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PTP-NP, a new member of the receptor protein tyrosine phosphatase family, implicated in development of nervous system and pancreatic endocrine cells

Ming-Ko Chiang and John G. Flanagan

Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115, USA

SUMMARY

The regulation of protein tyrosine phosphorylation is an important mechanism for developmental control. We describe here a new member of the protein tyrosine phosphatase (PTP) family, called PTP-NP (for neural and pancreatic). The cDNA sequence indicates a receptor-type transmembrane molecule. At early organogenesis, in situ hybridization with a probe for the PTP-NP extracellular region detects expression confined to the region of the developing pancreas, an organ of medical importance, but poorly understood with regard to molecular mechanisms of developmental control. This localized expression appears early, even before morphological differentiation of the pancreas, and is found in presumptive precursors of the endocrine cells by the earliest times that they can be distinguished. In neural development, an alternate RNA with a different or missing extracellular region is expressed transiently at early stages of neurogenesis and the full-length *PTP-NP* RNA appears later. To search for a ligand of PTP-NP, a fusion protein probe was made with the extracellular domain fused to an alkaline phosphatase tag. This probe bound strongly to pancreatic islets, providing evidence for a ligand-receptor interaction that could be involved in endocrine cell regulation. The results show PTP-NP is an especially early marker for pancreatic development and suggest it may be a receptor that could control the development of pancreatic endocrine cells.

Key words: receptor, tyrosine phosphatase, nervous system, pancreas, PTP-NP, mouse

INTRODUCTION

Protein tyrosine phosphorylation has been extensively characterized as a major mechanism of transducing signals within cells. The balance of tyrosine phosphorylation is maintained and modulated by two opposing sets of enzymes, the protein tyrosine kinases (PTKs) and the protein tyrosine phosphatases (PTPs). During embryonic development, several protein tyrosine kinases are known to have powerful and specific roles (Cantley et al., 1991; Fantl et al., 1993; Imamoto et al., 1994; van der Geer et al., 1994). Though the PTPs are less well characterized, there is some genetic evidence to indicate important functions in specific tissues during development. The *Drosophila* gene *corkscrew*, encoding an intracellular PTP, is required for the development of the head and tail of the embryo (Perkins et al., 1992). Mice homozygous for the *motheaten* (*me*) allele, which encodes a mutated version of the intracellular PTP, haematopoietic cell phosphatase (HCP, also known as SH-PTP1 and PTP1C), have a variety of defects in the immune system (Shultz et al., 1993; Tsui et al., 1993). Important roles for other PTPs are also indicated by biochemical studies. For instance, an intracellular PTP, FAP-1 (also known as PTP-BAS) has been found to be involved in the signal transduction pathway of apoptosis (Sato et al., 1995).

In addition to the phosphatase catalytic domain, many PTPs contain a transmembrane and extracellular domain (Cohen and Cohen, 1989; Hunter, 1989; Walton and Dixon, 1993; Brady-Kalnay and Tonks, 1995). Like the transmembrane PTKs, the transmembrane PTPs could be receptors with the potential to regulate the phosphorylation state of downstream targets in response to binding of extracellular ligands. However, there is as yet little evidence on ligands, or on the potential for ligand-induced signaling. Two transmembrane PTPs, PTPμ and PTPκ, have been demonstrated to exhibit homophilic binding which can cause cell-cell adhesion (Brady-Kalnay et al., 1993; Gebbink et al., 1993; Sap et al., 1994). Another transmembrane PTP, RPTPβ, was found to correspond to phosphacan, a proteoglycan that can interact with the adhesion molecules N-CAM, Ng-CAM and the extracellular matrix protein tenascin (Milev et al., 1991; Barnea et al., 1994; Grumet et al., 1994), and was also identified as a ligand of the neuronal cell surface molecule contactin (Peles et al., 1995).

In vertebrates and invertebrates, several receptor-type PTPs have been identified with restricted expression patterns in the developing nervous system. In *Drosophila*, DPTP99A, DPTP10D and DLAR are transmembrane PTPs with neuron-specific expression, and immunolocalization of these molecules on axons has led to proposals that they may be involved in axon outgrowth and guidance (Tian et al., 1991; Yang et al., 1991). In vertebrates, *RPTPβ* shows expression restricted to the developing nervous system (Carnoll et al., 1993; Levy et al., 1993). *LAR*, *RPTPσ* and *CRYPα* were also

found expressed in embryonic neuronal tissues (Yan et al., 1993; Stoker, 1994; Wang et al., 1995). In addition, *PTPα* was found to be induced during neuronal differentiation of P19 cells (den Hertog et al., 1993). Similarly, the expression of two other PTPs, *PC12-PTP1* and *LAR*, was induced in differentiating PC12 cells (Sharama and Lombroso, 1995; Zhang and Longo, 1995).

Unlike the nervous system, there is little information on molecular mechanisms for cell-cell signaling in pancreatic development and no cell-cell signaling molecules specific to the pancreatic lineage have yet been identified. The pancreas is of enormous medical importance, because of its role in widespread diseases, notably juvenile diabetes and pancreatic cancer. Formation of the pancreas during development has been well studied at the morphological and cellular level, but little is known about control of the induction, growth or differentiation of the pancreas at the molecular level (Slack, 1995). Previous studies have shown some transcription factors expressed in early developing pancreas. In particular, *PDX-1*(also known as *IPF-1*, *IDX-1* or *STF-1*: Ohlsson et al., 1993; Miller et al., 1994; Guz et al., 1995), a homeobox gene, is expressed in the pancreatic primordium and adjacent gut endothelium and has been shown by targeted mutagenesis to be critical for the development of the pancreas (Jonsson et al., 1994). However, even though extracellular signals to control pancreatic endocrine development could be clinically useful, the mechanisms of extracellular signaling that control pancreas formation and endocrine cell development are still unknown.

We describe here the identification and characterization of a novel transmembrane PTP, called PTP-NP. A new sequence motif in the extracellular domain, as well as strong phosphatase domain homology, identifies a new subfamily within the PTP family including PTP-NP and two other sequences. In embryos and in cultured cells, there are at least two forms of *PTP-NP* mRNA: type 1 (with extracellular sequences described here) and type 2 (lacking the extracellular region of type 1). Type 1 shows strong expression in pancreatic primordium from early stages of development. Its appearance in the midgut dorsal endothelium even precedes the formation of the pancreatic rudiment, making it a candidate for control at the earliest stages of pancreatic commitment. The type 2 transcript is expressed prominently and transiently at early stages of neurogenesis, while type 1 is expressed more weakly in the nervous system at later stages. A fusion protein probe consisting of the PTP-NP extracellular domain linked to an alkaline phosphatase tag detects a candidate ligand in pancreatic islets, suggesting a ligand-receptor signaling pathway that could mediate developmental control of pancreatic endocrine cells.

MATERIALS AND METHODS

PCR amplification of PTP fragments and identification of *PTP-NP* cDNA clones

Total RNA from dissected neural tube was isolated by the single-step RNA isolation method (Kingston et al., 1994). After reverse transcription to produce single strand cDNAs, PCR amplification was performed with degenerate primers corresponding to conserved sequences in the phosphatase domain of known PTPs. The sense primer was TT(C/T)TGG(A/C)(A/G)NATG(A/G)TNTGG, corresponding to the sequence FWRM(I/V)W, and the anti-sense primer was A(C/T)NCCNGCN(C/G)(A/T)(A/G)CA(A/G)TG, corresponding to the sequence HCSAG(I/V). PCR was carried out for 40 cycles: 94°C, 1 minute; 40°C, 5 minutes; 72°C, 30 seconds. PCR products in the expected size range of approximately 320-400 bp were then purified by agarose gel electrophoresis and subcloned into the Bluescript II KS(+) vector for sequence analysis. Among the clones analyzed, a novel phosphatase domain sequence of 359 bp was identified and used as a probe to screen a mouse brain cDNA library. Overlapping clones contained a sequence of 3200 bp, which covered the whole coding sequence of 1001 amino acids illustrated in Fig. 1A.

P19 in vitro induction of neuronal differentiation

P19 cells were maintained in alpha modification of MEM supplemented with 2.5% fetal calf serum and 7.5% calf serum. For induction of neuronal differentiation, cells were trypsinized and seeded at 10^5/ml in bacteriological grade Petri dishes with medium containing 1 μM retinoic acid (RA) (Jones-Villeneuve et al., 1982; MacBurney et al., 1982). The medium was replaced every 2 days and, 6 days after the induction, aggregated cells were plated onto tissue-culture-grade plates containing medium without RA.

RNA in situ hybridizations and northern blotting

Two separate PTP-NP probes were used for nothern blot and in situ hybridization (Fig. 1B). Probe 1 was a fragment of approximately 1.23 kb extending from the 5′ end of the cloned sequence to a unique *Xba*I site at nucleotide 1238; probe 2 was a 955 bp fragment from the *Xba*I site to nucleotide 2192 in the cytoplasmic domain. Northern blot hybridizations were performed by standard procedures, with two final high stringency washes in 0.2× SSPE, 1% SDS, at 65°C for 30 minutes. Embryos were isolated from outbred Swiss Webster mice and were considered to be 0.5 days of gestation at noon of the day that plugs were detected. In situ hybridization of whole mounts or sections was performed as described elsewhere using digoxigenin-labeled antisense riboprobes (Wilkinson, 1992; Wilkinson and Nieto, 1993; Cheng and Flanagan, 1994). For every in situ hybridization probe, labeled sense riboprobes were used as negative controls. Stringent washing for whole-mount in situ hybridization was performed three times in 50% formamide, 5× SSC, 1% SDS, at 70°C for 30 minutes; and three times in 50% formamide, 2× SSC, at 65°C for 30 minutes. For in situ hybridization of sections, slides were washed in 50% formamide, 2× SSC once at 60°C and once at 65°C for 30 minutes each and then three times in 10 mM Tris pH 8.0, 0.5 M NaCl, 5 mM EDTA at room temperature for 10 minutes. After treatment with RNase A (25 μg/ml) and RNase T1 (100U/ml) at 37°C for 45 minutes, slides were washed again twice in 50% formamide, 2× SSC at 65°C for 30 minutes.

