ZRP-1, a Zyxin-related Protein, Interacts with the Second PDZ Domain of the Cytosolic Protein Tyrosine Phosphatase hPTP1E*

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Protein-protein interactions play an important role in the specificity of cellular signaling cascades. By using the yeast two-hybrid system, a specific interaction was identified between the second PDZ domain of the cytosolic protein tyrosine phosphatase hPTP1E and a novel protein, which was termed ZRP-1 to indicate its sequence similarity to the Zyxin protein family. The mRNA encoding this protein is distributed widely in human tissues and contains an open reading frame of 1428 base pairs, predicting a polypeptide of 476 amino acid residues. The deduced protein displays a proline-rich amino-terminal region and three double finger LIM domains at its carboxyl terminus. The specific interaction of this novel protein with the second PDZ domain of hPTP1E was demonstrated both in vitro, using bacterially expressed proteins, and in vivo, by co-immunoprecipitation studies. Deletion analysis indicated that an intact carboxyl terminus is required for its interaction with the second PDZ domain of hPTP1E in the yeast two-hybrid system and suggested that other sequences, including the LIM domains, also participate in the interaction. The genomic organization of the ZRP-1 coding sequence is identical to that of the lipoma-preferred partner gene, another Zyxin-related protein, suggesting that the two genes have evolved from a recent gene duplication event.

Protein-protein interactions play a crucial role in maintaining the normal function of cells. Such interactions are important in the transmission of signals within the cell. Scaffolding, anchoring, and adaptor proteins, which bring together the various signaling molecules through such interactions, are determinant in fine-tuning these pathways and often contain modular structural domains mediating protein-protein interactions (1–3). A partial list of these domains include Src homology 2 (SH2 and SH3) domains (4), Tyr(P) binding domains (5), pleckstrin homology domain (6, 7), and PDZ domain (8, 9).

PDZ domains consist of a motif of approximately 90 amino acid residues, found in one or multiple copies in a variety of signaling proteins. This domain derives its name from the structural elements including a band 4.1 homology domain and the PDZ domains of dystrophin (11), and the epithelial tight junction protein, ZO-1 (12). Other PDZ domain-containing proteins include nitric oxide synthase (13), the Drosophila dishevelled protein, Dsh (14), the channel-interacting PDZ domain protein (15), etc. (for reviews see Refs. 9 and 16). PDZ domains have also been found in a subfamily of protein tyrosine phosphatases (PTPs), which includes PTPH1 (17), PTPMEG (18), and hPTP1E (19). The latter, also called PTP-BAS, PTPL1, and FAP-1, is a cytosolic PTPase and contains five PDZ domains in addition to a single tyrosine phosphatase catalytic domain. This protein also contains other distinct structural elements including a band 4.1 homology domain and five PEST regions (19–22). A recent study revealed that the second PDZ domain (PDZ2) of hPTP1E interacts with a sequence within the last 15 amino acids at the COOH terminus of Fas, a cell-surface receptor involved in the apoptotic pathway (22). In addition, a GTPase-activating protein (GAP) with activity toward Rho (PTPLI-associated RhoGAP 1; PARG-1) interacts with PDZ4 of hPTP1E (23). By using the yeast two-hybrid system and the PDZ2 of hPTP1E as bait, we have screened a HeLa cell cDNA library to identify other binding partners. We have cloned and characterized a novel protein (ZRP-1, zyxin-related protein-1) that interacts strongly with this domain. ZRP-1 is a 476-amino acid protein containing 3 LIM domains at its COOH terminus and a proline-rich NH2-terminal segment. The region of ZRP-1 involved in the interaction with the PDZ2 domain of hPTP1E has been characterized. The specificity as well as the in vitro and in vivo interactions of these proteins have also been demonstrated.

