Cloning and characterization of MUPP1, a novel PDZ domain protein

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Abstract Using the yeast two-hybrid system we isolated a cDNA clone encoding a novel protein interacting with the C-terminal domain of the 5-HT_{2C} receptor. The protein, named MUPP1 (multi-PDZ-domain protein), contains thirteen PDZ domains and no obvious catalytic domain; it is related to hINADL and a putative *C. elegans* polypeptide referred to as C52A11.4 containing six or ten PDZ domains, respectively. Domains highly similar to those of MUPP1 are arrayed in the same order in all three proteins. The MUPP1 gene is localized on human chromosome 9p24-p22. Transcripts encoding MUPP1 are abundant in the brain as well as in several peripheral organs.

Key words: PDZ domain; Yeast two-hybrid; Protein-protein interaction; Scaffold protein; Serotonin receptor

1. Introduction

Using the yeast two-hybrid system it has been shown that the postsynaptic density protein PSD-95 interacts with cytoplasmic tail sequences of ion channel subunits, including the NR2 subunits of the NMDA receptor and Shaker-type K^+ channels, via a novel protein-protein motif, the PDZ domain [1–3]. PSD-95 belongs to a family of structurally related receptor interacting proteins which are characterized by three PDZ domains in the N-terminal half and a SH3 and guanylate kinase-like domain in the C-terminal region. Due to the presence of the putative guanylate kinase domain, these proteins are also referred to as the MAGUK (membrane-associated guanylate kinase) superfamily. In mammals, this family includes PSD-95/SAP90 [4,5], SAP97/hdlg [6,7], PSD-93/chapsyn-110 [8,9], and SAP102 [10]. PSD-95 colocalizes with NMDA receptors and Shaker-type K^+ channels in rat central neurons and causes clustering when coexpressed in heterologous cells [1,11]. Evidence for an ion channel clustering function in vivo has been provided by genetic analysis of the MAGUK member Discs large (Dlg) in *Drosophila melanogaster*. Dlg also binds and clusters Shaker channels in vitro and colocalizes in larval neuromuscular junctions. In Dlg mutants, however, the synaptic clustering of Shaker channels is abolished [12] arguing that the members of the MAGUK proteins are directly involved in the localization and clustering of ion channels at synaptic sites. Recently, the C-terminus of AMPA receptors was shown to interact with a PDZ domain of a novel protein called GRIP, glutamate receptor interacting protein [13]. GRIP contains seven PDZ domains and is structurally not related to MAGUK. Interestingly, the concept of receptor C-terminal interactions with PDZ domains also extends to G-protein coupled receptors. Homer, a newly identified dendritic protein, specifically binds to the C-terminus of metabotropic glutamate receptors, mGluR1a and mGluR5, via its single PDZ-like domain [14].

The C-termini of the NMDA receptor and K^+ channel subunits share a common element, referred to as the t/SXV* (where asterisk indicates a carboxyl group) motif [2,1] which interacts with the PDZ domains of PSD-95. The t/SXV* motif is also present in C-termini of various G-protein coupled receptors [2] including the 5-HT_2 receptor subfamily comprising 5-HT_{2A}, 5-HT_{2B} and 5-HT_{2C} receptors [15]. We hypothesized that the 5-HT_2 receptor subfamily may also be associated with PDZ domain-containing proteins via its C-terminal tail region. In this study, we isolated a cDNA clone encoding a novel member of PDZ domain-containing proteins called MUPP1 (multi-PDZ-domain protein) interacting with the C-terminal 5-HT_{2C} receptor polypeptide in yeast.