RAP in situ analysis

To construct an NP-AP expression plasmid, encoding the extracellular domain of PTP-NP fused to an alkaline phosphatase tag, the cDNA sequence from nucleotide 33 to 1832 was amplified by polymerase chain reaction. The PCR product was cut with *Hin*dIII and *Bam*HI, and inserted into the same sites of the vector APtag-2 (Cheng et al., 1995). The resulting plasmid, pNP-AP, was transiently transfected

Fig. 1. Sequence of *PTP-NP*. (A) Nucleotide sequence of *PTP-NP*. The deduced amino acid sequence of *PTP-NP* is shown in single letter code above the nucleotide sequence. The open box indicates the approximate location of the protein tyrosine phosphatase domain. The putative signal peptide and the transmembrane domain are underlined. The cysteine residues in the extracellular domain are circled. (B) Kyte-Doolittle hydrophilicity plot of predicted PTP-NP polypeptide. A diagram of the predicted structure of PTP-NP is shown above the plot, indicating the secretion signal peptide (SP), the transmembrane domain (TM) and the *Xba*I restriction site at nucleotide 1238 which separates probe 1 and probe 2 used for northern and in situ hybridization. Accession number U57345.

A

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                                  M  G  P  P  L  P  L  L  L  L  L  L  L  P  P  P  L  P  R  A  L  P  A  P  A  S  A  R  G  R   30
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  Y  E  V  P  P  G  A  L  L  H  L  K  V  T  L  Q  K  L  S  R  T  G  F  T  W  Q  D  D  Y  T  Q  R  V  I  A  Q  E  L  A  N   110
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cgcatcaggaagagcaagaggactctaccaagttcatttgctcacctttcctctccattgcctgcatcctgggggttctcctggcttccagccttgcctactgcctccgccacaactcac  1920

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  E  V  N  A  I  L  K  A  L  P  Q  *                                                                                    1030
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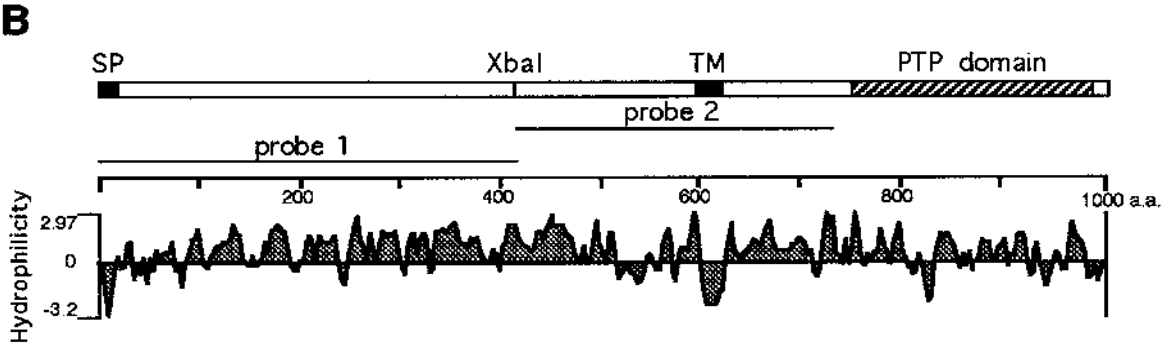
B



with Lipofectamine (GIBCO BRL) into COS cells grown in DMEM with 10% bovine calf serum. Medium was changed 24 and 48 hours after transfection and the supernatant was harvested after a further 4-6 days. The supernatant was centrifuged, 0.45 μm filtered and stored at 4°C with 20 mM HEPES (pH 7.0) and 0.05% sodium azide.

RAP (receptor affinity probe) in situ analysis was carried out as described (Cheng et al., 1995) with the NP-AP fusion protein as probe. Briefly, frozen sections of pancreas were rinsed with HBHA buffer (Hank's balanced salt solution with 0.5 mg/ml BSA, 0.1% NaN3, 20 mM Hepes [pH 7.0]) and then were overlaid with NP-AP fusion protein supernatant for 75 minutes at room temperature, washed six times for 5 minutes each in HBHA, treated with acetone/formaldehyde fixative for 2 minutes, washed three times with HBS and heated for 30 minutes at 65°C to inactivate endogenous phosphatase activities but not the characteristically heat stable AP activity of the fusion protein. Sections were then stained for 2 hours with NBT and BCIP.

Immunohistochemistry and double-staining

Guinea pig antibodies against insulin, rabbit antibodies against somatostatin and pancreatic polypeptide were purchased from Dako corporation; rabbit antibodies against glucagon from Chemicon International Inc.; and rabbit antibodies against amylase from Accurate Chemical. For double staining, the samples were first processed for

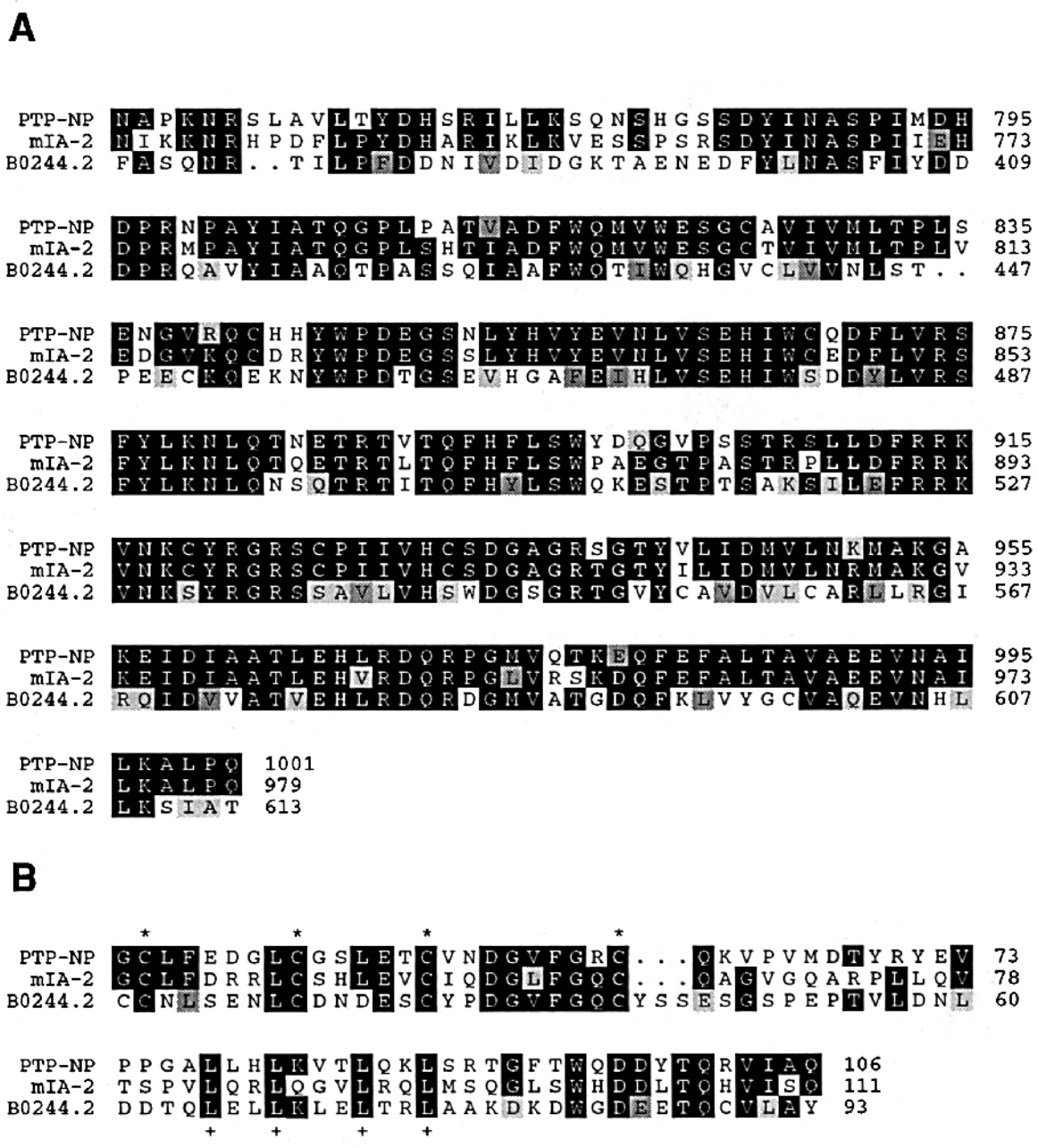


Fig. 2. Alignment of the phosphatase domain (A) and the N-terminal cysteine-conserved region (B) of PTP-NP and other members in this phosphatase subfamily (mIA-2 and *C. elegans* B0244.2 gene product). Black boxes indicate identical amino acid residues in two or more sequences at that position. Gray boxes indicate residues that are conservative changes with respect to the residues in the black boxes. (*) and (+) signs indicate conserved cysteine and leucine residues mentioned in the text, respectively. Sequences were aligned with the PILEUP program and displayed using the PRETTYBOX program in the University of Wisconsin Genetics Computer Group package.

in situ hybridization or RAP in situ analysis as indicated above, except that during the in situ hybridization, the time for the proteinase K treatment was reduced from 6.5 minutes to 2 minutes to preserve antigens on the slides. After development for the in situ hybridization or RAP in situ analysis, the sections were then fixed in 4% paraformaldehyde for 20 minutes, washed three times for 10 minutes each in PBS, preblocked in PBS containing 10% goat serum for 30 minutes and then incubated with diluted primary antibodies in 2% goat serum for 30 minutes at room temperature. The slides were then washed three times for 5 minutes in PBS, incubated with 1:200 diluted biotinylated secondary antibody (Vector Labs) in PBS with 2% goat serum for 30 minutes, followed by three washes for 5 minutes each in PBS. After incubation with 10 μg/ml streptavidin conjugated to the fluorochrome Cy3 (Jackson Immunoresearch Laboratories) in PBS for 30 minutes, the slides were given three more 5 minutes washes in PBS and mounted for viewing (mounting media from Accurate Chemical & Scientific).