MATERIALS AND METHODS

Identification of Interacting Proteins Using the Two-hybrid System

The yeast two-hybrid system was employed to identify novel proteins interacting with the PDZ domains of hPTP1E. The MATCHMAKER (CLONTECH Laboratories) HeLa cell cDNA library was screened essentially following the protocol outlined by the manufacturers. All yeast transformations were performed using the lithium acetate method of Gietz et al. (24). Plasmid DNA was prepared by the method of Nasmuth and Reed (25). The inserts were amplified by PCR using the oligos GAD1F (5′-TACCACTACAATGGATGATG-3′) and M13 “Universal” primer flanking the insert in the plasmid pGADGH. The amplified PCR products were sequenced directly using an ABI377 “Prism” automated DNA sequencer.

For β-galactosidase liquid assays, the assays were done essentially as outlined (26). In brief, colonies of SFY526 containing the various constructs of ZRP-1 in pGADGH along with PDZ2 in pGBT-9 were grown overnight in Leu-, Trp− medium at 30 °C. The cells were pelleted and the cell wall disrupted with liquid nitrogen. The released β-galactosidase was assayed using O-nitrophenyl-β-D-galactopyranoside as substrate. The resultant color was measured at 420 nm and the β-galactosidase activity calculated.

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† The abbreviations used are: PTP, protein tyrosine phosphatases; PCR, polymerase chain reaction; GST, glutathione S-transferase; HA, hemagglutinin; bp, base pair; MBP, maltose-binding protein; oligo, oligonucleotide; kb, kilobase pair; GAP, GTPase-activating protein.

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Plasmid Constructs and Interaction Studies

Two-hybrid Screening—The PDZ domains of hPTP1E (PDZ1) (amino acid 1092–1184), PDZ2 (amino acid 1361–1461), and PDZ4 (amino acid 1787–1968) were amplified by PCR from a hPTP1E clone isolated previously and inserted into the BamHI site of pGBT9. A BgII site was introduced in the oligos to bring the sequence in frame with that of the GAL4 DNA binding domain. Clones with the correct orientation were selected by colony PCR using a forward oligonucleotide (5′-TACATCG-GAAAGAGTAG-3′) specific to the plasmid and an internal oligonucleotide from the hPTP1E sequence.

In Vitro Interaction Studies—To confirm in vitro the protein–protein interaction observed in the yeast two-hybrid system, the carboxy-terminal portion of ZRP-1 containing the 3 LIM domains was expressed as a fusion protein with the maltose-binding protein (MBP). The carboxyl-terminal portion (amino acids 278–476) was amplified from the cDNA clone isolated from the two-hybrid system using specific oligos containing an EcoRI and an XhoI site in the forward and reverse oligos, respectively, for cloning purposes. The fragment was inserted in frame into an EcoRI/XhoI-digested plasmid pMal-C2 (New England Biolabs). The DNA fragments encoding the PDZ domains of hPTP1E as amplified for the two-hybrid constructs were fused in frame with the glutathione S-transferase (GST) fusion protein in pGEX-3X (Amersham Pharmacia Biotech). The DNA sequences of all plasmid constructs was verified on an Applied Biosystems Inc. DNA sequencer. The GST-PDZ and the maltose-bind- ing protein-LIM (MBP-LIM) fusion proteins were expressed in DH5α and used for in vitro interactions. The proteins in the bacterial pellet from 1.5 ml of culture were solubilized in 250 μl of Buffer 1: Tris-buffered saline, 1% Nonidet P-40, 0.5% sodium deoxycholate (0.1 M NaCl), 10 μg/ml aprotinin, 10 mM benzamidine, 10 mM sodium fluoride, 10 mM potassium chloride, 5 mM magnesium chloride, and sonicated. 50 μl of diluted lysis buffer (25 μl of SDS-polyacrylamide gel electrophoresis buffer, analyzed on a 10% polyacrylamide gel, and blotted onto a nitrocellulose membrane according to standard protocols. The blots were blocked with 5% non-fat milk powder in Tris-buffered saline, 0.1% Tween 20. The immunoprecipitates obtained using anti-PDZ2 antibody were detected using anti-HA antibody (1:5000 dilution), and those precipitated by the anti-HA antibody were detected using anti-PDZ2 antibody (1:3,000 dilution). The bound first antibody was detected using horseradish peroxidase-labeled goat anti-rabbit IgG (1:5000 dilution) or goat anti-mouse IgG (1:5000 dilution), and the secondary antibody was detected using the enhanced chemiluminescence (ECL) system from Amersham Pharmacia Biotech.