2. Materials and methods

2.1. Yeast two-hybrid system

The coding sequence for the C-terminal 90 (residues 369–458) amino acids of the human 5-HT_{2C} receptor was cut out from the plasmid pXMD1-hu2C [16] *Acc*I-*Eco*RI and ligated into the blunt *Nde*I-*Eco*RI site of the yeast vector pAS2-1 in frame with the GAL4 DNA binding domain. Plasmid DNA for yeast transformation was purified by two subsequent CsCl gradients. Yeast two-hybrid screening was performed using the CG1945 strain harboring the reporter genes HIS3 and β-galactosidase under the control of upstream GAL4 binding sites [32]. The yeast culture was transformed with the bait plasmid pAS2-1/hu2C and selected on medium lacking tryptophan. The transformed yeast cells were used for a sequential transformation with a human fetal brain cDNA library (Clontech) harboring 5×10^6 independent clones. The calculated transformation efficiency was also 5×10^6. Positive clones were selected on triple minus plates (−Trp, −Leu, −His) and 5 days after transformation assayed for β-galactosidase activity. False positive clones were eliminated by cotransforming either the bait vector pAS2-1 or the original pACT2 vector into yeast. Isolated plasmids from positive yeast colonies were re-introduced into yeast and again assayed for HIS3 and β-galactosidase activity.

2.2. cDNA cloning

The plasmid pACT2/huMUPP1 was digested *Eco*RI/*Xho*I releasing an insert of 1778 bp which was radiolabelled and used to screen a rat brain cDNA library [17]. Screening approximately 1×10^6 colonies [18], we identified 26 positives, which were further analyzed by Southern blot of *Sal*I-digested DNA minipools. The largest clone isolated contained an insert of 5348 bp (pXMD1/rMUPP1). Sequence analysis revealed an open reading frame of 4178 bp and a 3′ end of 1170 bp. The 5′ end was obtained by sequential 5′ RACE steps using the 'Marathon' ready rat brain cDNA (Clontech).

2.3. Northern blot

A human multiple tissue Northern blot (Clontech) with 2 μg poly(A)$^+$ RNA per lane was hybridized to random primed radiolabelled DNA (Boehringer Mannheim). The plasmid pACT2/MUPP1 was digested with *Bam*HI to release a 669 bp (corresponding to nucleotide 4797–5469 of the rat cDNA) fragment. Fifty ng were radio-

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labelled with [α-^{32}P]dCTP to a specific activity of 1×10^9 counts per μg. The blot was hybridized for 2 h in ExpressHyb solution (Clontech). The filter was washed for 40 min with 2×SSC, 0.05% SDS at room temperature, and then 40 min with 0.1×SSC, 0.1% SDS at 50°C and exposed for 3 days at −80°C to an X-ray film.

2.4. Radiation hybrid and somatic cell hybrid mapping

Primers AAT GCA CTG GTC CTG ACA AT (plus strand) and AAT GAT GTT AGG TTG TCA GT (minus strand) comprise nucleotides 1429–1448 and 1534–1555 of the human MUPP1 nucleotide sequence (EMBL accession number AJ001319). A PCR-product was obtained with human genomic DNA but not with hamster or mouse DNA. PCR was performed in 50 μl with 100 ng DNA, 10 pmol of each primer, 1U Taq-polymerase (Boehringer Mannheim), 0.2 mM of each dNTP and 1 μCi [α-^{32}P]dCTP with 36 cycles of 55°C for 30 s, 70°C for 30 min and 94°C for 1 min.

