Cloning and characterization of MUPP1, a novel PDZ domain protein

Christoph Ullmer, Karin Schmuck[1], Andrea Figge, Hermann Lübbert[1,*]

Therapeutic Area Nervous System, NOVARTIS Pharma AG, S-360.6.12, CH-4002 Basel, Switzerland

Received 9 January 1998; revised version received 3 February 1998

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.
© 1998 Federation of European Biochemical Societies.

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-

*Corresponding author at present address. Fax: +49-2171759015.

[1]Present address: Biofrontera Pharmaceuticals GmbH, Gründer- und Innovationszentrum, Hemmelratherweg 201, D-51377 Leverkusen, Germany.

0014-5793/98/$19.00 © 1998 Federation of European Biochemical Societies. All rights reserved.
PII S0014-5793(98)00141-0