Isolation of ZRP-1 cDNAs

A human breast cancer cDNA library in agt-10 (CLONTECH Laboratories) (~500,000 clones) was screened using a 367-bp PCR product containing the first and the second LIM domains of ZRP-1 as probe. The probe was labeled with [α-32P]dCTP, using the “Ready-To-Go” random labeling kit from Amersham Pharmacia Biotech. Hybridization and washes were performed according to the manufacturer’s instructions.

Isolation of cDNA by Anchored PCR

Anchored PCR was performed using the 5′- rapid amplification of cDNA ends system from Life Technologies, Inc. DNA was prepared from total RNA using random hexamer DNA primers. The reverse transcribed single-stranded cDNA was tailed with deoxythymidine (dCTP) using deoxynucleotide terminal transferase. Anchored PCR was performed essentially as described by Loh et al. (29) with a forward primer consisting of a unique sequence (the anchor sequence) followed by a series of 14 d(C) (the anchor polyG) primer) and a second forward primer containing only the anchor sequence. The reverse primer was a nested primer specific for ZRP-1. The fragments were cloned into the pGEM-T vector and sequenced. Subsequently the entire sequence was rechecked by sequencing directly both strands of PCR-generated products amplified from cDNA without subcloning the DNA fragments.

Western Blot

The proteins bound to the glutathione beads were separated by SDS-polyacrylamide gel electrophoresis and blotted onto a nitrocellulose membrane using standard protocols. The co-precipitation of the MBP-LIM fusion protein with GST-PDZ2 was detected using a polyclonal anti-MBP antibody (New England Biolabs) at a dilution of 1:10,000. The bound antibody was detected using a horseradish peroxidase-conjugated goat anti-rabbit IgG antibody (Bio-Rad) (dilution 1:5000) and the ECL system from Amersham Pharmacia Biotech.

Northern Blot

The expression of ZRP-1 mRNA was studied with a human multiple tissue Northern blot (CLONTECH Laboratories). The probe used was a 367-bp PCR fragment containing the first and part of the second LIM domains of ZRP-1 labeled with [α-32P]dCTP, using the “Ready-To-Go” random labeling kit from Amersham Pharmacia Biotech. Hybridization and washes were performed according to the manufacturer’s instructions.

Isolation and Characterization of Human ZRP-1 Genomic Sequences

The upstream and downstream regions of the human ZRP-1 gene were amplified from total genomic DNA using the Genomic Walking Kit from CLONTECH. To obtain the 5′-flanking sequence of the gene, the following gene-specific oligonucleotides were used: primer 1, (ZRP-R9) 5′-GCCCCGACATGCGCTGGAAAAG, and primer 2, (ZRP-R10) 5′-CCCCGACCTGCGCGTATTCC. The 3′ portion of the gene was obtained using the following primers: 1, (ZRP-F2) 5′-CTGAGGCCCCCTGAGGACTCC. The expression region of the gene was amplified by long PCR using the Expand DNA polymerase mix from Roche Molecular Biochemicals, with the following oligonucleotides: ZRP-F3, 5′-TACAGGCGCTTCTCCTGAGCC, and ZRP-R8, 5′-CTGAAACTGAGCCAGGGTGA. With the exception of two gaps within intron 4 and intron 7, respectively, the sequence of the entire ZRP-1 gene has been determined and deposited in GenBankTM with the following accession numbers: AF093834, AF093835, and AF093836.