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          5        10        15        20        25        30        35        40        45        50
   1 M L E T I D K N R A L Q A A E R L Q S K L K E R G D V A N E D K L S L L K S V L Q S P L F S Q I L S
  51 L Q T S L Q Q L K D Q V N V A T L A T A N A D H A H T P Q F S S A I I S N L Q S E S L L L S P S N G
 100 N L E A I S G P G A P P A M D G K P A C E E L D Q L I K S M A Q G R H V E I F E L L K P P C G G L G
 151 F S V V G L R S E N R G E L G I F V Q E I Q E G S V A H R D G R L K E T D Q I L A I N G Q V L D Q T  1
 201 I T H Q Q A I S I L Q K A K D T I Q L V I A R G S L P H I S S P R I S R S P S A A S T V S A H S N P
 251 T H W Q H V E T I E L V N D G S G L G F G I I G G K A T G V I V K T I L P G G V A D Q H G R L C S G
 301 D H I L K I G D T D L A G M S S E Q V A Q V L R Q C G N R V K L M I A R G A V E E T P A P S S L G I  2
 351 T L S S S T S T S E M R V D A S T Q K N E E S E T F D V E L T K N V Q G L G I T I A G Y I G D K K L
 401 E P S G I F V K S I T K S S A V E L D G R I Q I G D Q I V A V D G T N L Q G F T N Q Q A V E V L R H  3
 451 T G Q T V R L T L M R K G A S Q E A E I T S R E D T A K D V D L P A E N Y E K D E E S L S L K R S T
 501 S I L P I E E E G Y P L L S T E L E E T E D V Q Q E A A L L T K W Q R I M G I N Y E I V V A H V S K
 551 F S E N S G L G I S L E A T V G H H F I R S V L P E G P V G H S G K L F S G D E L L E V N G I N L L  4
 601 G E N H Q D V V N I L K E L P I D V T M V C C R R T V P P T A L S E V D S L D I H D L E L T E K P H
 651 I D L G E F I G S S E T E D P M L A M S D V D Q N A E E I Q T P L A M W E A G I Q A I E L E K G S R
 701 G L G F S I L D Y Q D P I D P A N T V I V I R S L V P G G I A E K D G R L F P G D R L M F V N D I N  5
 751 L E N S T L E E A V E A L K G A P S G M V R I G V A K P L P L S P E E G Y V S A K E D T F L C S P H
 801 T C K E M G L S D K A L F R A D L A L I D T P D A E S V A E S R F E S Q F S P D N D S V Y S T Q A S
 851 V L S L H D G A C S D G M N Y G P S L P S S P P K D V T N S S D L V L G L H L S L E E L Y T Q N L L
 901 Q R Q H A G S P P T D M S P A A T S G F T V S D Y T P A N A V E Q K Y E C A N T V A W T P S Q L P S
 951 G L S T T E L A P A L P A V A P K Y L T E Q S S L V S D A E S V T L Q S M S Q E A F E R T V T I A K
1001 G S S S L G M T V S A N K D G L G V I V R S I I H G G A I S R D G R I A V G D C I L S I N E E S T I  6
1051 S L T N A Q A R A M L R R H S L I G P D I K I T Y V P A E H L E E F R V S F G Q Q A G G I M A L D I
1101 F S S Y T G R D I P E L P E R E E G E G E E S E L Q N A A Y S S W S Q P R R V E L W R E P S K S L G
1151 I S I V G G R G M G S R L S N G E V M R G I F I K H V L E D S P A G K N G T L K P G D R I V E V D G  7
1201 M D L R D A S H E Q A V E A I R K A G S P V V F M V Q S I V N R P R K S P L P S L P H S L Y P K C S
1251 F S S T N P F A E S L Q L T S D K A P S Q S E S E S E K A T L C S V P S S S P S V F S E M S S D Y A
1301 Q P S A T T V A E D E D K E D E F G Y S W K N I Q E R Y G T L T G Q L H M I E L E K G H S G L G L S
1351 L A G N K D R T R M S V F I V G I D P T G A A G R D G R L Q I A D E L L E I N G Q I L Y G R S H Q N  8
1401 A S S I I K C A P S K V K I I F I R N A D A V N Q M A V C P G S A A D P L P S T S E S P Q N K E V E
1451 P S I T T S A S A V D L S S L T N V Y H L E L P K D Q G G L G I A I C E E D T L N G V T I K S L T E
1501 R G G A A K D G R L K P G D R I L A V D D E L V A G C P I E K F I S L L K T A K T T V K L T V G A E  9
1551 N P G C Q A V P S A A V T A S G E R K D S S Q T P A V P A P D L E P I P S T S R S S T P A I F A S D
1601 P A T C P I I P G C E T T I E I S K G Q T G L G L S I V G G S D T L L G A I I I H E V Y E E G A A C
1651 K D G R L W A G D Q I L E V N G I D L R K A T H D E A I N V L R Q T P Q R V R L T L Y R D E A P Y K 10
1701 E E D V C D T F T V E L Q K R P G K G L G L S I V G K R N D T G V F V S D I V K G G I A D A D G R L
1751 M Q G D Q I L M V N G E D V R N A T Q E A V A A L L K C S L G T V T L E V G R I K A A P F H S E R R 11
1801 P S Q S S Q V S E S S L S S F S L P R S G I H T S E S S E S S A K K N A L A S E I Q G L R T V E I K
1851 K G P A D A L G L S I A G G V G S P L G D V P I F I A M M H P N G V A A Q T Q K L R V G D R I V T I 12
1901 C G T S T D G M T H T Q A V N L M K N A S G S I E V Q V V A G G D V S V V T G H Q Q E L A N P C L A
1951 F T G L T S S T I F P D D L G P P Q S K T I T L D R G P D G L G F S I V G G Y G S P H G D L P I Y V
2001 K T V F A K G A A A E D G R L K R G D Q I I A V N G Q S L E G V T H E E A V A I L K R T K G T V T L 13
2051 M V L S  2054
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Fig. 1. Deduced amino acid sequence of the rat MUPP1 cDNA. MUPP1 is a 2054 amino acid protein that contains thirteen PDZ domains and no obvious catalytic domain. The PDZ domains are shaded and are numerically marked to the right.

