Short sequence-paper

Molecular cloning of a mammalian homologue of the yeast vesicular transport protein vps45

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Abstract

We have identified the rat homologue (rvps45) of the yeast vps45 protein, a member of the Sec1 family of proteins involved in intracellular vesicle trafficking. Sequence analysis of the full-length rvps45 cDNA obtained from a rat brain library predicts a protein of 570 amino acids which shares 36% identity with the yeast vps45 protein. The sequence shows less homology with other mammalian Sec1 family proteins. Northern blotting identified a 2.3 kb mRNA highly expressed in brain and testis. RT-PCR analysis showed that the rvps45 gene product is expressed throughout the brain. The homology of this protein with the yeast vps45 together with its high expression in brain suggests a role for rvps45 in transport from the Golgi complex to synaptic vesicles. © 1997 Elsevier Science B.V. All rights reserved.

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The Sec1 family of proteins are involved in vesicular transport and synaptic transmission [1]. Four distinct Sec1 proteins have been identified in the yeast Saccharomyces cerevisiae. Sly1p is necessary for transport from the endoplasmic reticulum to the Golgi complex [2]. Sec1p mediates transport from the Golgi complex to the cell membrane [3], while vps45 is required for transport from the Golgi to a late endosomal, prevacuolar compartment [4,5]. Slp1p (vps33p) appears to mediate transport between the prevacuolar compartment and the lysosome-like vacuole [6,7].

Recently, homologues of some of the Sec1-family proteins have been identified in various species, including mammals, suggesting that the molecular machinery for vesicular transport is well conserved. The nematode Sec1 gene, unc-18, interacts with the synaptic vesicle protein syntaxin, and unc-18 mutations result in the accumulation of acetylcholine at the nerve terminal [8]. The mammalian Sec1p homologue, Munc-18, which was identified from rat brain (also known as n-Sec1 or rbSec1A), can also interact with syntaxin, and is involved in the regulation of transmitter release from synaptic vesicles [9–11]. In chromaffin cells it binds syntaxin 1A and is associated with chromaffin granules [12]. A number of Munc-18 isoforms have now been cloned from mammalian tissues, and each may have a specialized function in vesicle transport [13–16]. The mammalian homologue to yeast Sly1p was recently cloned.
from rat liver [17] and a similar Sly1p protein was identified from a rat brain cDNA library [18]. Given the existence of these mammalian homologues of the yeast Sec1 family, mammalian versions of the other yeast family members might exist. Here we report the molecular cloning and characterization of another member of the mammalian Sec1 family, rVps45, which is expressed at high levels in the testis and brain.

A clone (1.8 kb) was obtained during the course of screening a rat brain cDNA library (Clontech) on an unrelated project. Preliminary sequencing identified the clone as a potential homologue of the yeast vps45. Sequencing of both strands of the entire clone using the dideoxy chain-termination method (Sequenase 2.0, United States Biochemical) showed that this cDNA contained a single open reading frame of 1710 bp, corresponding to a full-length rVps45 cDNA (Fig. 1). The cDNA contained a predicted open reading frame coding for 570 amino acids. Analysis of the cloned rVps45 gene product showed 36% amino acid identity with the yeast vps45 (Fig. 2). In contrast, the identity of this sequence with the other mammalian Sec1 family members was less than 20% (Fig. 2). Northern blot analysis detected a single transcript of approx. 2.3 kb. The highest expression of the rVps45 mRNA was detected in rat brain and testis, with moderate to low levels in kidney, lung, spleen, heart and liver (Fig. 3).

For reverse-transcriptase-PCR analysis, total RNA was extracted from various tissues of adult male Sprague–Dawley rats [19]. One hundred nanograms of total RNA was reverse-transcribed using 200 units of MMLV reverse transcriptase (BRL) in a buffer containing a final concentration of 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3.0 mM MgCl₂, 10 mM dithiothreitol, 5% dimethyl sulfoxide, 19 units RNase inhibitor (Pharmacia), 0.01% bovine serum albumin, deoxynucleotide triphosphates (dNTPs; Pharmacia) at 0.5 mM each and 0.25 μg of RT primer: 5′-AGCTACAGCTGAGCTGAGCTCAGT-3′ in a final volume of 20 μL. RT primers were used to amplify an 878 bp PCR product: forward primer: 5′-TTTCATCAGAGGCTGCAA-3′; reverse primer: 5′-CATGCAGAAAAGGCTGGT-3′. For amplification, one-twentieth of the RT reaction was used in the PCR mixture containing 10 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM MgCl₂, 200 μM of each dNTP, 2 units of Taq polymerase (BRL) and 30 pmol of each of the forward and reverse primers in a final volume of 50 μL. The mixture incubated in a thermal cycler for 32 cycles in case of rVps45 and 26 cycles in case of GAPDH. Fifteen microliters of the PCR product was run on 1% agarose gels stained with 0.2 μg/ml ethidium bromide and then visualized with UV light. A PCR product of the predicted size was obtained, subcloned into pCRII vector version 2.1; Invitrogen and sequenced. This confirmed the sequence information obtained from the cDNA obtained from the rat brain library. As shown in Fig. 4, RT-PCR indicated

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Fig. 1. Nucleotide and deduced amino acid sequence of rat vesicular transport protein vps45 (rVps45). The start and the stop codons are in bold-face.

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that rvp45 is expressed in all the brain regions examined.

The Sec1 family of proteins plays an important role in the regulation of vesicle trafficking and secretory processes. Here we report the identification of the mammalian homologue of the yeast vps45, a member of the Sec1 family. Recent studies have shown that the mammalian Sly1 protein can interact with syntaxin 5 to regulate transport from the endoplasmic reticulum to the Golgi complex [17]. The various mammalian Sec1p-like proteins, the Munc-18s, are known to interact with a number of syntaxin isoforms and a role for these proteins in regulating synaptic vesicle docking or release has been suggested [9–12,14,16]. Binding of the Sec1 proteins to syntaxin appears to require the entire protein rather than its membrane-association domain. This suggests that the Sec1 proteins may be important for the regulation of syntaxin function at the synaptic vesicle membrane.

Fig. 2. Comparison of the amino acid sequence of rvp45 from rat brain with its yeast homologue vps45 [4,5], and the other mammalian Sec1 protein family members, rslp1 [17]; munc18b [16], munc 18 [9] and munc18c [16]. Identical amino acids in all sequences are underlined, while conserved residues are in bold-face. Initial sequence alignments were performed using the ClustalW program.
than a discrete domain [10]. Given the overall homology of the rat vps45 to the other mammalian Sec1 proteins (Fig. 2), and their similar predicted secondary structure (data not shown), it is likely that rvps45 also interacts with particular syntaxin isoforms.

The exact role and subcellular localization of the mammalian rvps45 are not known yet, but studies conducted on its yeast homologue show that vps45 is involved in the regulation of vesicle trafficking between the Golgi apparatus and the vacuole [4,5]. Null mutations of the yeast vps45 lead to the accumulation of membrane vesicles and defects in vacuolar protein sorting [4,5]. These results suggest that the site of action of the mammalian rvps45 may also be in transport from the Golgi complex into secretory vesicles. It may therefore interact with the recently identified syntaxin 6, which is localized to the Golgi apparatus [20]. The identification of the mammalian rvps45 will provide us with the tools to determine the role of this protein in mammalian secretory pathways. In particular, the high expression of this protein in the brain suggests that rvps45 could play an important role in synaptic vesicle trafficking and neurotransmission.

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References