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RESULTS

Identification of a Novel LIM Domain Containing Protein Interacting with the Second PDZ Domain of hPTP1E in a Yeast Two-hybrid Screen—The PDZ2 domain of hPTP1E was fused to the Gal4 DNA binding domain containing plasmid pGBT9 and used as bait to screen a HeLa cell cDNA library using the yeast two-hybrid system (30). Analysis of a total of $1.7 \times 10^7$ clones resulted in the identification of approximately 400 candidates as determined by the His$^+$ screen. Most of these were, however, eliminated upon testing for their ability to activate the β-galactosidase gene. Among the 20 positive clones, two overlapping sequences derived from the same gene were found to interact strongly with the PDZ2 domain of hPTP1E as determined by the intensity of the blue color indicator. The longest clone, C90, possessed an open reading frame of 261 amino acids and interacted more strongly than its shorter counterpart (194 amino acids open reading frame). By using the C90 cDNA sequence, a human breast cDNA library was screened, yielding 7 clones, the longest of which was 1.5 kb and contained the sequence encoding the last 432 amino acid residues. The remainder of the sequence was obtained by rapid amplification of cDNA ends-PCR performed on total RNA from HeLa cells.

The composite nucleotide sequence of the ZRP-1 cDNA was established and deposited with GenBank™ (accession number AF000974). The human ZRP-1 cDNA sequence is 1755 base pairs long and displays an open reading frame of 1428 bp with a translational initiation codon at nucleotide positions 160–162 and a stop codon at positions 1588–1590. The sequence around the ATG matches the Kozak consensus initiation sequence (31), and two stop codons are present in this reading frame within the upstream 5'-noncoding region. The open reading frame predicts a 476-amino acid polypeptide with a calculated mass of 50.3 kDa (Fig. 1). The amino-terminal sequence of the predicted protein is enriched in proline residues that account for nearly 20% of all amino acid residues. The second half of the protein contains three cysteine-rich zinc binding domains referred to as LIM domains. These domains are protein motifs of approximately 55 amino acid residues containing the consensus peptide sequence $\text{C}_X\text{C}_X\text{C}_{16-23}\text{H}_X\text{C}_X\text{C}_X\text{C}_X\text{C}_X\text{C}_X$ (C,H,D), where $X$ represents any amino acid (32–36). Alignments of the LIM domain sequences of ZRP-1 and those of the related proteins of the Zyxin family of proteins that includes Zyxin (37, 38), LPP (39), and Enigma (40) are shown in Fig. 2.

Despite the fact that all four proteins possess a proline-rich amino-terminal region, their actual amino acid sequences differ significantly in this region. The region of highest homology is that comprising the three LIM domains. Indeed, within this region, ZRP-1 displays 71.9 and 57.5% identity with LPP and Zyxin, respectively. Of the three LIM domain, LIM3 is the most conserved, showing 77% identity between ZRP-1 and the corresponding region of LPP. The similarity between ZPP and ZRP-1 is also evident when the genomic organization of the two genes is compared. As shown in Fig. 3, the ZRP-1 gene coding region consists of 9 exons distributed over approximately 5 kilobases of DNA. As for the LPP protein, the amino-terminal proline-rich region of ZRP-1 is encoded by 5 exons, and the first two LIM domains are encoded by separate exons, whereas the third LIM domain is coded for by the last two exons. Table I summarizes the nucleotide sequences of the splice sites. Sequence comparisons of the ZRP-1 and the LPP genes revealed that the locations of the splice sites are identical suggesting that the two genes result from a recent gene duplication event.

The tissue distribution of the ZRP-1 mRNA was examined by Northern blot analysis (Fig. 4). A strong signal was observed in most tissues including heart, placenta, lung, liver, kidney, and pancreas. A weaker signal was obtained in brain and skeletal muscle. The observed size of the message is approximately 2 kb suggesting that the full-length sequence has been cloned.
ZRP-1 and the PDZ2 of hPTP1E, its interaction was tested with other PDZ domains of hPTP1E. The cysteine and histidine residues characteristic of LIM domains are in **bold letters**, and the conserved amino acids are **shaded**. A conserved alanine at position –6 with respect to the conserved histidine is **underlined**.

![Diagram](image)

**Fig. 2.** Sequence comparison of the LIM domains of ZRP-1 with those of human LPP, Zyxin, and Enigma. The cysteine and histidine residues characteristic of LIM domains are in **bold letters**, and the conserved amino acids are **shaded**. A conserved alanine at position –6 with respect to the conserved histidine is **underlined**.