3. Results

3.1. Yeast two-hybrid screen with the C-terminus of the 5-HT_{2C} receptor

To identify proteins that interact with the C-terminus of the human 5-HT_{2C} receptor we used the C-terminal 90 amino acids as bait to screen a human fetal brain cDNA library using the yeast two-hybrid system [21]. Two identical clones were isolated containing a 1.8 kb partial cDNA (clone 1; EMBL accession number AJ001319) encoding a 454 amino acid peptide. Searching the sequence database identified a partial mouse cDNA referred to as 9BP-1 [22] (GenBank accession number AF000168) displaying 93.5% similarity to clone 1 on the protein level. Clone 1 was then used to isolate the rat homologue from a rat brain cDNA library [17]. The 5′ end of 2166 bp was obtained by sequential RACE steps. The assembled full-length rat cDNA (EMBL accession number AJ001320) has an overall length of 7516 bp, and encodes a protein of 2054 amino acids (Fig. 1) exhibiting 96.6% similarity to the partial human amino acid sequence. The inferred rat protein sequence contains thirteen PDZ domains separated by segments of unique protein sequence and no obvious catalytic domain (Fig. 2A). Therefore we have called this protein MUPP1 for multi-PDZ-domain protein 1. Multiple sequence alignment of all thirteen PDZ domains (Fig. 2B) contained within the MUPP1 protein sequence indicated that each domain contains the structural characteristics of a prototypical PDZ motif [23,24], including the conservation of most residues thought to be important for target binding [23].

3.2. Sequence and structural similarities

Database searches revealed similarities to other PDZ domain-containing proteins. The most significant score was obtained for the recently cloned human INADL (INAD-like) protein [25] and to a lesser extent for the putative polypeptide C52A11.4 in *C. elegans* [19], previously described as '9-PDZ' [20] where homology is restricted to PDZ domains and short adjacent regions (data not shown). The open reading frame C52A11.4 represents a genomic DNA sequence. Ten PDZ domains were identified and assembled by comparison to MUPP1 PDZ domains. Protein alignment of all PDZ domains from INADL and C52A11.4 with MUPP1 PDZ domains revealed that all three proteins share highly identical PDZ domains (Fig. 2A). Only PDZ10 of C52A11.4 showed no greater identity to a particular MUPP1 PDZ domain. Pairwise comparison revealed that the most identical PDZ domains are arrayed in the same order resulting in a similar organization in each predicted protein (Table 1). Each PDZ

