

TABLE I. **N-terminal amino sequences of heparin binding proteins separated by two-dimensional gel electrophoresis and their identification by DDBJ homology search.**

Spots	N-terminal sequence	Identification after data base search
A	N-terminal block	
B	N-terminal block	
C	22 amino acids	Unknown protein
D	PKSKELVSSSS	Transcriptional coactivator P15 (*17*)
E	ASASKSAKTATTGPGTT-KTA	Neuraxin fragment starting from amino acid number 721 (*18*)
F	N-terminal block	
G	N-terminal block	
H	AQVRIGGKGTARRKK-KV	BTF3a fragment starting from amino acid number 60 (*19*)
I	MKETIMNQEK	Transcription factor BTF3b (*20*)
J	***RKKKKQLKP***	Zep
K	15 amino acids	Unknown protein
L	VLSGEDKSNIAACG	Hemoglobin alpha chain (*21*)
M	NDKKKGPKVT	Mouse peptidyl-prolyl *cis-trans* isomerase B precursor (*22*)
N	VHFTDEEKT	Hemoglobin epsilon chain (*23*)

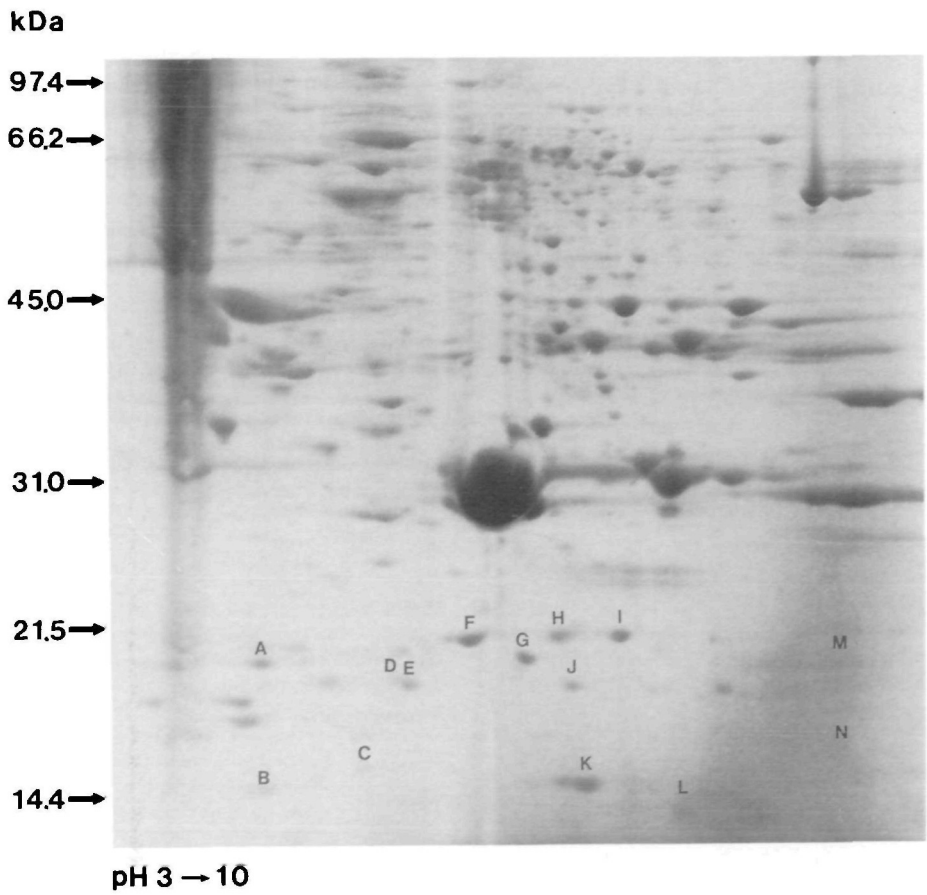

Fig. 1. **Two-dimensional electrophoresis of total heparin binding proteins from day-13 mouse embryo brains.** Proteins (1.0 mg) eluted from the heparin-Sepharose column by 0.5 M NaCl in 20 mM sodium phosphate were analyzed. Spot J is Zep.

antisense cRNA probes were prepared by *in vitro* transcription with a DIG RNA labeling kit (Boehringer Mannheim, Germany).