RESULTS

Cloning and sequence analysis of *PTP-NP*

To search for new PTPs with developmentally regulated expression, we used PCR to amplify PTP cDNAs from dissected neural tubes of day 9.5 embryos. Amplification was carried out with degenerate oligonucleotides corresponding to two conserved regions of the PTP domain: FW(R/Q)M(I/V)W and HCSAGV. A PCR product encoding a novel phosphatase domain was identified and this 359 bp sequence was used to screen a cDNA library from new-born mouse brain. 20 cDNA clones were isolated and overlapping clones cover a sequence of 3200 base pairs of cDNA sequence (Fig. 1A). The nucleotide sequence contains an open reading frame that could encode a polypeptide of 1001 amino acids, which was named PTP-NP. This open reading frame starts with a methionine codon in a nucleotide sequence context consistent with a translation initiation site (Kozak, 1987), followed by a typical hydrophobic signal sequence for peptide secretion (von Heijne,

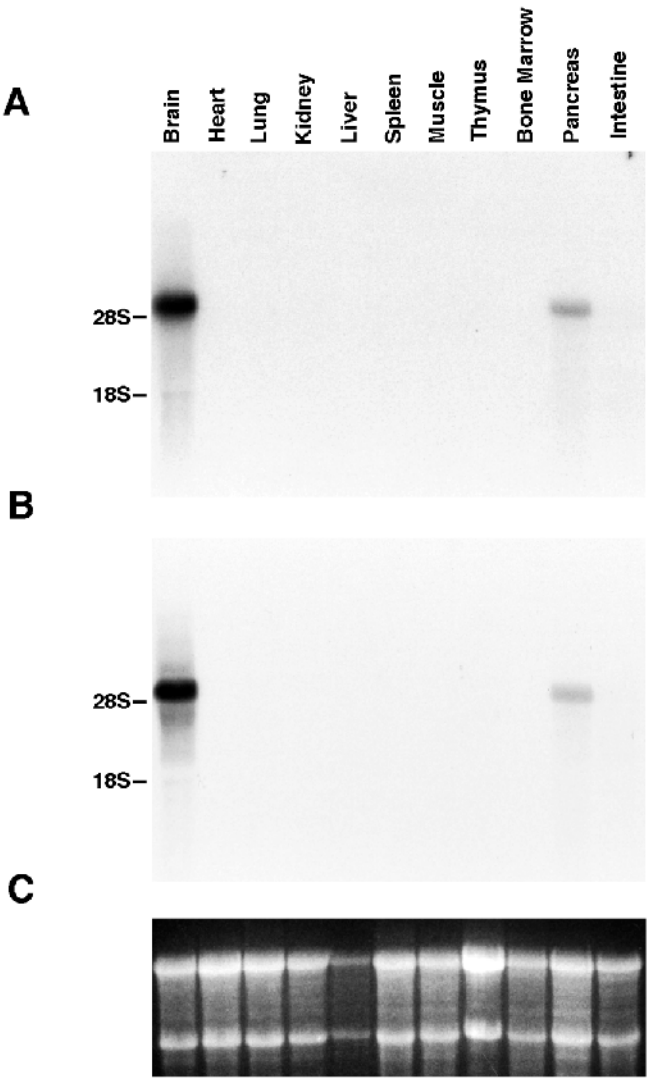


Fig. 3. Northern blot analysis of *PTP-NP* mRNA in adult tissues. 20 μg of total RNA obtained from various adult tissues were used for the northern analysis. Probes are illustrated in Fig. 1. The blot was first hybridized with the cDNA probe 1 (A), then was stripped and rehybridized with the cDNA probe 2 (B). Ethidium bromide staining of 28S and 18S ribosomal RNA is shown in C as a control for sample loading.

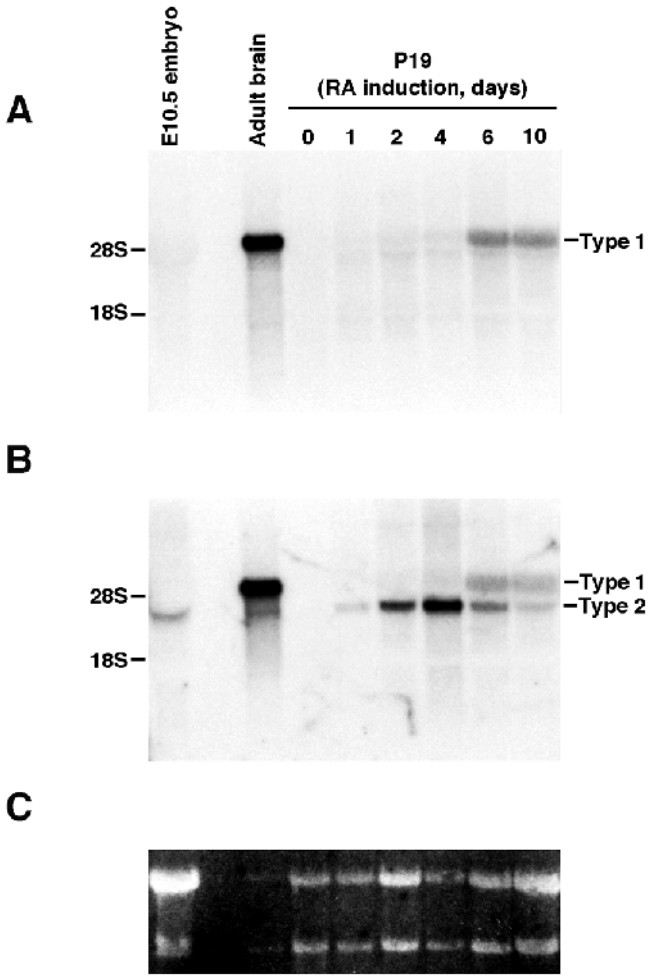


Fig. 4. Northern blot analysis of *PTP-NP* mRNA during P19 neuronal differentiation. 20 μg total RNA from day 10.5 embryos, 4 μg total RNA from adult brain and 15 μg total RNA from P19 cells at different stages after RA induction were used for the Northern analysis. The blot was first hybridized with the cDNA probe 1 (A), then was stripped and rehybridized with the cDNA probe 2 (B). Ethidium bromide staining of 28S and 18S ribosomal RNA is shown in C.