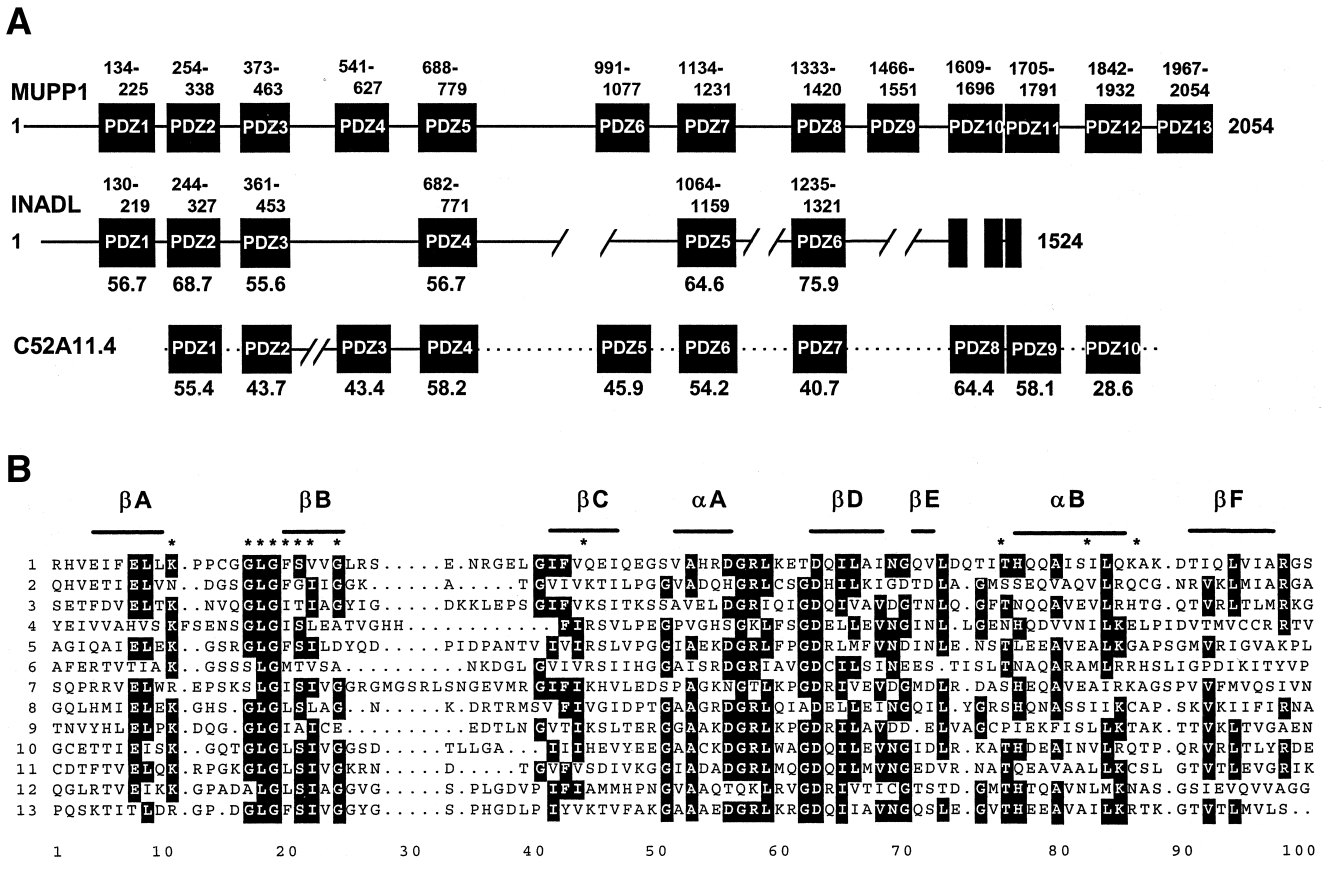

Fig. 2. A: Diagram of the domain structures of MUPP1, INADL and C52A11.4. PDZ domains are shown as closed boxes. The PDZ organization of human INADL protein and C52A11.4 are illustrated relative to the rat MUPP1 protein structure. Numbers above indicate the amino acid position of each PDZ domain and numbers below the percent identity of INADL and C52A11.4 with the respective rat MUPP1 PDZ domain. Dotted lines indicate unknown distances between PDZ domains as the linker sequences of C52A11.4 could not be precisely predicted from the genomic DNA sequence. B: Multiple sequence alignment of all PDZ domain sequences contained within the MUPP1 protein sequence. Black boxes indicate amino acid identities that are conserved (>50%). Insertions/deletions are denoted by dots. The known secondary structure of PDZ domains is shown above the alignment [23,24]. Asterisks denote the position of residues that contact the ligand in the rat PSD-95 PDZ3 structure [23].