Expression of the Zep-GFP Fusion Protein in COS7 Cells—PCR was carried out with template cDNA containing the whole of the Zep protein-coding sequence (nucleotide numbers 111-1491) using synthesized oligonucleotide primers. The 5′-primer, 5′-ATAT**CTCGAG**ACCACC*ATGGGTCGCAAGAAG*-3′, spanned the Zep start codon (in italics) and included an *Xho*I site (bold). The 3′-primer, 5′-GTCA**GGATCC**TCGTAACGGCCACCTTGCGA-3′, also contained a *Bam*H1 site (bold). Following digestion with *Xho*I and *Bam*H1, the PCR fragment was inserted into pEGFP-N1, a Green fluorescent protein (GFP)-fusion protein expression vector with a CMV immediate-early promoter (Clontech). In the fusion protein, GFP located in the C-terminal side. COS7 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum. For DNA transfection, 2×10^4 cells were plated in the center of a 22×22 mm glass cover slip. Plasmid DNA was transiently transfected into COS7 cells using LipofectAMINE (Gibco BRL), and the cells were cultured for 24 h at 37°C following the manufacturer's instructions. Cells were fixed in 0.4% paraformaldehyde for 20 min at room temperature and observed under a fluorescence microscope (BX60 OLYMPUS) at an excitation wavelength of 490 nm. To visualize nuclei, the cells were treated with 0.1% Triton X-100 and soaked in 4′,6-diamidino-2-phenylindole (DAPI) at 1 μg/ml in phosphate-buffered saline containing 1% bovine serum albumin.

Heparin Binding Property of FLAG-Tagged Zep—A cDNA with the whole Zep protein-coding sequence (nucleotide numbers 111-1491) and the FLAG epitope (Asp-Tyr-Lys-Asp-Asp-Asp-Asp-Lys) tagged at the C-terminal end was produced by PCR and subcloned in frame into the CMV expression vector, pcDNA3. The cDNA was transfected into COS7 cells (2×10^6) using LipofectAMINE, after which the cells were cultured for 24 h at 37°C and then lysed with 1 ml of buffer B (20 mM Tris-HCl pH 7.5, 0.15 M NaCl, 1% Triton X-100, and 1 mM PMSF). The lysates were centrifuged at $13,000\times g$ for 10 min, and the supernatant was mixed with 0.2 ml heparin-Sepharose and agitated for 1 h at 4°C. The resin was washed with 1 ml of 0.2 M NaCl in buffer B, and the bound proteins were eluted with 0.5 M NaCl, 1.0 M NaCl, and 2.0 M NaCl in 1 ml of buffer B. The proteins were recovered from aliquots of each fraction (100 μl) by ethanol precipitation, subjected to 10% SDS-PAGE, and electrophoretically transferred to a nitrocellulose membrane (*16*). The membrane was incubated first in 5% skim milk in Dulbecco's phosphate buffered saline (PBS) and then with diluted anti-FLAG M2 antibody (SIGMA, 10 μg/ml in 5% skim milk) for 2 h at room temperature. After washing with PBS containing 0.1% Tween 20, the membrane was incubated with affinity-purified anti-mouse IgG conjugated with horseradish peroxidase, and the bound antibodies were visualized by ECL (Amersham).

Fig. 2. Nucleotide sequence of Zep cDNA and its predicted amino acid sequence. Numbering of the bases starts at the first nucleotide, while amino acid numbering starts at the potential start site of the open reading frame. * indicates the in frame termination codon. The poly(A) addition consensus sequence is indicated by the dotted line. In the amino acid sequence, the zinc finger motifs are boxed, and the consensus Cys and His residues are indicated in bold. The nuclear localization signal (NLS) is indicated by the shaded box at amino acids 93-109. The acidic region is underlined (amino acids 110-120) and the proline-rich region (amino acids 129-452) is marked by arrows. The serine/threonine-rich region is double underlined.

RESULTS

Analysis of Heparin Binding Proteins Expressed in the Embryonic Brain—Heparin binding proteins from day-13 mouse embryonic brains were analyzed by two-dimensional gel electrophoresis (Fig. 1). Considering that lower molecular mass proteins might be easier to analyze and clone, we concentrated our efforts on proteins with molecular masses below 22 kDa. Fourteen spots in this range were either absent or present in only small amounts on the two-dimensional gel electrophoretogram of heparin binding proteins from adult brain (data not shown). These spots (A-N) were cut out from the gel (Fig. 1) and their N-terminal sequences were determined (Table I). By comparing the sequence data with known protein sequences using the DDBJ homol-