**Fig. 3.** Genomic organization of the human ZRP-1 gene. The various domains of the ZRP-1 protein are shown above a schematic representation of the human ZRP-1 locus. The **boxes** represent the 9 exons, and the **shaded** portions correspond to the coding sequence.

**TABLE I**

| Exon | Size (bp) | Splice donor | Splice acceptor | Intron Size (bp) |
|------|----------|--------------|-----------------|-----------------|
| 1    | ~240     | gtaagggcccttgtg. . . . | cc CAC GCA G | 254 |
| 2    | 138      | gtaagggcccttgtg. . . . | cag CAC AGG CAG | 119 |
| 3    | 128      | gtaagggcccttgtg. . . . | gacgccctgccctct. . . | 261 |
| 4    | 472      | gtaagggcccttgtg. . . . | gacgccctgccctct. . . | 884 |
| 5    | 372      | gtaagggcccttgtg. . . . | gacgccctgccctct. . . | 700 |
| 6    | 170      | gtaagggcccttgtg. . . . | gacgccctgccctct. . . | 425 |

ZRP-1 and the PDZ2 of hPTP1E, its interaction was tested with other PDZ domains of hPTP1E. The PDZ domains of ZRP-1 (amino acids 1092–1184), 4, and 5 (amino acids 1787–1968) of hPTP1E were cloned into pGBT9 in frame with the DNA binding domain of GAL4 and co-transformed with ZRP-1 (in pGADGH) into SFY526. Neither of the domains activated the β-galactosidase gene, suggesting a lack of interaction (data not shown). Additionally, no interaction was observed when the PDZ domains of two related PTPases, PTPH1 (17) and PTPMEG (18), were assayed under the same conditions (results not shown), thus substantiating the specificity of the interaction of ZRP-1 with the PDZ2 of hPTP1E.

To determine the contribution of the various domains of ZRP-1 and that of its carboxyl-terminal residues to its interaction with the PDZ2 domain of hPTP1E, several constructs containing various combinations of LIM domains were prepared in the plasmid pGADGH (Fig. 5A). The yeast strain SFY526 was co-transformed with these LIM constructs and the PDZ2-containing plasmid. The yeast colonies obtained after transformation were plated, and the intensity of interaction was assessed by both filter-lift and liquid β-galactosidase assays. The results obtained from filter-lift assays are presented in Fig. 5A. An interaction was observed only in constructs containing the carboxyl-terminal fragment of ZRP-1 (constructs 1–4). No interaction was observed with the other constructs suggesting that an intact carboxyl terminus is required for this interaction. The intensity of the interaction was dependent on the number of LIM domains present, being strongest in the presence of all three LIM domains and weakest when only one LIM domain was present (compare constructs 1 and 4). Clone C90 (the longest clone obtained from the two-hybrid screen), which contains 63 extra amino acids in addition to the...
three LIM domains and the carboxyl-terminal tail, gave the strongest signal. The variation in the intensity of interactions was not due to variations in the level of expression of the different constructs as demonstrated by a Western blot of the Gal4-activating domain fusion proteins using a Gal4AD monoclonal antibody (Fig. 5B) showing that all constructs were expressed at comparable levels. Liquid assays for β-galactosidase activity were carried out using the various LIM domain constructs, and the results are shown in Fig. 5C. The results obtained parallel those obtained with filter-lift assays and show that the β-galactosidase activity decreases with a reduction in the number of LIM domains, and the activity is close to background in the constructs containing the three LIM domains but lacking the last 11 carboxyl-terminal amino acid residues. The data suggest that in addition to the carboxyl-terminal sequence, the rest of the protein molecule may be important in stabilizing the interaction.