Table 1
Amino acid sequence identities of MUPP1 PDZ domains with MUPP1-related proteins, INADL and C52A11.4

MUPP1 PDZ	1	2	3	4	5	6	7	8	9	10	11	1213	
INADL													
PDZ1	**56.7**	34.1	33.7	23.3	28.4	29.5	26.9	29.4	25.6	38.3	35.0	29.5	38.4
PDZ2	38.6	**68.7**	40.5	27.5	33.3	34.2	28.9	32.1	32.1	33.3	44.6	31.7	34.6
PDZ3	29.8	40.0	**55.6**	26.5	21.3	30.3	37.6	18.8	29.4	29.6	30.6	25.0	28.2
PDZ4	26.9	28.0	29.2	32.1	**56.7**	26.9	29.2	30.0	31.3	31.3	32.1	26.4	32.2
PDZ5	33.0	25.6	30.3	34.7	29.2	21.6	**64.6**	28.6	29.9	38.1	29.1	33.7	33.0
PDZ6	37.2	32.5	29.9	36.7	31.0	25.0	29.7	**75.9**	28.0	34.5	33.3	26.4	32.6
C52A11.4													
PDZ1	38.6	**55.4**	35.4	30.0	30.1	25.0	31.7	32.9	33.3	28.4	39.0	24.1	35.0
PDZ2	30.7	30.9	**43.7**	27.4	23.0	31.2	32.6	28.2	26.8	28.9	29.1	25.3	32.6
PDZ3	26.2	26.3	24.2	**43.4**	25.6	20.7	27.4	28.6	26.7	29.7	26.5	24.7	30.5
PDZ4	31.8	36.3	29.9	26.6	**58.2**	22.6	28.1	35.4	31.3	33.3	40.5	20.2	33.7
PDZ5	27.7	29.5	34.5	17.9	27.7	**45.9**	19.5	28.0	22.8	27.2	34.6	25.3	34.9
PDZ6	29.5	26.8	28.1	35.0	33.8	27.8	**54.2**	26.2	23.8	32.1	30.2	34.8	38.6
PDZ7	28.6	27.2	27.2	31.9	21.9	25.0	29.0	**40.7**	19.5	27.5	27.8	26.3	21.3
PDZ8	32.9	36.1	34.5	32.1	32.2	32.1	34.5	37.9	36.4	**64.4**	39.2	32.5	46.5
PDZ9	32.9	39.8	37.3	26.3	34.5	30.4	28.4	29.8	39.0	40.0	**58.1**	22.0	22.0
PDZ10	33.8	35.6	36.7	19.5	31.6	21.1	30.4	19.0	29.5	23.8	36.7	28.6	30.3

Amino acid sequences of PDZ domains were aligned using the BestFit program (Genetics Computer Group, Inc., Madison, USA). Percentage identities over the complete amino acid sequence of each PDZ domain was calculated. The highest identity is shown in bold letters. EMBL accession numbers: INADL (human), AJ001306; C52A11.4 (*C. elegans*), Z46792. The following nucleotide positions of genomic sequences encoding PDZ domains from C52A11.4 [19,20] were identified: PDZ1 1769–1883, 2199–2275, 2661–2718; PDZ2 6577–6657, 7036–7127, 7543–7645; PDZ3 7784–8063, 8237–8244; PDZ4 8455–8522, 8833–8966, 9199–9270; PDZ5 13747–13890, 14185–14291, 14455–14467; PDZ6 17570–17772, 18037–18132; PDZ7 18895–18961, 19121–19305; PDZ8 23975–24238; PDZ9 24274–24535; PDZ10 26135–26356, 26658–26724.

domain appears to have a unique sequence identity which is highly conserved in different species (human, rat and *C. elegans*). Fig. 2A shows that the segments between each PDZ domain can display variable lengths in INADL. For the C52A11.4 protein these spacer sequences can not be precisely predicted from the genomic DNA sequence. The PDZ3 domain of INADL has not been described previously [25]. The authors also speculate about an additional PDZ domain at the C-terminus of INADL spanning amino acid residues 1433–1523. Protein alignment with MUPP1 revealed that this domain covers 24 amino acid residues of MUPP1-PDZ11 and MUPP1-PDZ10 lacking the 28 internal amino acid residues (Fig. 2A). The resulting domain does not have the characteristics of a prototypical PDZ domain.