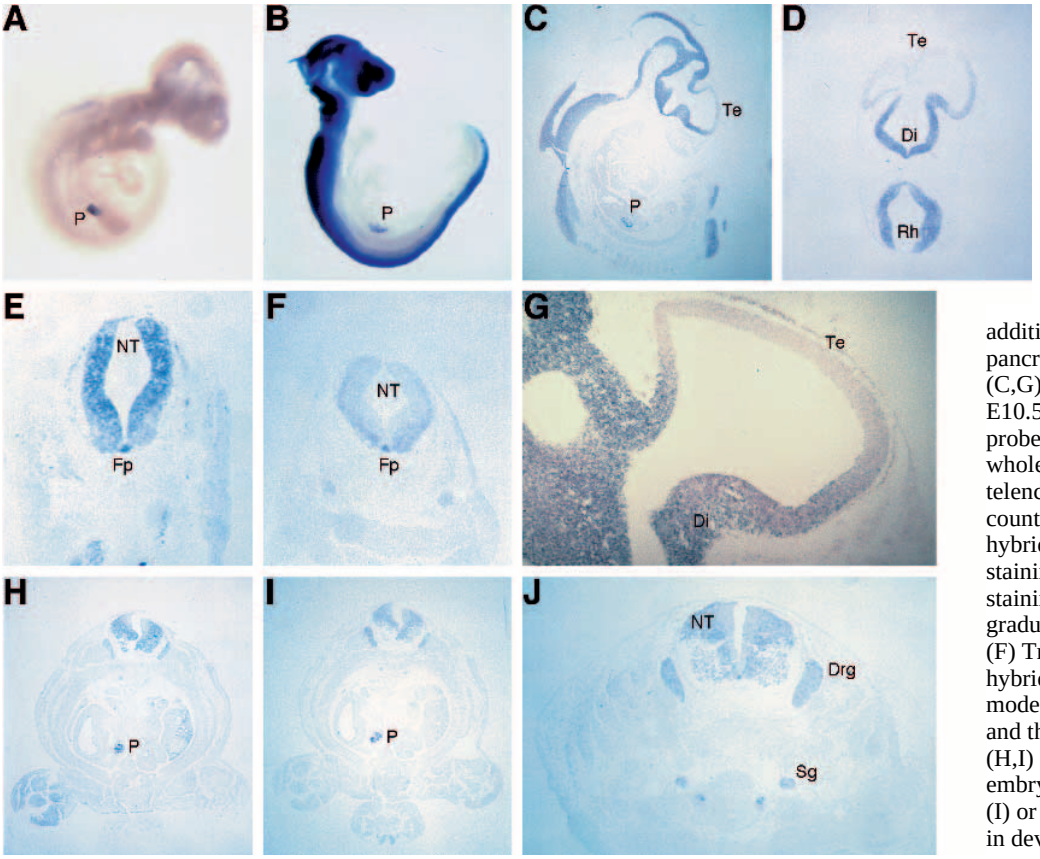


Fig. 5. Expression of *PTP-NP* in early embryos. (A) Whole-mount in situ hybridization of an E9.5 embryo with probe 1, showing *PTP-NP* expressed in pancreatic primordium. (B) An E9.5 embryo hybridized with probe 2, showing the prominent expression of type 2 *PTP-NP* in neural tube (detected specifically by probe 2) in addition to the expression of *PTP-NP* in pancreatic primordium. Sagittal sections (C,G) and transverse sections (D,E) of E10.5 embryos were hybridized with probe 2, showing strong signals in the whole neural tube except in telencephalon. (G) The section was counterstained with neutral red after hybridization, to confirm the absence of staining in the telencephalon. The staining for *PTP-NP* RNA decreases gradually toward telencephalon. (F) Transverse section of E10.5 embryo hybridized with probe 1, showing moderate signals only in the floor plate and the marginal region of neural tube. (H,I) Transverse sections of E12.5 embryo hybridized with either probe 1 (I) or probe 2 (H), showing same signals in developing nervous system and pancreatic primordium. (J) Expression of *PTP-NP* in peripheral neurons. Abbreviations: P, pancreatic primordium; Te, telencephalon; Di, diencephalon; Rh, Rhombencephalon; NT, neural tube; Fp, floor plate; Drg, dorsal root ganglion; Sg, sympathetic ganglion.

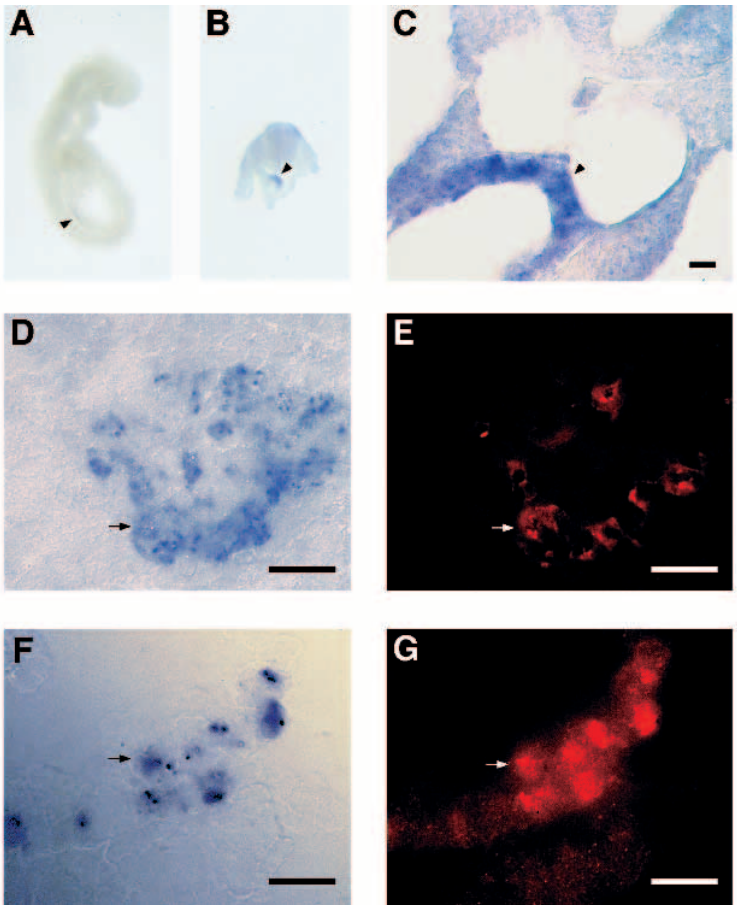


Fig. 6. Expression of *PTP-NP* in early pancreatic development. (A) Whole-mount in situ hybridization of an E8.5 embryo treated with antisense probe 1. After hybridization, the stained E8.5 embryos were sectioned either by manual dissection (B) or by cryostat frozen sectioning (C) at the midgut region. Arrowheads show the expression of *PTP-NP* in the dorsal midgut region. (D-G) E9.5 embryos were sectioned through the region containing the pancreatic primordium, transversely in D and E, or sagittally in F and G. Sections were then hybridized with the antisense probe 1 and double stained with either anti-glucagon (E) or anti-insulin (G) antibodies. E and G are fluorescent micrographs showing the same fields as in D and F, respectively. Arrows illustrate cells expressing both *PTP-NP* and endocrine markers (glucagon or insulin). Scale bars, 20 μm.

1984) (Fig. 1A,B). Functionality of these sequences to generate a secreted protein is confirmed by production of a soluble fusion protein with the PTP-NP extracellular domain fused to an alkaline phosphatase tag, as described further below. A stretch of hydrophobic amino acids is located between amino acid residues 601 and 625, and is a predicted transmembrane domain (Fig. 1A,B). Thus, PTP-NP has the typical structural features of a receptor-like transmembrane molecule with a single cytoplasmic PTP domain.

A new subgroup in the PTP sequence family

When the Genbank database was searched with the PTP-NP sequence, the phosphatase domain of PTP-NP was found to be similar to previously reported PTPs and shows a particularly close relationship with two other members of the family. One is IA-2, identified from insulinoma cells (Lan et al., 1994; Lu et al., 1994). The other close homolog is B0244.2, identified as a cDNA sequence in a genome project for the nematode *C. elegans*. An alignment of PTP-NP and mIA-2 gives an amino acid identity of 81% in the phosphatase domain and each of these two genes shows amino acid similarity of 69% in the phosphatase domain when compared to the *C. elegans* gene. In marked contrast to the close intracellular homology, the three sequences show little obvious similarity in their extracellular regions. However, in a region near the N-terminal ends of the extracellular domains, the three genes show a short region of sequence conservation (Fig. 2B). Particularly noteworthy in this region are four cysteine residues with exactly conserved spacing, consistent with a conserved disulfide bonded structure. These are the only four cysteines in the PTP-NP extracellular region. Also, four leucine residues are conserved with a spacing of three to four residues and could form a leucine zipper-like amphipathic region mediating protein-protein interactions. The close similarity of the PTP domains and the conserved motif in the extracellular domain, defines a new subfamily of PTP sequences.

Northern blot analysis of *PTP-NP* RNA in adult tissues

Expression of *PTP-NP* in adult tissues was analyzed by northern blot hybridization. Two different parts of the gene were used as probes: probe 1 is derived from the N-terminal end of the extracellular region, while probe 2 contains the C-terminal part of the extracellular domain and a partial cytoplasmic region (Fig. 1B). Both probes detect a major transcript migrating at approximately 5.3 kb expressed in brain and pancreas (Fig. 3A,B). An additional faint band at approximately 4.5 kb was also detected, at a size consistent with the type 2 transcript described further below, but only in brain and only when probe 2 was used (Fig. 3B). No expression of PTP-NP was detected in any other tissues tested.