In Vitro and in Vivo Demonstration of Interaction between ZRP-1 and hPTP1E—The interaction of the PDZ2 of hPTP1E with ZRP-1 was confirmed by both in vitro and in vivo studies. For in vitro studies the PDZ domain was expressed as a GST fusion protein (GST-PDZ), and the carboxyl-terminal portion containing the LIM domains of ZRP-1 (amino acid residues 278–476) was expressed as a maltose-binding protein fusion (MBP-LIM). Both fusion proteins were induced with isopropyl-D-galactopyranoside, and the results are shown in Fig. 5C. The deletion of the last cysteine residue of ZRP-1 did not prevent the interaction of the two proteins (Fig. 5A, lane 2). The deletion of the carboxyl-terminal 11 amino acid residues, however, completely abolished the interaction (Fig. 5A, lane 4). Lanes 1 and 5 correspond to controls with no HA-ZRP transfected (−) and after transfection of the cells (+) but immunoprecipitated with preimmune serum. To demonstrate the level of transiently expressed HA-ZRP in these cells, an aliquot of each cell lysate was analyzed using an anti-HA antibody (Fig. 5B). A distinct band in the 27-kDa region was observed only in the HA-ZRP sample. The other bands observed in the negative control represent the background of the HA antibody used.

The reciprocal experiment, i.e., the ability of the transiently expressed HA-ZRP to immunoprecipitate endogenous hPTP1E, was also evaluated (results not shown). HA-ZRP was immunoprecipitated using anti-HA antibody, and the presence of hPTP1E in the precipitate was assayed using anti-PDZ2 antibody. A clear band in the 275-kDa region was detected in the sample prepared from the HA-ZRP-expressing cells with no distinct protein band in the pcDNA3 control. The results of these studies clearly demonstrate that ZRP-1 interacts with hPTP1E in vivo.

Finally, since an earlier study (22) had shown that the cytoplasmic tail of the membrane receptor protein Fas interacts with PDZ2 of hPTP1E (FAP-1), we were concerned that none of the clones isolated in our screenings corresponded to the Fas sequence. To verify the presence of the Fas sequence in our HeLa cDNA library, we used PCR. This experiment demonstrated that Fas cDNAs were indeed present in the library (results not shown). We then cloned the carboxyl-terminal part of Fas, amino acid residues 219–319 (41), as used by Sato et al. (22) in pGADH and studied its interaction with PDZ2 (pGADT9). Under these conditions, both filter-lift and liquid β-galactosidase assays suggested that this interaction was much weaker in comparison to that elicited by ZRP-1 (Fig. 8 and results not shown). Indeed, under our assay conditions, the strength of the interaction with Fas was roughly one-tenth of the intensity of the interaction observed with ZRP-1.

**DISCUSSION**

By using the second PDZ domain of hPTP1E as a bait in the yeast two-hybrid system, we have isolated a novel protein (ZRP-1) that interacts strongly with PDZ2 of hPTP1E. ZRP-1 contains three cysteine-rich zinc binding LIM domains. These latter domains are themselves protein interacting modules present in a large number of proteins with diverse functions (32–35). ZRP-1 belongs to a group of LIM proteins which includes Zyxin, a member of the cell adhesion complex (37, 38, 42), LPP, a preferred fusion partner of HMG1C in lipomas (39), and Enigma (40). The LIM domains of ZRP-1 display a high degree of sequence similarity with two proteins belonging to this group, namely LPP (39) and Zyxin (42). These proteins each contain three carboxyl-terminal LIM domains that represent the region of highest similarity between them. The overall identity between ZRP-1 and LPP in the three LIM domains is 71.9%, with the highest identity being within the last two LIM domains (77%). A similar pattern was observed between ZRP-1 and Zyxin. The overall identity to ZRP-1 in the three LIM domains is 57.5%, with the highest identity being within the last two LIM domains, 61.5% in LIM-2 and 64.5% in LIM-3. These three proteins also possess a proline-rich NH2-terminal region. However, the identity be-