3.3. Spatial distribution of MUPP1 transcripts

The tissue distribution of human MUPP1 mRNA was examined by Northern blot analysis. A human Northern blot hybridized with human MUPP1 (clone 1) revealed a prominent mRNA of 8.5 kb present in heart, brain, placenta, liver, skeletal muscle, kidney and pancreas, but not in lung tissue (Fig. 3). The sequences of a 5.0 kb transcript seen in heart, liver and kidney, and of a 4.0 kb transcript in brain, are too short to encode a full-length MUPP1 protein. However, it cannot be excluded that these mRNAs are alternative transcripts of MUPP1 which encode smaller but still functional variants of MUPP1. The blot was stripped and rehybridized with a probe for human β-actin to control the relative amounts of poly(A)$^+$ RNA per lane.

3.4. Chromosomal localization of MUPP1

Human MUPP1-specific oligonucleotides were used to determine the exact chromosomal location of the MUPP1 gene on the Stanford G3 radiation hybrid panel (Research Genetics). Linkage analysis was carried out using the RHMAP software package [26] to interpret the results of the radiation hybrid screen. The statistical evaluation showed strong linkage to STS markers located on chromosomes 9 and X. To determine which chromosome contains the MUPP1 gene, more PCR amplifications were performed on the UK HGMP somatic cell hybrid DNA panel [27] for the human chromosomes 1, 9, 10, 20 and X. Chromosomal MUPP1 DNA is only present in the cell line GM10611, which contains human DNA only from chromosome 9 (data not shown). The somatic cell hybrid DNAs for human chromosomes 1, 10 and

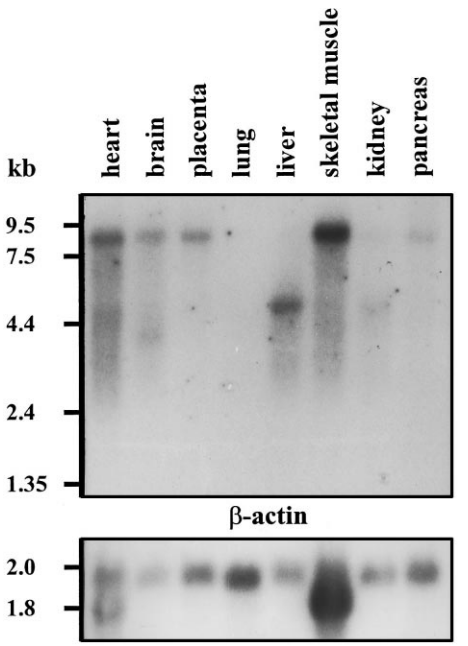

Fig. 3. Northern blot analysis of human MUPP1 mRNA expression. Human multiple tissue Northern blot with 2 μg poly(A)$^+$ RNA per lane was hybridized to a random primed ^{32}P-labelled probe of human MUPP1.

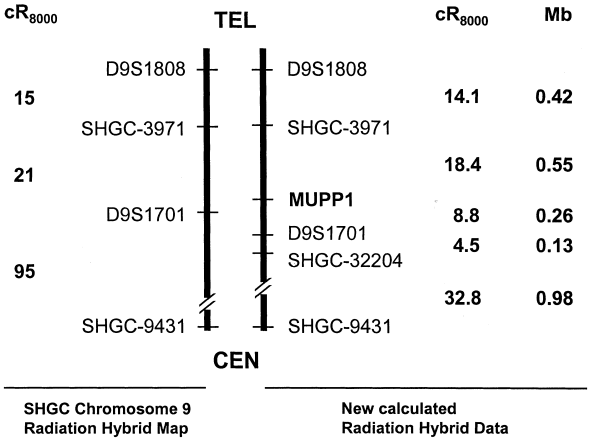

Fig. 4. Comparison of the radiation hybrid data with the SHGC chromosome 9 radiation hybrid map (G3) for the MUPP1 gene on chromosome 9p24-p22. Chromosomal regions are shown as thick lines. Distances of STS markers are given in centirays (cR_{8000}) and megabases (Mb), based on the estimate of 0.03 Mb/cR [31]. TEL, telomer; CEN, centromer.