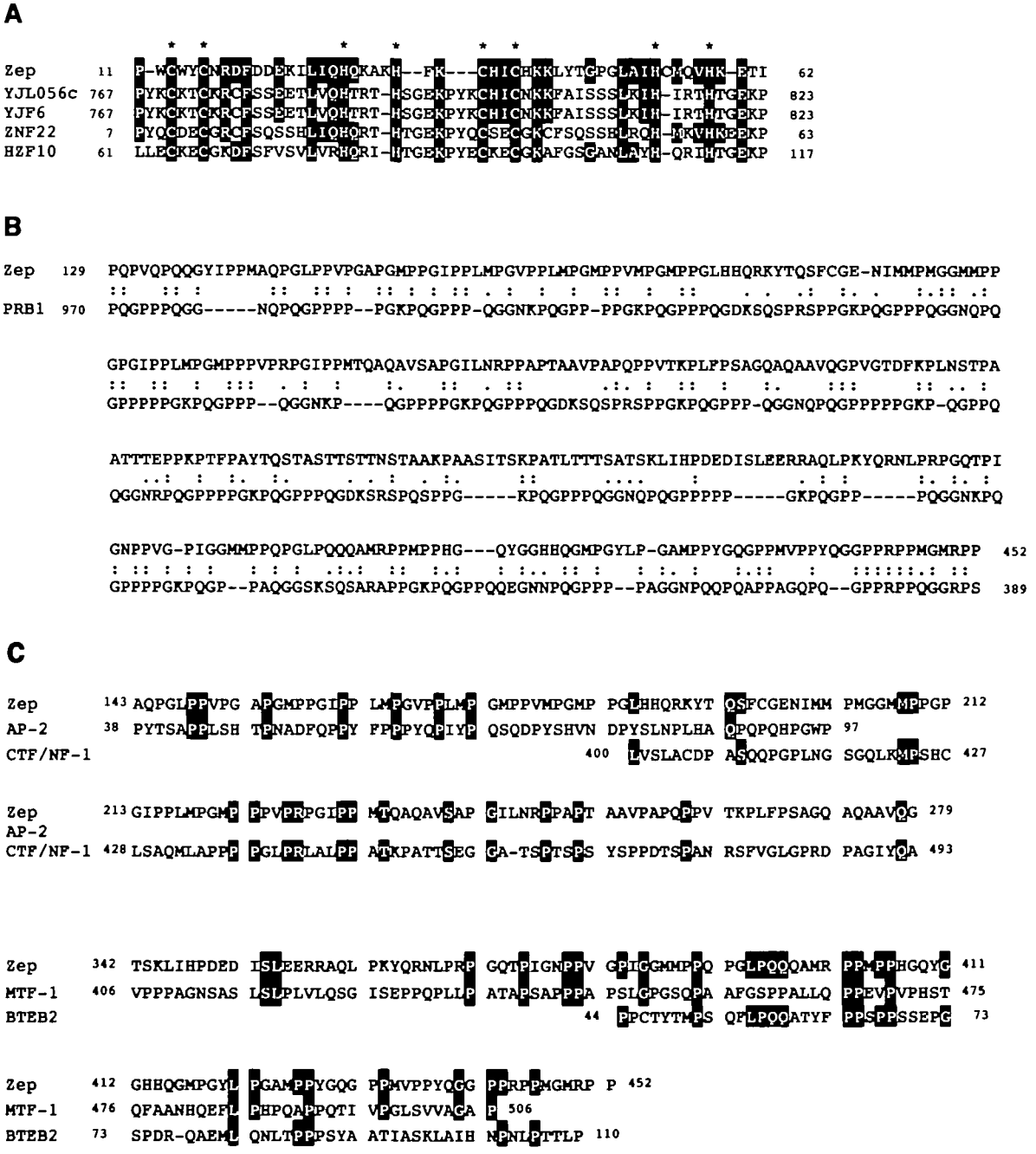

Fig. 3. **Sequence comparison of Zep with other proteins.** (A) Sequence similarities among Zep, YJL056c (PIR S56828), YJF6 (Swiss-prot P47043), ZNF22 (PIR C56409), and HZF10 (PIR S47072) in their zinc finger domain. The amino acid sequences of Zep, YJL056c, YJF6, ZNF22, and HZF10 are aligned. Portions conserved among all sequences are boxed. The consensus C2H2 motifs are indicated by asterisks. (B) Sequence similarities between Zep and salivary proline-rich precursor protein PRB1. The homology is 32%. (C) Sequence similarities among Zep, AP-2 (*31*), CTF/NF-1 (*26*), MTF-1 (*10, 27*), and BTEB2 (*28*). The length of each proline-rich domain and the homology between Zep and each transcription factor are as follows: AP-2, 60 amino acids, 13%; CTF/NF-1, 100 amino acids, 17%; MTF-1, 101 amino acids, 17%; BTEB2, 67 amino acids, 22%.