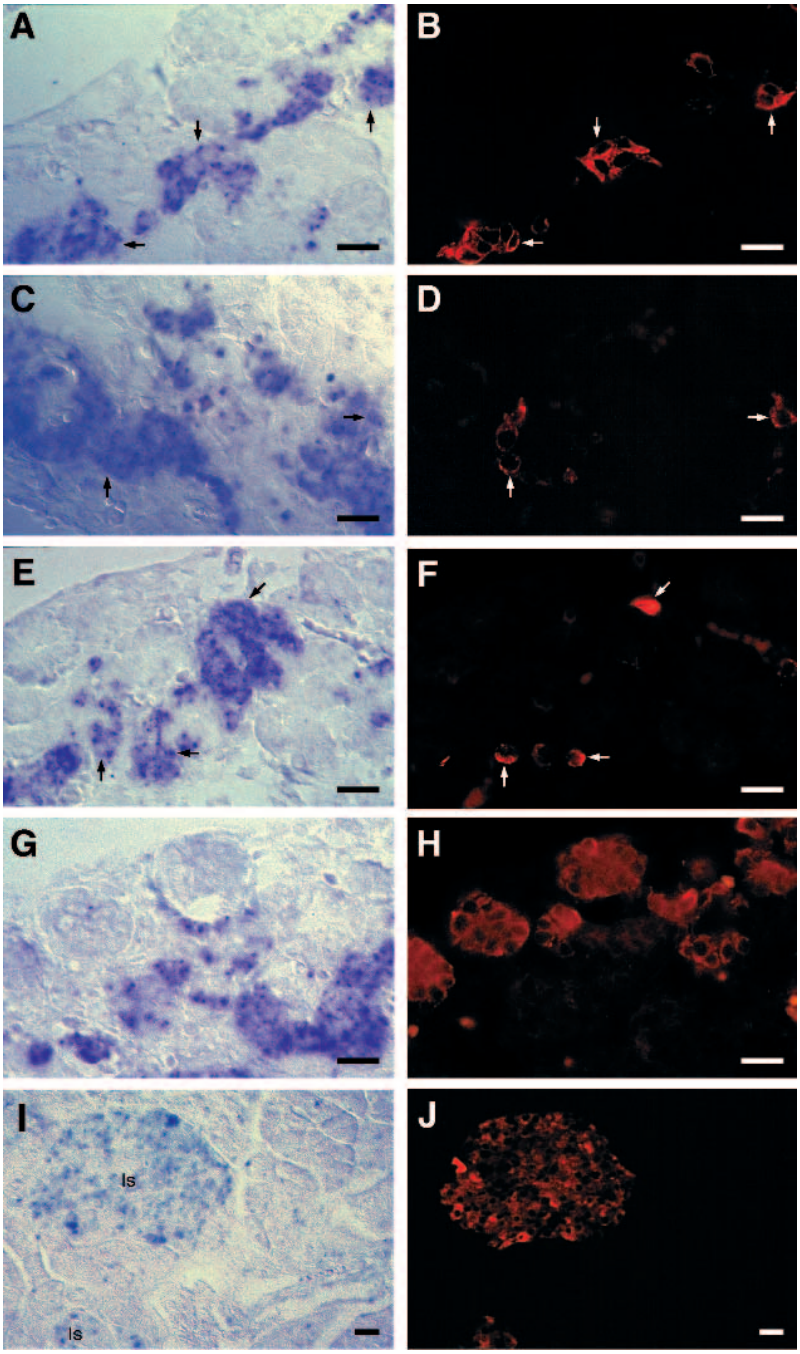


Fig. 7. Expression of *PTP-NP* in late pancreatic development. (A-H) Transverse sections of E15.5 embryonic pancreas were hybridized with the antisense probe 1 (A,C,E,G) and double stained with anti-insulin (B), anti-glucagon (D), anti-somatostatin (F), or anti-amylase (H) antibodies, showing *PTP-NP* is expressed in cells of endocrine lineages, but not in exocrine cells. An adult pancreas section was also hybridized with the same probe (I) and double stained with anti-insulin antibodies (J) to show the expression of *PTP-NP* in pancreatic islets; some weak staining seen outside the islets is also present in controls with sense strand probe (not shown) and is therefore likely to be nonspecific background. B,D,F,H and J are fluorescent micrographs showing the same field as in A,C,E,G and I, respectively. Arrows illustrate cells expressing both *PTP-NP* and endocrine markers. Is, pancreatic islets. Scale bars, 20 μm.

Northern analysis of *PTP-NP* expression during neural differentiation of P19 cells and in embryos

Since *PTP-NP* had been amplified from the neural tube of day 9.5 embryos, and additionally was found to be expressed strongly in adult brain, we were interested to explore the possibility of a role in neurogenesis. The P19 mouse embryonal carcinoma cell line provides an in vitro model system to analyze regulation of neuronal differentiation. These multipotent cells can be maintained in tissue culture in an undifferentiated state and, when aggregated, can be induced by retinoic acid to differentiate and express characteristics of neurons (Jones-Villeneuve et al., 1982; MacBurney et al., 1982; Bain et al., 1994).

Northern blot analysis was used to observe the temporal expression pattern of *PTP-NP* in P19 differentiation. Again, probes 1 and 2 were used separately. No obvious hybridization signal was detected in undifferentiated P19 cells before retinoic acid induction (Fig. 4A,B). At early time points after induction, by 24 hours, probe 2 detected a single prominent band migrating at approximately 4.5 kb (Fig. 4B). The intensity of this band reached a peak by day 4 after induction, then began to decline at day 6, at which time a lower level of hybridization was seen, with an additional band migrating at 5.3 kb. The size of the 5.3 kb RNA corresponds to the major transcript detected in adult brain (Figs 3, 4). When probe 1 was used, there was no obvious hybridization with the 4.5 kb band and only the 5.3 kb band appeared prominently (Fig. 4A). When day 10.5 embryos were tested by northern blot, consistent with the early appearance of the 4.5 kb band in P19 differentiation, a 4.5 kb RNA was the major band detected at this stage and it hybridized only with probe 2. The transcript that hybridizes with both probes 1 and 2 is defined here as type 1, while the transcript that hybridizes only to probe 2 is defined as type 2. The two transcripts appear to be produced from a single gene, since high-stringency conditions were used for the RNA hybridizations and a single gene is detected by Southern blot analysis with multiple restriction enzymes (data not shown). Since the type 2 transcript does not hybridize effectively with probe 1, it is presumably missing most or all of the extracellular domain sequence shown in Fig. 1A. At this point, we do not know whether it is truncated and lacks an extracellular domain, or whether it might encode a different extracellular domain.

Expression of *PTP-NP* in embryonic nervous system

To test expression in embryos, whole-mount in situ hybridization was initially performed at day 9.5 of gestation. Strikingly different patterns were observed with probes 1 and 2. When probe 1 was used in the hybridization, obvious staining was seen only in the pancreatic primordium (Type 1 RNA; Fig. 5A). When probe 2 was used, similar pancreatic hybridization was seen and there was an additional strong expression in the neural tube (Type 2 RNA; Fig. 5B).

The expression pattern was examined further by hybridization on sections from day 10.5 to 15.5 embryos. At E10.5, probe 2 detected prominent expression in the neural tube, as well as in the pancreatic primordium (Fig. 5C,D,E,G). Expression was seen in all parts of the neural tube, except in the telencephalon, where staining was weak or absent (Fig. 5C,D,G). By E10.5, probe 1 detects weak expression in the floorplate and the marginal regions of the neural tube (Fig. 5F). The appearance of staining with probe 1 in the marginal neural tube and the floor plate at this stage approximately correlates with the time and position of neuronal differentiation, and is consistent with the appearance of the type 1 RNA in P19 cells as they progress to more differentiated stages (Fig. 4A,B).

By E12.5, no major differences in expression patterns were observed with the use of the two probes: the intensity of hybridization detected by probe 2 in the neural tube decreases, while moderate expression detected by probe 1 in the neural tube expands to cover a broader domain (Fig. 5H,I). By E12.5, *PTP-NP* was also found to be expressed in cells of the peripheral nervous system, including the sympathetic ganglia and dorsal root ganglia (Fig. 5J), Later, at E13.5 to E15.5, the moderate expression in the neural tube is sustained (data not shown).

Taken together, the results from in situ hybridization and northern blot analysis indicate that, in the developing nervous system, expression of type 1 *PTP-NP* RNA (detected by probes 1 and 2) begins after the initial stages of neural tube development and may be correlated with the differentiation of neurons. In contrast, type 2 *PTP-NP* mRNA (detected by probe 2 only) is expressed strongly but transiently at early stages of neural development, as the neural tube is initially formed.

Expression of PTP-NP in developing pancreas

In the pancreatic region, in contrast to the neural tube, probes 1 and 2 detected similar patterns and hybridization intensities at all stages tested, implying that the type 1 RNA is a major form throughout pancreatic development (Fig. 5A,B,H,I and data not shown). This conclusion is consistent with the observation that the type 1 RNA at 5.3 kb is the only form detected in adult pancreas (Fig. 3). Expression in the region of the developing pancreas is first seen by E8.5, in the dorsal part of the midgut endoderm, which is believed to give rise to the pancreatic primordium (Fig. 6A-C). The expression of *PTP-NP* at this early stage precedes the formation of the pancreatic rudiment and also precedes the expression of known markers that distinguish the exocrine or endocrine lineages (Slack, 1995). At E9.5, *PTP-NP* is expressed in the pancreatic rudiment, which is now morphologically distinct (Fig. 5A,B). The expression of PTP-NP in the pancreatic primordium was sustained at later stages of development (E10.5-E15.5; Figs 5H,I, 7).

Within the newly formed pancreatic rudiment at E9.5, not all the cells express *PTP-NP* (Fig. 6D and data not shown). To determine whether the *PTP-NP*-expressing cells might include precursors of the pancreatic endocrine cells, we performed double staining, combining in situ hybridization for *PTP-NP* with immunolocalization for markers of endocrine cell types that first appear at E9.5. As shown in Fig. 6D-G, all cells expressing either insulin or glucagon also expressed *PTP-NP*. This result strongly implies that *PTP-NP* is expressed in the population of early endocrine progenitor cells. Some additional cells expressed *PTP-NP* but not insulin or glucagon (Fig. 6D-G, and data not shown); these could be cells of a different lineage, or cells that have not yet begun to express the hormonal markers. At later stages of development, *PTP-NP* is still expressed in all cells of endocrine lineage. At E15.5, as shown in Fig. 7A-F, cells expressing insulin, glucagon or somatostatin were all positive for *PTP-NP* expression. *PTP-NP* was also found in cells staining weakly for pancreatic

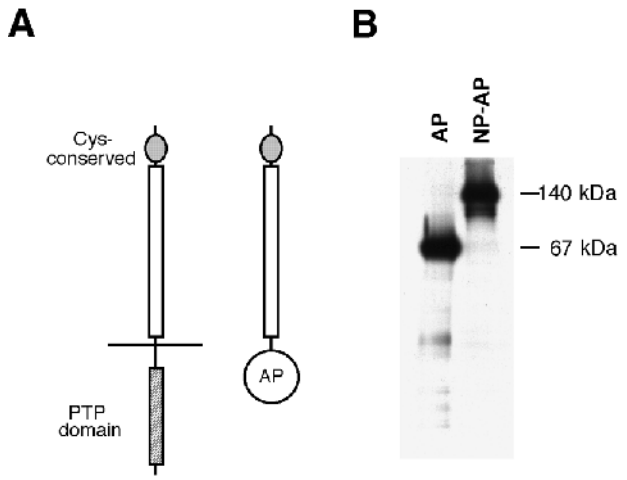


Fig. 8. Construction and expression of NP-AP as a soluble receptor affinity reagent. (A) The structure of PTP-NP is illustrated on the left and the diagram to the right illustrates the structure of the NP-AP soluble receptor affinity reagent which consists of the receptor extracellular domain fused to an alkaline phosphatase tag. (B) Expression of the NP-AP fusion protein and of unfused AP in the supernatants of transfected COS cells. Cells were metabolically labeled with [^{35}S]methionine and then the supernatants were immunoprecipitated with a monoclonal antibody against human placental AP, separated on a 8% polyacrylamide gel and autoradiographed.

polypeptide at this stage (data not shown). On the contrary, exocrine cells that express amylase were not found to express *PTP-NP* (Fig. 7G,H). In adult pancreas too, *PTP-NP* is expressed in endocrine cells within morphologically distinct pancreatic islets (Fig. 7I,J).