20, all of which contain parts of human chromosome X, and the cell line HORL9X, containing exclusively human chromosome X, were negative for the human MUPP1 gene (data not shown).

The exact locus order as determined with RHMINBRK showed that the MUPP1 gene is linked to STS markers D9S1808, SHGC-3971, D9S1701 and SHGC-32204, which are located on 9p24-p22 in a total region of 78.6 cR_{8000} or approximately 2.36 Mb. Physical distances have been obtained from multipoint maximum likelihood analysis in RHMAXLIK (branch and bound ordering, equal retention model). STS markers displaying strong linkage to MUPP1 are illustrated (Fig. 4). The MUPP1 gene is located 18.4 cR_{8000} or 0.55 Mb centromeric of STS marker SHGC-3971 and 8.8 cR_{8000} or 0.26 Mb telomeric of STS marker D9S1701.

4. Discussion

This study describes the characterization of a novel cDNA, MUPP1, which codes for a protein containing thirteen PDZ domains, making it the numerical leader among the known proteins containing PDZ domains.

Structurally, MUPP1 is organized similar to the PDZ domain proteins INAD from *Drosophila melanogaster*, the proposed human homologue hINAD-like (hINADL), and GRIP, the glutamate receptor interacting protein. All three proteins have multiple PDZ domains and no obvious catalytic domain. No interacting proteins have been described for hINADL (six PDZ domains) [25] which displays highest similarities to MUPP1 in structure and amino acid sequences of the respective PDZ domains. The protein GRIP, which contains seven PDZ domains, has been shown to interact with AMPA receptor subunits GluR2 and GluR3 [13], and the protein InaD containing five PDZ domains assembles the store operated calcium channel (TRP) [28], protein kinase C and phospholipase C-β [29]. In Drosophila, InaD serves as a scaffold for components of the phototransduction cascade. A structural significance of INAD is obvious by the fact that the inactivation no after-potential D ($inaD^{215}$) mutation, which disrupts the PDZ3-TRP interaction, induces retinal degradation due to mislocation of TRP [30,29]. TRPs no longer localize to rhabdomers, but instead are found randomly distributed throughout the plasma membrane. Furthermore, TRP protein levels decline with age in $inaD^{215}$ mutants [29]. These findings suggest that INAD-like PDZ proteins might serve as multivalent adapter proteins which contribute to the formation of macromolecular complexes for signal transduction, but may also have an indirect or direct regulatory influence on the function of their target proteins.

We have shown that INADL and C52A11.4 share highly identical PDZ domains with MUPP1 which can unequivocally be assigned to a distinct MUPP1 PDZ domain in a conserved structural organization. Each PDZ domain has a unique sequence identity which is highly preserved through different species (human, rat and *C. elegans*). This suggests common interacting candidate proteins exerting similar functions within the macromolecular complex. It appears that a precisely organized arrangement of interacting proteins through ordered PDZ domains would be the principal function of multivalent adapter proteins to efficiently funnel extracellular signals into the proper receptor-activated signal transduction pathway. In light of these findings we suggest that these proteins are classed in a common family termed multi-PDZ-domain protein family (MUPP).

MUPP1 was cloned in a yeast two-hybrid screen using the C-terminal 5-HT_{2C} receptor polypeptide as bait. This suggests that 5-HT_{2C} receptors have the potential to interact in vivo with PDZ domains of MUPP1 and/or other proteins containing PDZ domains. The multivalent nature of MUPP1, with its thirteen PDZ domains, allows for a large diversity of potential interactions. Identification of these interacting partners will help to clarify the functional implications of MUPP1 which might be involved in the mechanisms in G-protein coupled receptor signalling e.g. the 5-HT_{2C} receptor-activated phosphoinositide-linked second messenger system.

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