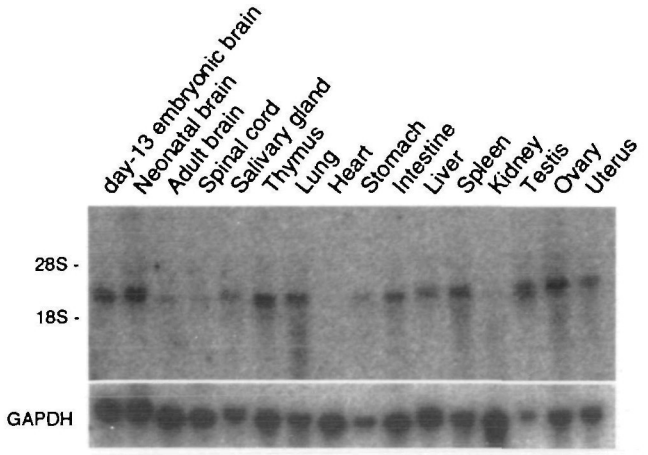

Fig. 4. Expression of Zep as determined by Northern blot analysis. The size of the transcript is 2.2 kb. From left to right, the lanes (5 μg total RNA per lane) correspond to day-13 embryonic brain, neonatal brain, adult brain, spinal cord, salivary gland, thymus, lung, heart, stomach, intestine, liver, spleen, kidney, testis, ovary, and uterus. Size markers represent 28S and 18S RNA.

ogy search system, we found 7 spots to be known proteins and 3 (C, J, K) to be novel (Table I). Spot J was investigated in the present study.

Molecular Cloning and Structure of Zep—The N-terminal sequence of spot J was RKKKKQLKP. We found a sequence homologous to the predicted nucleotide sequence in the EST data base. After obtaining the 399 bp EST sequence by PCR, we screened a day-13.5 embryonic cDNA library using this cDNA fragment as a probe, and obtained a cDNA of 2,100 bp. The nucleotide sequence of the cDNA insert revealed a single open reading frame consisting of 460 amino acids, with a molecular mass of 49 kDa (Fig. 2). In support of the predicted open reading frame, the sequence surrounding the initiation codon (CAGTTATGG) showed six matches with the eukaryotic initiator consensus sequence (CCA/GCCATGG) (*24*), and the upstream region contained an in-frame stop codon. The protein detected on two-dimensional gel electrophoresis (20 kDa) is probably a processed or a proteolytically degraded form. The N-terminal sequence of spot J is the same as amino acids 3-11 of the predicted protein. A polyadenylation signal sequence was found 13 nucleotides upstream of the 3′ poly(A) tail.