Detection of a candidate ligand for PTP-NP

Since the structure of PTP-NP suggests it could be a receptor, we were interested to test for the possible existence of a ligand. We have previously described the use of fusion proteins, consisting of receptor extracellular domains joined to an AP tag, as probes that can be used to detect ligands (Flanagan and Leder, 1990; Cheng and Flanagan, 1994; Chiang and Flanagan, 1995). The AP tag provides the fusion probe with an intrinsic marker activity that is sensitive and easy to use and avoids the need for purification of the probes or the use of secondary reagents or radioactive labeling. This approach appears to be widely applicable, and has been used to identify or characterize a variety of ligands (for example: Flanagan and Leder, 1990; Cheng and Flanagan, 1994; Chiang and Flanagan, 1995; Cheng et al., 1995).

To search for a ligand of PTP-NP, we constructed a fusion protein consisting of the extracellular domain of PTP-NP linked to an AP tag (Fig. 8A). This fusion protein, referred to as NP-AP, is a secreted protein and is produced as a single major polypeptide with the expected apparent molecular mass of approximately 140 kDa (Fig. 8B). To test for a ligand directly in tissue, we performed AP (affinity probe) in situ analysis as described previously by applying NP-AP as an affinity reagent directly to frozen sections of adult pancreas and then testing for bound AP activity with standard histochemical stains.

NP-AP showed strong staining in the pancreas and the staining was localized to the pancreatic islets, where the endocrine cells are located (Fig. 9A). When AP by itself was used as a negative control, the pancreatic islets showed only background binding comparable to the level seen in the acinar tissue around the islets (Fig. 9B). To characterize the NP-AP stained cells further, we used double labeling with antibodies against endocrine cell markers. The results showed NP-AP bound specifically over the insulin-expressing β cells and did not bind detectably to α or δ cells (Fig. 9C-H). We have not detected homophilic binding of PTP-NP in vitro, in experiments where the extracellular domain was marked with two separate tags (AP and immunoglobulin tags), or where the NP-AP probe was tested for binding to cells transfected with PTP-NP (data not shown). Our results therefore seem most consistent with the existence of a heterophilic ligand for PTP-NP found in pancreatic islets.

DISCUSSION

Identification of PTP-NP and a new family of PTPs

The family of PTPs has not yet been well characterized in development. However, several observations, including the large size and diversity of the family, their potential for controlling tyrosine phosphorylation, their highly restricted expression patterns and genetic evidence on some of them, indicates that, like the tyrosine kinases, they are likely to have important and specific roles in developmental control.

We describe here the identification and characterization of a new member of the PTP family, called PTP-NP. The cDNA sequence of *PTP-NP* indicates it is a receptor type PTP with a single tyrosine phosphatase domain. Comparison of PTP-NP with the other known PTPs reveals a cysteine-conserved motif in the extracellular domain and, together with their homology in the phosphatase domain, this defines a new subclass of receptor type PTPs. One other member of this class of PTPs, IA-2, was identified from an insulinoma cell line and, like PTP-NP, was found to be expressed in adult brain and pancreas (Lan et al., 1994; Lu et al., 1994). However, its expression and potential role during embryonic development are not known. PTP-NP shows close homology with the murine mIA-2 molecule in the whole phosphatase domain but only distant homology in the extracellular domain, which suggests these two PTPs might bind different ligands but share the same downstream target molecules. These two genes are also closely related to the *C. elegans B0244.2* gene, identified from a genome project, implying the possibility of conservation of the functions of this subfamily during evolution and suggesting the *C. elegans* gene may represent the prototype of PTP-NP and IA-2.

Two forms of *PTP-NP* are expressed differentially in the developing nervous system

We initially identified *PTP-NP* in a screen for potential cell-cell signaling molecules involved in early development of the nervous system. Consistent with our initial identification of *PTP-NP* in RNA from embryonic neural tube, *PTP-NP* mRNA was found to be expressed highly in neural tube from early stages of neurulation and was also found to be expressed in adult brain. To analyze the potential role of PTP-NP in neural development further, we used the P19 mouse embryonal

carcinoma cell line, a well-characterized in vitro model system for neuronal differentiation (Jones-Villeneuve et al., 1982; MacBurney et al., 1982; Bain et al., 1994). During P19 neuronal differentiation, two different types of *PTP-NP* transcripts were detected and are under different temporal regulation. Type 1 *PTP-NP*, containing the extracellular domain described here, is expressed at relatively late stages, when the induced P19 cells begin to resemble neurons morphologically and express markers of late neuronal differentiation. In contrast, the RNA expression of type 2 *PTP-NP*, which lacks the extracellular domain of type 1, shows a rapid induction within 24 hours of RA treatment. This expression of the type 2 RNA is transient and is down regulated dramatically as the type 1 RNA is subsequently expressed at lower levels. The early expression of type 2 *PTP-NP* in P19 induction appears even before that of the proneural gene *MASH-1* (Johnson et al., 1992), suggesting that it could be involved at very early stages of neuronal cell fate determination.

These observations from P19 cells are consistent with the expression patterns of type 1 and type 2 *PTP-NP* in early embryos. At early stages of neural tube development type 2 PTP-NP is expressed strongly in the neural tube. Expression of type 1 *PTP-NP* begins to appear later, by E10.5 in the marginal zone of the neural tube and the floor plate, where neuronal differentiation is relatively advanced and, by E12.5, it becomes a major form of *PTP-NP* expressed in the neural tube. Taken together with the P19 experiments, these results indicate that type 2 *PTP-NP* is induced strongly and transiently in early neurogenesis, and type 1 *PTP-NP* is expressed more weakly at later stages of neuronal differentiation.

Type 1 *PTP-NP* is expressed in early developing pancreas

Despite the importance of pancreatic endocrine cells in physiology and disease, little is known at the molecular level about the developmental control of the pancreas and no cell-cell signaling molecules have yet been identified as specific regulators of pancreatic development. We were therefore intrigued to find that, in addition to its neural expression, *PTP-NP* appeared specifically in one other tissue, the pancreas. Moreover, at early organogenesis, as the pancreas begins to form, prominent expression of the full-length *PTP-NP* type 1 RNA is confined specifically to the pancreas.

At the site of pancreatic development, the expression of *PTP-NP* was first observed as early as E8.5, in the endodermal layer of the dorsal region of the gut while it was still open to the yolk sac. The site within the endoderm that gives rise to the pancreatic rudiment has previously been identified from morphological descriptions of early pancreatic development and from in vitro explant culture experiments (Wessells and Cohen, 1967). In these studies, it was found that pancreatic tissue could be cultured from a specific region of the gut from 10- and 11-somite embryos, was formed less efficiently when the tissue was obtained from 7- to 9-somite embryos and was not produced from earlier embryos. The site of *PTP-NP* expression identified in our experiments appears to be localized to the region within the endoderm that becomes committed to form the pancreas and moreover the time of appearance of *PTP-NP* RNA appears to be similar to the time of initial pancreatic commitment.