![Fig. 4. Tissue distribution of ZRP-1 mRNA. A human multiple tissue Northern blot (CLONTECH) was probed with a 32P-labeled 367-bp fragment containing the first and part of the second LIM domains of ZRP-1.](image-url)
From the crystal structures of the peptide bound and free PDZ3 domains of PSD-95 (44) and the human homologue of Drosophila Dlg, DlgA (45), it was determined that the carboxy-terminal sequence (S/T)XV in the PDZ domain binding protein was involved in the interactions. In keeping with the hypothesis that the carboxy-terminal fragment of ZRP-1 may be involved in the interaction with PDZ2 of hPTP1E, we observed no interaction in the absence of the carboxy-terminal 11 amino acid residues of ZRP-1, in both yeast or mammalian cells. The carboxy-terminal sequence of ZRP-1, VTTDC-COOH, is significantly different from the consensus sequence of (S/T)XV originally suggested to be the required peptide motif at the carboxyl terminus for interaction with PDZ domains (44–45). By using oriented peptide libraries, Songyang et al. (46) have suggested that the carboxy-terminal requirement for interaction with the PDZ2 of hPTP1E is -(E/V)(T/S)XVTCOOH. This library was biased since it did not contain any cysteine or tryptophan residues at the COOH terminus. ZRP-1 on the other hand contains a cysteine at the COOH terminus. This again is different from the sequence (S/T)XV-COOH suggested to be the conserved sequence required for interaction with PDZ domains. These observations would further suggest that other carboxy-terminal sequences could also define the specificity for particular PDZ domains. Deletion of the last cysteine residue abolished the interaction in the yeast two-hybrid system where the specific interaction of hPTP1E PDZ2 domain and the ZRP-1 protein is assayed. This suggests that the carboxy-terminal amino acid residue is indeed involved in the interaction. However, removal of this last cysteine residue did not affect the interaction of ZRP-1 with endogenous hPTP1E. This apparent inconsistency could result from several factors. It is indeed conceivable that in mammalian cells, the constraints for the interaction of the two proteins are different than those in the yeast cells and that the presence of the complete hPTP1E protein stabilizes the interaction. Finally, by deleting the last cysteine residue from ZRP-1, a novel carboxyl terminus is created whose sequence (. . . VTTDC-COOH) may represent a novel PDZ-binding motif capable of interacting with any one of the five hPTP1E PDZ domains. Further studies will be necessary to clarify these observations.

Similar observations have been made recently (48) in the course of a study of the interaction of a LIM domain-containing protein, RIL, with PTP-BL, the murine homologue of the human hPTP1E. The RIL protein interacts with both the PDZ2 and PDZ4 domains. The carboxy-terminal sequence of RIL.
contains the sequence VELV-COOH, which does not match with either the consensus (S/T)XV-COOH or the sequences -E(T/T)S/X(V/I)-COOH (for PDZ2) and -(I/Y/V)YYV-COOH (for PDZ4) identified by Songyang et al. (46). For the interaction of RIL with PDZ4 to occur, the carboxy-terminal sequence is required. In addition, the interaction is much stronger in the presence of the LIM domain, an observation similar to that seen with ZRP-1. These observations are consistent with the concept of the LIM domains also playing an important role in stabilizing the interaction with the PDZ domain of hPTP1E. The importance of upstream peptide sequences in the interactions with PDZ domains has also been suggested in another study with neuronal nitric oxide synthase (47). These authors observed that the preference of Asp at position -2 is determined by tyrosine 77 of neuronal nitric oxide synthase. Mutation of Tyr-77 and Asp-78 to His-77 and Glu-78 results in a change in the specificity from Asp-Xaa-Val to Thr-Xaa-Val. The fusion proteins were expressed in bacteria and assayed for interaction as outlined under “Materials and Methods.” The protein complex was precipitated using glutathione-Sepharose beads.

The binding of ZRP-1 to the PDZ domain of hPTP1E is much stronger than the binding of Fas to the same PDZ domain via its consensus (S/T)XV carboxy-terminal sequence. The biological significance of this observation remains to be established.

The broad tissue distribution of ZRP-1, as demonstrated by Northern analysis and reflected by the large number of related sequences in the EST data base (NCBI), suggests a ubiquitous role for this protein in cellular function. A search through the GenBank™ data base revealed that ZRP-1 was identical to a part of the TRIP6 sequence (GenBank™ accession number L40374) (55), identified as a thyroid receptor interacting protein. This sequence contained only two LIM domains and the carboxy-terminal portion of the protein. However, these authors suggested that it would be unlikely that TRIP6 is involved in thyroid receptor function and that its similarity to Zyxin probably reflects a common subcellular localization. In the course of the preparation of this manuscript, the complete sequence of the human TRIP6 mRNA was reported (GenBank™ accession number AJ001902) (56). The sequence of this gene product is identical to that of ZRP-1. The gene has been assigned to a segment of human chromosome 7q22 between the erythropoietin and the plasminogen activator inhibitor-1 precursor genes (56). This region of chromosome 7 is often deleted in malignant myeloid diseases and uterine leiomyoma. Since the molecular mechanisms of these diseases are not yet clearly understood, the involvement of ZRP-1 in these cancers needs to be evaluated.