The predicted protein contains two C2H2-type zinc finger

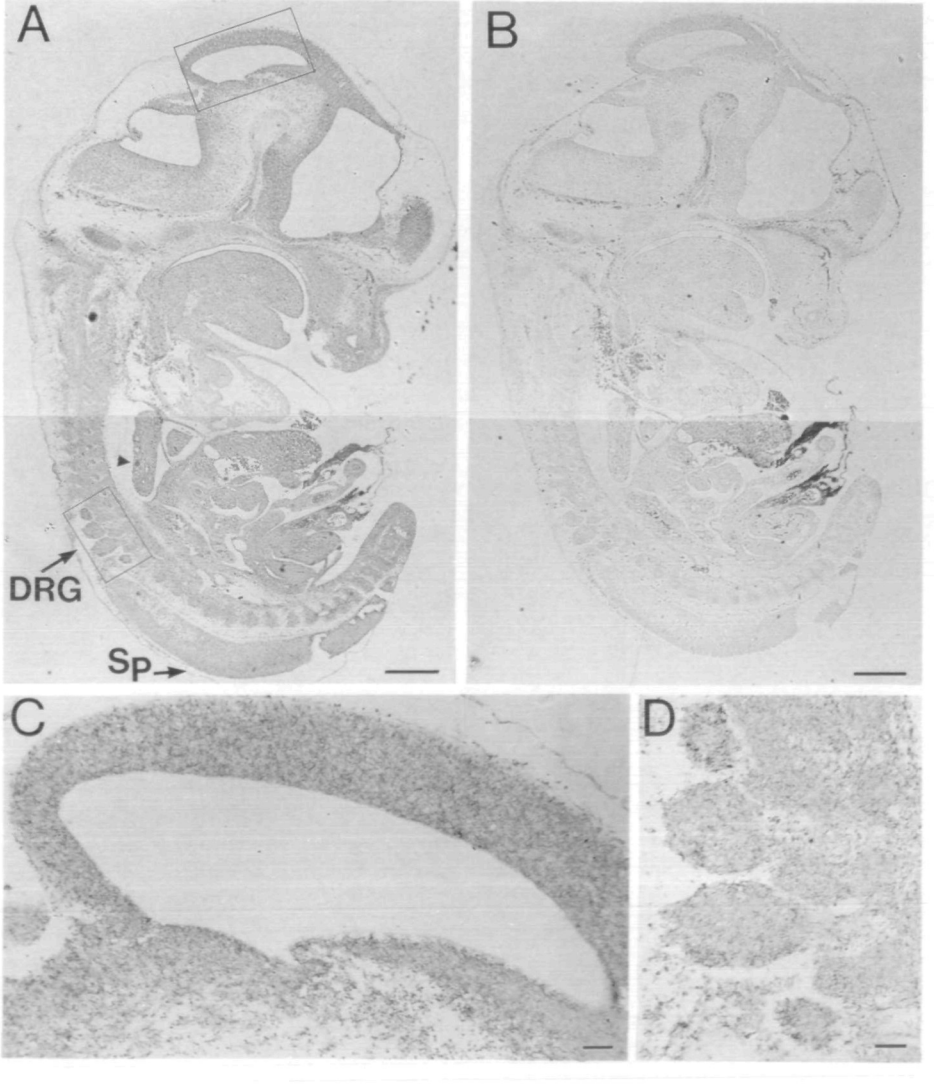

Fig. 5. Expression of Zep mRNA in the day-13 embryo as determined by *in situ* hybridization. Antisense (A, C, D) or sense (B) digoxigenin-labeled RNA probes were used. A and B, sagittal sections of the day-13 embryo. DRG, dorsal root ganglion; Sp, spinal cord. Lung epithelial cells are indicated by the arrowhead. Bars, 500 μm. C, a higher power view of the cerebral cortex. Bar, 100 μm. D, a higher power view of the DRG. Bar, 100 μm.