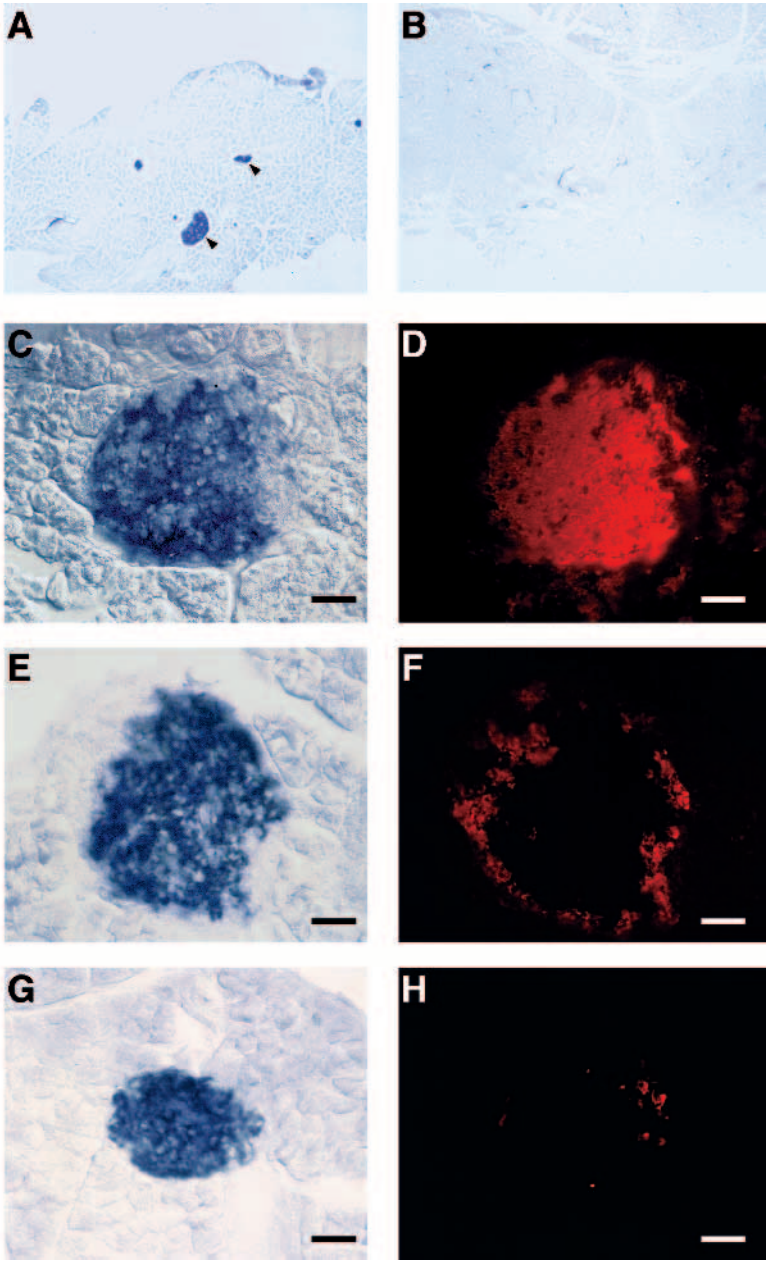


Fig. 9. Detection of a candidate PTP-NP ligand with NP-AP fusion protein in pancreatic islets. (A) A frozen section of adult pancreas was treated with supernatant containing NP-AP and then was washed, fixed and stained for bound AP activity. Arrowheads show specific staining over the islets. (B) When a pancreas section was treated with supernatant containing AP alone, no specific staining was observed. (C-H) Pancreas sections stained with NP-AP (C,E,G) were further double stained with anti-insulin (D), anti-glucagon (F) or anti-somatostatin (H) antibodies, showing the regions that bind NP-AP co-localize with the cells expressing insulin but not with the cells expressing glucagon or somatostatin. C,E,G are fluorescent micrographs showing the same fields as in B,D,F. Scale bars, 20 μm.

Later, by E9.5 and after, as the pancreatic rudiment becomes morphologically distinguishable, cells containing *PTP-NP* appeared to be located only within the pancreatic rudiment and not in adjacent areas of the gut. The temporal and spatial expression of *PTP-NP* in developing pancreas is somewhat similar to that of *PDX-1* (also known as *IPF-1*, *IDX-1* or *STF-1*), a homeobox gene that is the earliest known marker for pancreatic development (Ohlsson et al., 1993; Miller et al., 1994; Guz et al., 1995). However, unlike *PDX-1*, *PTP-NP* is not expressed in the adjacent duodenum, making it a more specific marker at the site of the early developing pancreas. *PTP-NP* type 1 is thus noteworthy as a particularly early and specific marker of pancreatic development.

Within the developing pancreas, *PTP-NP* expression is not seen in all cells. To determine which lineages might express *PTP-NP*, we performed double-labeling experiments with antibodies against hormonal markers, and against amylase, a marker for exocrine cells. By E15.5 distinct types of pancreatic endocrine cell have appeared each expressing a single hormonal marker (Slack, 1995). At this stage, we found that, of cells stained with any of the four hormonal markers insulin, glucagon, somatostatin or pancreatic polypeptide, all were positive for *PTP-NP* expression. On the contrary, amylase-positive cells did not express *PTP-NP*. Similarly, in adult pancreas, PTP-NP is found in the islets. These results indicate *PTP-NP* expression is specific for all endocrine lineages and is not found in exocrine cells.

At earlier stages of pancreatic development, the endocrine markers, insulin and glucagon, were previously reported to be co-expressed in the same cells in the pancreatic rudiment and, although not formally proven, it is believed that these hormone-expressing cells are likely to be precursors of the endocrine cells (Teitelman et al., 1993; Slack, 1995). At E9.5, the earliest stage at which these two hormonal markers have been detected by antibodies, we found that mRNA for *PTP-NP* is expressed in all the insulin- or glucagon-producing cells. An additional population of cells was found to express *PTP-NP* RNA but did not stain obviously for the hormonal markers. Those additional cells could represent an additional population of endocrine progenitor cells. In particular, in view of the very early developmental onset of *PTP-NP* expression, it is plausible that this population could represent stem cells or other undifferentiated progenitors that have not yet begun to produce hormonal markers.

The identification over the last few years of a large number of orphan receptor tyrosine kinases and phosphatases implies the existence of hitherto unidentified ligands that could be important regulators of developmental processes. To test for the possible existence of a ligand for PTP-NP, we used a technique that we have described previously to identify the ligands of other orphan receptors, making a soluble fusion probe consisting of the extracellular domain of the receptor joined to alkaline phosphatase. This NP-AP fusion protein binds strongly to pancreatic islets, identifying a candidate ligand and suggesting a ligand-receptor signaling pathway that could be involved in control of pancreatic endocrine cells. Since both receptor and ligand are apparently associated with pancreatic endocrine cells, they may mediate local control, acting within the pancreas. This possibility is particularly intriguing, considering that there does not appear to be an efficient systemic control mechanism that can, for example, promote regeneration of endocrine cells in diabetic states. Instead, pancreatic endocrine cell number and regeneration is believed to be primarily under the local control of factors acting within the pancreas (Slack, 1995). Further characterization of the candidate ligand and the PTP-NP receptor may help to understand the early development of the pancreas and could lead to reagents that could modulate the behavior of pancreatic endocrine cells in the context of normal development or disease.

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REFERENCES

Bain, G., Ray, W. J., Yao, M. and Gottlieb, D. I. (1994). From embryonal carcinoma cells to neurons: The P19 pathway. *BioEssays* **16**, 343-348.

Barnea, G., Grumet, M., Milev, P., Silvennoinen, O., Levy, J., Sap, J. and Schlessinger, J. (1994). Receptor tyrosine phosphatase β is expressed in the form of proteoglycan and binds to the extracellular matrix protein tenascin. *J. Biol. Chem.* **269**, 14349-14352.

Brady-Kalnay, S. M., Flint, A. J. and Tonks, N. K. (1993). Homophilic binding of PTPμ, a receptor-type protein tyrosine phosphatase, can mediate cell-cell aggregation. *J. Cell Biol.* **122**, 961-972.

Brady-Kalnay, S. M. and Tonks, N. K. (1995). Protein tyrosine phosphatases as adhesion receptors. *Curr. Opin. Cell Biol.* **7**, 650-657.

Canoll, P. D., Barnea, G., Levy, J. B., Sap, J., Ehrlich, M., Silvennoinen, O., Schlessinger, J. and Musacchio, J. M. (1993). The expression of a novel receptor-type tyrosine phosphatase suggests a role in morphogenesis and plasticity of the nervous system. *Brain Res. Dev. Brain Res.* **75**, 293-298.

Cantley, L. C., Auger, K. R., Carpenter, C., Duckworth, B., Graziani, A., Kapeller, R. and Soltoff, S. (1991). Oncogenes and signal transduction. *Cell* **64**, 281-302.

Cheng, H.-J. and Flanagan, J. G. (1994). Identification and cloning of ELF-1, a developmentally expressed ligand for the Mek4 and Sek receptor tyrosine kinases. *Cell* **79**, 157-168.

Cheng, H.-J., Nakamoto, M., Bergemann, A. D. and Flanagan, J. G. (1995). Complementary gradients in expression and binding of ELF-1 and Mek4 in development of the topographic retinotectal projection map. *Cell* **82**, 371-381.

Chiang, M.-K. and Flanagan, J. G. (1995). Interaction between the Flk-1 receptor, vascular endothelial growth factor, and cell surface proteoglycan identified with a soluble receptor reagent. *Growth Factors* **12**, 1-10.

Cohen, P. and Cohen, P. T. W. (1989). Protein phosphatase come of age. *J. Biol. Chem.* **264**, 21435-21438.

den Hertog, J., Pals, C. E. G. M., Peppelenbosch, M. P., Tertoolen, L. G. J., de Laat, S. W. and Kruijer, W. (1993). Receptor protein tyrosine phosphatase α activates pp60c-*src* and is involved in neuronal differentiation. *EMBO J.* **12**, 3789-3798.

Fantl, W. J., Johnson, D. E. and Williams, L. T. (1993). Signaling by receptor tyrosine kinases. *Annu. Rev. Biochem.*. **62**, 453-481.

Flanagan, J. G. and Leder, P. (1990). The *kit* ligand: a cell surface molecule altered in Steel mutant fibroblasts. *Cell* **63**, 185-194.

Gebbink, M., Zondag, G., Wubbolts, R., Beijersbergen, R., VanEtten, I. and Moolenaar, W. H. (1993). Cell-cell adhesion mediated by a receptor-like protein tyrosine phosphatase. *J. Biol. Chem.* **268**, 16101-16104.