Group 3 LIM proteins, to which ZRP-1 belongs, have been shown to be involved in a number of interactions involving both the LIM domains and the non-LIM domain portion of the molecule. In the case of Zyxin, the first LIM domain binds cysteine-

Fig. 6. In vitro interaction of PDZ2 with the carboxy-terminal LIM domain containing region of ZRP-1. The PDZ2 domain of hPTP1E and the carboxy-terminal region of ZRP-1 containing all three LIM domains were expressed as a GST fusion and MBP fusion protein, respectively. The fusion proteins were expressed in bacteria and assayed for interaction as outlined under “Materials and Methods.” The protein complex was precipitated using glutathione-Sepharose beads. The proteins bound to the beads were analyzed by Western blot (WB) analysis using an anti-maltose binding protein antibody. Lanes are labeled as follows: 1, GST-PDZ2 + MBP-LIM; 2, MBP-LIM alone; 3, GST-PDZ2 alone; and 4, GST + MBP-LIM.

Fig. 7. In vivo interaction of the carboxy-terminal portion of ZRP-1 with hPTP1E. ZRP-1 was expressed as a HA-tagged protein in 293-T cells and used for immunoprecipitation studies. The co-precipitation of ZRP-1 with PDZ2 of hPTP1E is shown in A. The endogenous hPTP1E was precipitated with PDZ2 antibody, and the co-precipitated HA-ZRP-1 was detected by anti-HA antibody. The co-immunoprecipitation of HA-ZRP-1 with hPTP1E is shown in lane 2. Immunoprecipitation experiments using the AC476 construct is shown in lane 3 and that with 4 amino acids (Saa) 466–476 is presented in lane 4. The background of the system is represented by lane 1 with no HA-ZRP-1 transfected and lane 5 for which the preimmune serum was used. B, the amount of HA-LIM proteins expressed after transfection of the various constructs is shown. WB, Western blot.
rich protein, and its proline-rich amino-terminal region binds α-actin (57) as well as the human proto-oncogene product VAV, an SH3 adaptor protein (58). Enigma, another member of this group, recognizes the active endodycopic codes of the insulin receptor, a region characterized by two tyrosine-containing tight turn motifs through the third LIM domain (40) and the receptor tyrosine kinase, Ret, through its second LIM domain (59). Furthermore, the mitogenic signaling by Ret/ptc2, a papillary thyroid cancer oncogene product, was shown to require the association with Enigma via the LIM 2 domain (60). In addition to the interactions with heterologous regions of proteins, LIM domains have been shown to function as protein dimerization domains. For example, LIM domains of cysteine-rich protein can efficiently homodimerize (61). The various dimerization domains. For example, LIM domains have been shown to function as protein interaction modules. In a recent study of this type, the Zyxin and Enigma domains showed significant similarity indicating a common origin by gene duplication. Interestingly, there are also major differences between the two genes. Whereas the LPP gene is located on chromosome 3, between 3q27 and 3q28 (39), the ZRP-1 gene is located on chromosome 7 (56). The major difference between the two genes resides, however, in their respective size. Whereas ZRP-1 sequence is contained within a 6-kilobase segment of genomic DNA, the LPP gene was estimated to be dispersed over at least 400 kb of genomic DNA (39).

In conclusion we have identified a novel protein that interacts strongly with PDZ2 of hPTP1E. This protein also contains other potential protein interacting modules making it a potential scaffolding protein. Identification of other proteins interacting with the various domains of hPTP1E and of ZRP-1 is required for a better understanding of the role of these proteins in cellular function.

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