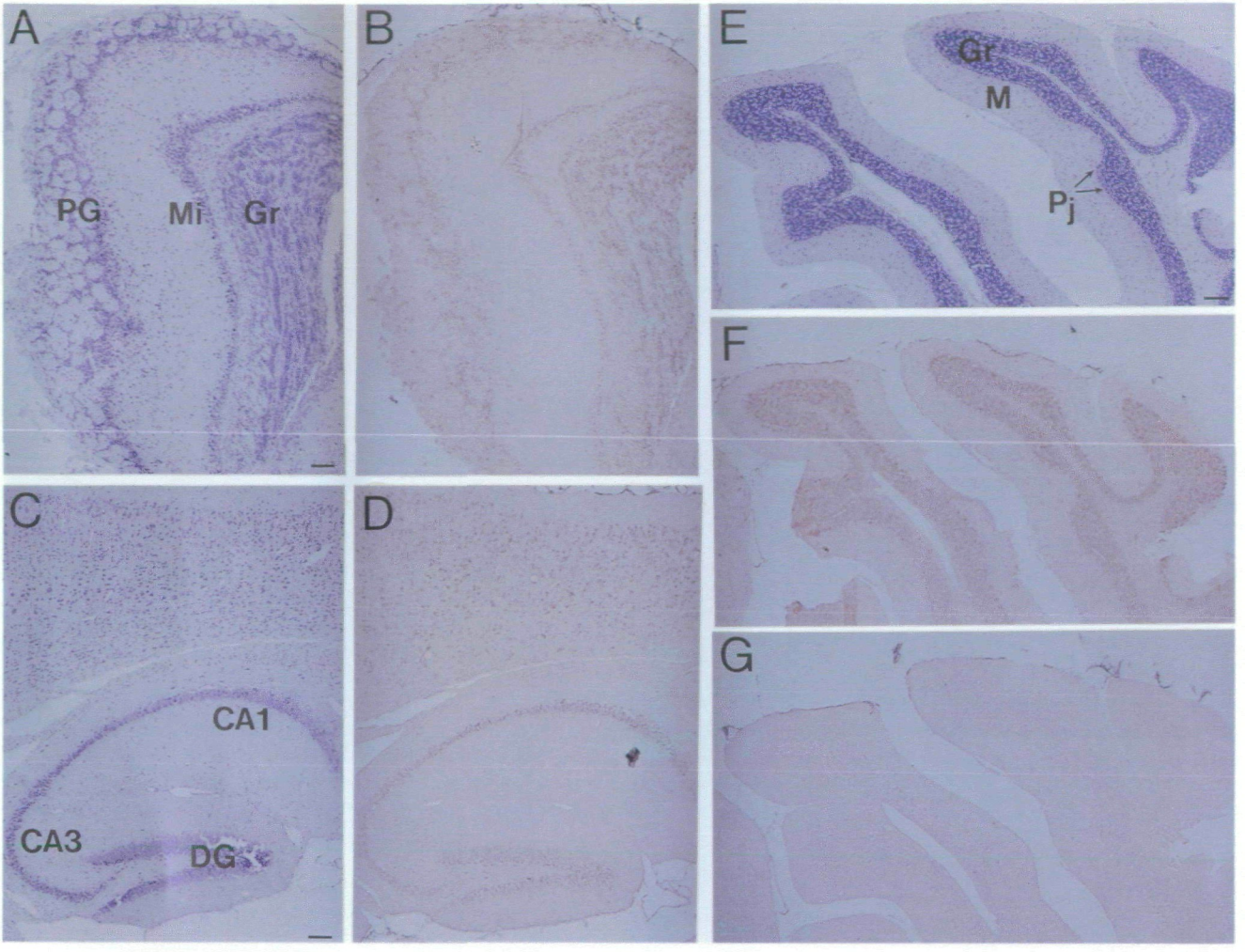

Fig. 6. **Expression of Zep mRNA in adult mouse brain as determined by *in situ* hybridization.** Nissl stained sections are shown in A, C, E. Antisense (B, D, F) or sense (G) digoxigenin-labeled RNA probes were used. A and B, olfactory bulb. Gr, granule cell layer; Mi, mitral cell layer; PG, periglomerular cell layer. C and D, hippocampus. CA1 and CA3, CA1 and CA3 regions of the hippocampal pyramidal cell layer; DG, dentate gyrus. E, F, and G, cerebellum. Gr, granule cell layer; M, molecular layer; Pj, Purkinje cell layer. Bars, 100 μm.

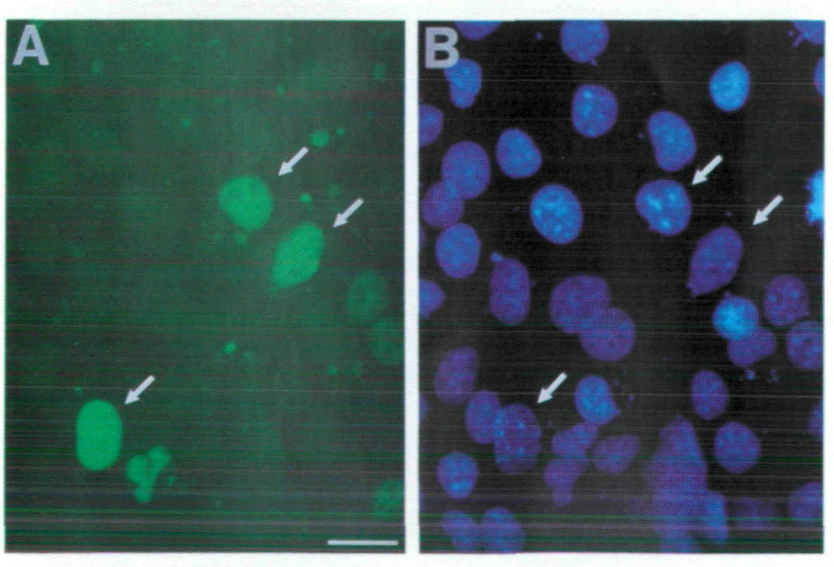

Fig. 7. **Localization of the Zep-GFP fusion protein in COS7 cells.** The same cells are viewed by fluorescence microscopy for the localization of the Zep-GFP fusion protein (A) and by DAPI staining for the localization of nuclei (B). Arrows indicate cells strongly positive for both Zep-GFP and DAPI staining. Bar, 25 μm.

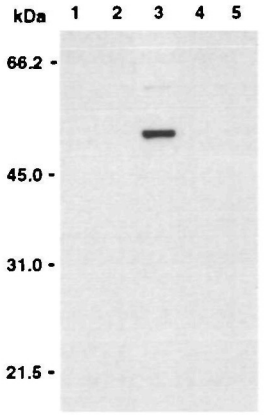

Fig. 8. **Heparin binding activity of FLAG-tagged Zep.** Extracts of COS7 cells containing FLAG-tagged Zep were fractionated by heparin-Sepharose, subjected to 10% SDS-PAGE, and electrophoretically transferred to a nitrocellulose membrane. Staining with anti-FLAG antibody reveals a 50 kDa band in Lane 3. Lane 1, the pass through fraction; Lane 2, the nonabsorbed fraction removed by washing with 0.2 M NaCl in 20 mM Tris-HCl, pH 7.5; Lane 3, the fraction eluted with 0.5 M NaCl in 20 mM Tris-HCl, pH 7.5; Lane 4, the fraction eluted with 1.0 M NaCl in 20 mM Tris-HCl, pH 7.5; Lane 5, the fraction eluted with 2.0 M NaCl in 20 mM Tris-HCl, pH 7.5.

motifs in the N-terminal region (amino acids 13-34 and 37-58). Among the various C2H2-type zinc fingers reported to date, the present one is most homologous to motifs in two yeast sequences, YJL056c and YJF6 (40% homology at the amino acid level) (Fig. 3A).

Most of the rest of the molecule (amino acids 129-452) is proline-rich, with proline accounting for 26% of the amino acids in the region examined. Significant homology was found between this segment and the salivary proline-rich precursor protein, PRB1 (*25*) (Fig. 3B). Proline-rich domains have also been found in many transcription factors, such as CTF/NF-1 (*26*), MTF-1 (*11, 27*), and BTEB2 (*28*), although the lengths of the proline-rich domains are shorter. There is also homology between segments of the proline-rich domain of the present protein and those of other transcription factors (Fig. 3C). Because of the presence of the zinc finger motifs and the extended proline-rich domain, the protein was named Zep (Zinc finger protein with extended proline-rich domain).