Grumet, M., Milev, P., Sakurai, T., Karthikeyan, L., Bourdon, M., Margolis, R. K. and Margolis, R. U. (1994). Interactions with tenascin and differential effects on cell adhesion of neurocan and phosphacan, two major chondroitin sulfate proteoglycans of nervous tissue. *J. Biol. Chem.* **269**, 12142-12146.

Guz, Y., Montiminy, M. R., Stein, R., Leonard, J., Gamer, L. W., Wright, C. V. E. and Teitelman, G. (1995). Expression of murine STF-1, a putative insulin gene transcription factor, in β cells of pancreas, duodenal epithelium and pancreatic exocrine and endocrine progenitors during ontogeny. *Development* **121**, 11-18.

Hunter, T. (1989). Protein-tyrosine phosphatases: the other side of the coin. *Cell* **58**, 1013-1016.

Imamoto, A., Soriano, P. and Stein, P. L. (1994). Genetics of signal transduction: tales from the mouse. *Curr. Opin. Gen. Dev.* **4**, 40-46.

Johnson, J. E., Zimmerman, K., Saito, T. and Anderson, D. J. (1992). Induction and repression of mammalian *achaete-scute* homologue (MASH) gene expression during neuronal differentiation of P19 embryonal carcinoma cells. *Development* **114**, 75-87.

Jones-Villeneuve, E. M. V., Mcburney, M. W., Rogers, K. A. and Kalnins, V. I. (1982). Retinoic acid induces embryonal carcinoma cells to differentiate into neurons and glial cells. *J. Cell Biol.* **94**, 253-262.

Jonsson, J., Carlsson, L., Edlund, T. and Edlund, H. (1994). Insulin-promoter-factor 1 is required for pancreas development in mice. *Nature* **371**, 606-609.

Kingston, R. E., Chomczynski, P. and Sacchi, N. (1994). Guanidinium methods for total RNA preparation. In *Current Protocols in Molecular Biology* (ed. F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith and K. Struhl), pp. 4.2.1-4.2.8. New York: John Wiley & Sons, Inc.

Kozak, M. (1987). An analysis of 5′-noncoding sequences from 699 vertabrate messanger RNAs. *Nucleic Acids Res.* **15**, 8125-8148.

Lan, M. S., Lu, J., Goto, Y. and Notkins, A. L. (1994). Molecular cloning and identification of a receptor-type protein tyrosine phosphatase, IA-2, from human insulinoma. *DNA and Cell Biol.* **13**, 505-514.

Levy, J. B., Canoll, P. D., Silvennoinen, O., Barnea, G., Morse, B., Honegger, A. M., Huang, J.-T., Cannizzaro, L. A., Park, S.-H., Druck, T., Huebner, K., Sap, J., Ehrlich, M., Musacchio, J. M. and Schlessinger, J. (1993). The cloning of a receptor-type protein tyrosine phosphatase expressed in the central nervous system. *J. Biol. Chem.* **268**, 10573-10581.

Lu, J., Notkins, A. L. and Lan, M. S. (1994). Isolation, sequence and expression of a noval mouse brain cDNA, mIA-2, and its relatedness to members of the protein tyrosine phosphatase family. *Biochem. Biophys. Res. Commun.* **204**, 930-934.

MacBurney, M. W., Jones-Villeneuve, E. M. V., Edwards, M. K. S. and Anderson, P. J. (1982). Control of muscle and neuronal differentiation in a cultured embryonal carcinoma cell line. *Nature* **299**, 165-167.

Milev, P., Friedlander, D. R., Sakurai, T., Karthikeyan, L., Flad, M., Margolis, R. K., Grumet, M. and Margolis, R. U. (1994). Interactions of the chondroitin sulfate proteoglycan phosphacan, the extracellular domain of a receptor-type protein tyrosine phosphatase, with neuron, glia and neural cell adhesion molesules. *J. Cell Biol.* **127**, 1703-1715.

Miller, C. P., McGehee, R. E. Jr. and Habener, J. F. (1994). IDX-1: a new homeodomain transcription factor expressed in rat pancreatic islet and duodenum that transactivates the somatostatin gene. *EMBO J.* **13**, 1145-1156.

Ohlsson, H., Karlsson, K. and Edlund, T. (1993). IPF-1, a homeodomain-containing transactivator of the insulin gene. *EMBO J.* **12**, 4251-4259.

Peles, E., Nativ, M., Campbell, P. L., Sakurai, T., Martinez, R., Lev, S., Clary, D. O., Schilling, J., Barnea, G., Plowman, G. D., Grumet, M. and Schlessinger, J. (1995). The carbonic anhydrase domain of receptor tyrosine phosphatase β is a functional ligand for the axonal cell recognition molecule contactin. *Cell* **82**, 251-260.

Perkins, L. A., Larsen, I. and Perrimon, N. (1992). *Corkscrew* encodes a putative protein tyrosine phosphatase that functions to transduce the terminal signal from the receptor tyrosine kinase torso. *Cell* **70**, 225-236.

Sap, J., Jiang, Y. P., Friedlander, D., Grumet, M. and Schlessinger, J. (1994). Receptor tyrosine phosphatase R-PTP-κ mediates homophilic binding. *Mol. Cell. Biol.* **14**, 1-9.

Sato, T., Irie, S., Kitada, S. and Reed, J. C. (1995). FAP-1: a protein tyrosine phosphatase that associates with Fas. *Science* **268**, 411-415.

Sharama, E. and Lombroso, P. J. (1995). A neuronal protein tyrosine phosphatase induced by nerve growth factor. *J. Biol. Chem.* **270**, 49-53.

Shultz, L. D., Schweitzer, P. A., Rajan, T. V., Yi, T., Ihle, J. N., Matthews, R. J., Thomas, M. L. and Beier, D. R. (1993). Mutations at the murine *motheaten* locus are within the hematopoietic cell protein-tyrosine phosphatase (Hcph) gene. *Cell* **73**, 1445-1454.

Slack, J. M. W. (1995). Developmental biology of the pancreas. *Development* **121**, 1569-1580.

Stoker, A. W. (1994). Isoforms of a novel cell adhesion molecule-like protein tyrosine phosphatase are implicated in neural development. *Mech. Dev.* **46**, 201-217.

Teitelman, G., Alpert, S., Polak, J. M., Martinez, A. and Hanahan, D. (1993). Precursor cells of mouse endocrine pancreas coexpress insulin, glucagon and the neuronal proteins tyrosine hydroxylase and neuropeptide Y, but not pancreatic polypeptide. *Development* **118**, 1031-1039.

Tian, S. S., Tsoulfas, P. and Zinn, K. (1991). Three receptor-linked protein-tyrosine phosphatases are selectively expressed on central nervous system axons in the *Drosophila* embryo. *Cell* **67**, 675-685.

Tsui, H. W., Siminovitch, K. A., de Souza, L. and Tsui, F. W. (1993). *Motheaten* and *viable motheaten* mice have mutation in the haematopoietic cell phosphatase gene. *Nat. Gen.* **4**, 124-129.

van der Geer, P., Hunter, T. and Lindberg, R. A. (1994). Receptor protein-tyrosine kinases and their signal transduction pathways. *Annu. Rev. Cell Biol.* **10**, 251-337.

von Heijne, G. (1984). How signal sequences maintain cleavage specificity. *J. Mol. Biol.* **173**, 243-251.

Walton, K. M. and Dixon, J. E. (1993). Protein tyrosine phosphatase. *Annu. Rev. Biochem.* **62**, 101-120.

Wang, H., Yan, H., Canoll, P. D., Silvennoinen, O., Schlessinger, J. and Musacchio, J. M. (1995). Expression of receptor protein tyrosine phosphatase-σ (RPTP-σ) in the nervous system of the developing and adult rat. *J. Neurosci. Res.* **41**, 297-310.

Wessells, N. K. and Cohen, J. H. (1967). Early pancreas organogenesis: morphogenesis, tissue interactions, and mass effects. *Dev. Biol.* **15**, 237-270.

Wilkinson, D. G. (1992). *In situ Hybridization : a Practical Approach*, New York: Oxford University Press.

Wilkinson, D. G. and Nieto, M. A. (1993). Detection of messenger RNA by in situ hybridization to tissue sections and whole mounts. *Meth. Enzymol.* **225**, 361-373.

Yan, H., Grossman, A., Wang, H., D'Eustachio, P., Mossie, K., Musacchio, J. M., Silvennoinen, O., Schlessinger, J. (1993). A novel receptor tyrosine phosphatase-σ that is highly expressed in the nervous system. *J. Biol. Chem.* **268**, 24880-24886.

Yang, X., Seow, K. T., Bahri, S. M., Oon, S. H. and Chia, W. (1991). Two *Drosophila* receptor-like tyrosine phosphatase genes are expressed in a subset of developing axons and pioneer neurons in the embryonic CNS. *Cell* **67**, 661-673.

Zhang, J. S. and Longo, F. M. (1995). LAR tyrosine phosphatase receptor: alternative splicing is preferential to the nervous system, coordinated with cell growth and generates novel isoforms containing extensive CAG repeats. *J. Cell. Biol.* **128**, 415-431.

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Note added in proof

A partial amino acid sequence of PTP-NP was recently described as IA-2β and was found to be recognized by antisera of a high proportion of insulin-dependent diabetes mellitus patients (Lu et al. (1966) *Proc. Natl. Acad. Sci. USA* **93**, 2307-2311).