Two other domains frequently found in transcription factors are also present in the Zep protein, *i.e.*, two serine/threonine-rich domains (amino acids 310-321 and 328-343) and an acidic domain (amino acids 111-120). The Zep protein also has a nuclear localization signal (NLS) that corresponds to a bipartite motif (*29*) located at amino acids 93-109 (Fig. 2).

No significant homology in amino acid sequence was observed between the whole Zep molecule and other known zinc finger proteins published in the literature.

Expression of Zep mRNA—Northern blot analysis revealed high levels of Zep transcripts in embryonic and neonatal brains, but only low levels of Zep mRNA are expressed in the adult brain (Fig. 4). Therefore, the level of mRNA expression correlates with the amount of processed or degraded Zep protein as revealed by two-dimensional gel electrophoresis. The size of the transcript is 2.2 kb, indicating that the cDNA obtained in the present study is close to complete. Among adult mouse organs examined, Zep is strongly expressed in the thymus, lung, spleen, testis, and ovary, and expressed moderately in the liver and uterus (Fig. 4).

In situ hybridization analysis at embryonic day-13 revealed strong signals for Zep mRNA uniformly throughout the brain, with moderate signals in the spinal cord and dorsal root ganglia (Fig. 5). Moderate levels of the signal were found in other organs. In the adult brain, the strong signals are restricted to the cerebellum (Fig. 6F) and the olfactory bulb (Fig. 6B); granule cells are stained intensely in both cases. Moderate signals were detected in the hippocampus and the cortex (Fig. 6D).

Localization of Zep-GFP Fusion Protein in COS7 Cells—We investigated the localization of Zep to know whether it is present in the nucleus as expected for its putative role as a transcription factor. For that purpose, cDNA encoding Zep-GFP fusion protein was produced and transfected into COS7 cells. The fusion protein was predominantly expressed in the nucleus (Fig. 7). In contrast, the GFP protein expressed in COS7 cells was found to be diffusely distributed in both the cytoplasm and nucleus (data not shown).

Heparin Binding Properties of FLAG-Tagged Zep—Although the 20 kDa Zep fragment was found in the heparin binding protein fraction, it was not clear whether 49 kDa Zep has heparin binding activity. To clarify this point, we expressed Zep labeled with FLAG-tag at the C-terminus. The FLAG-tag itself is not expected to have heparin binding activity. Western blot analysis using an anti-FLAG antibody revealed that the FLAG-tagged Zep bound to heparin-Sepharose and was eluted by 0.5 M NaCl (Fig. 8). Therefore, the heparin binding property of intact Zep is similar to that of the 20 kDa Zep fragment.

DISCUSSION

We have found a new zinc finger protein in the embryonic mouse brain. The protein has zinc finger motifs in the N-terminal region, and a large proline-rich domain. The sequences of the two zinc finger regions are typical of the C2H2-type, which has the sequence Cys-X_{2or4}-Cys-X_{12}-His-X_{3-5}-His (*30*). Two cysteine residues, two histidine residues, and several hydrophobic residues, characteristic of C2H2-type motifs are all present in both zinc fingers (Fig. 3A). A distinctive characteristic of the zinc finger motifs of Zep is the short interval between them (Fig. 3A); *i.e.*, 2 amino acids in Zep in contrast to 6 amino acids in the majority of cases. The highest degrees of homology (40% at the amino acid level) were found to the putative zinc fingers YJL056c (PIR S56828) and YJF6, a 98.9 kDa yeast zinc finger protein (Swiss-prot P47043) (Fig. 3A).

The proline-rich domain (26% proline) makes up most of Zep. Proline-rich domains are found in many DNA-binding transcription factors such as AP-2 (*31*), CTF/NF-1 (*26*), MTF-1 (*10, 27*), and BTEB2 (*28*), and are involved in transcriptional activation (*32*). This domain of CTF1 interacts directly to TFIIB and facilitates TFIIB recruitment during the assembly of the preinitiation complex (*33*). The proline-rich domain of Zep shows at most 32% sequence identity to salivary proline-rich precursor protein PRB1 (Fig. 3B), which is not a transcription factor (*25*). Furthermore the size of the domain is the largest among

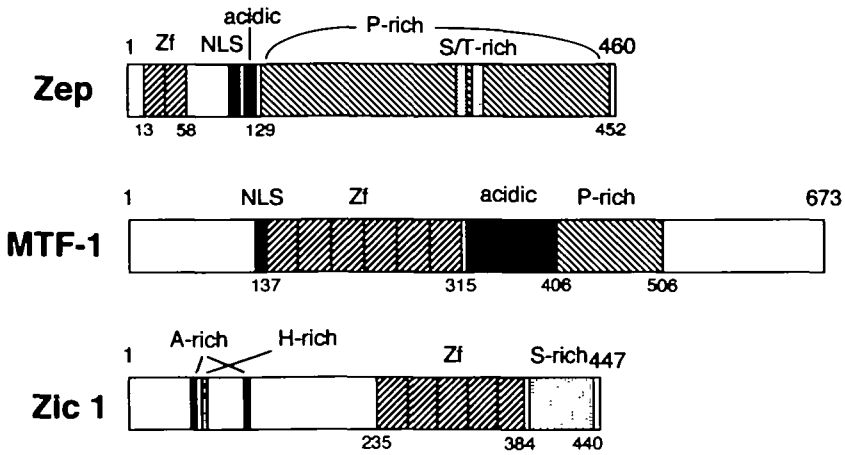

Fig. 9. **Schematic diagram showing the zinc finger and putative transcription activation domains.** Zep, MTF-1 (*10, 27*), and Zic 1 (*39*). Zf, zinc finger motif; acidic, acidic domain; P-rich, proline-rich domain; S/T-rich, serine/threonine-rich domain; A-rich, alanine-rich domain; H-rich, histidine-rich domain; NLS, nuclear localization signal.

transcription factors examined. However, segments of the proline-rich domain of Zep show homology to segments in other transcription factors (Fig. 3C).

In addition to the proline-rich domain, two domains implicated in transactivation, *i.e.* an acidic domain and a serine/threonine-rich domain, are detected in Zep. Acidic domains have been found in C2H2-type zinc finger proteins such as Zfy-2 (*8*), rKr1 (*9*), and MTF-1 (*10*), and are involved in transcriptional activation by interacting directly with TFIIB and TATA-binding protein (TBP) (*34*).

Serine/threonine-rich domains are present in AP-1 (*35*), ATBF-1 (*36*), and Sp1 which has 3 zinc fingers, 2 glutamine-rich domains, and a serine/threonine-rich domain (*37*). The role of the serine/threonine-rich domain has been clarified in FosB (*35*), a member of the Fos family. FosB contains a basic region-leucine zipper (bZIP) motif and a cluster of serine residues adjacent to the proline-rich motif in the C-terminus. Phosphorylation of the cluster of serine residues is required for transcriptional activation and neoplastic transformation activities (*35*).

The over-all organization of Zep is distinct from other transcription factors, even though Zep has two zinc finger motifs and the three segments implicated in transcriptional activation (Fig. 9). Furthermore, the short interval between the two zinc finger motifs and the extended proline-rich domain make Zep unique among the many zinc finger-containing proteins. We are aware that not all zinc finger-containing proteins are transcription factors (*38*). However, the presence of the three sequence domains potentially involved in transcriptional activation and the occurrence of NLS suggest that Zep is a transcriptional regulator. The localization of the Zep-GFP fusion protein also suggests that Zep is a nuclear protein. The 20 kDa Zep fragment was found in cell extracts devoid of the nuclear fraction. Probably, during the procedure of cell disruption and extraction, the subcellular distribution of Zep or its fragment was changed.

The heparin binding property of Zep suggests its DNA binding activity. Based on the hypothesis that Zep is the prototype of a novel class of transcriptional regulator proteins, we are now studying DNA binding capability and the specificity of Zep. Clarification of the DNA binding specificity of Zep will enable us to construct test DNAs to examine whether Zep actually has transcriptional regulator activity.

Zep is strongly expressed in embryonic and neonatal brain, but only weakly in adult brain. *In situ* hybridization analysis showed strong Zep signals in the brains of day-13 embryos. In the adult brain, Zep expression is strong only in granule cells of the cerebellum and olfactory bulb. This mode of expression suggests that Zep plays a role in the development of the nervous system. A number of C2H2-type zinc finger proteins, such as Zic (*39*), Krox 20 (*11*), and Krox 24 (*40*), have been found in the developing central nervous system. Zic is a zinc finger protein expressed in the dorsal region of the neural tube at a specific embryonic stage, whereas it is restricted to the cerebellar granule cells in adults (*39*). This developmental pattern of Zic expression is similar to that of Zep. Olfactory nerve axons and granule cells in the hippocampus show axonal growth even in adults due to continued cell division (*41, 42*); the persistent presence of Zep in the adult brain may be related to this capability.

The expression of Zep is not restricted to the nervous system. In day-13 embryos, moderate levels of expression are observed in other organs (Fig. 5). In adults, strong Zep expression is detected in the thymus, spleen, testis, and ovary, all of which contain dividing cells. Therefore it is possible that Zep plays a general role in dividing cells, and it remains to be elucidated whether the role of Zep is the same in various tissues.

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