Presenilins interact with Rab11, a small GTPase involved in the regulation of vesicular transport

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Presenilin 1 (PS1) mutations account for the majority of early-onset dominant cases of familial Alzheimer’s disease. Presenilins (PSs) are located in many intracellular compartments such as the endoplasmic reticulum, Golgi apparatus, nuclear region and vesicular structures. These proteins include from seven to nine putative transmembrane domains, with the N- and C-terminal ends and a large hydrophilic loop orientated towards the cytoplasm. We report an interaction between the human PS1 or PS2 hydrophilic loop and Rab11, a small GTPase belonging to the Ras-related superfamily. Interaction domains were mapped to codons 374–400 for PS1 and to codons 106–179 for Rab11, a region including the fourth GTP-binding domain. Considering the implication of Rab proteins in vesicular transport pathways, the PS–Rab11 interaction suggests that PSs might be involved in amyloid precursor protein vesicular routing.

INTRODUCTION

Alzheimer’s disease (AD), the first cause of neurodegenerative dementia, is characterized by two cerebral lesions, senile plaques resulting from the deposition of the extracellular Aβ peptide and intracellular neurofibrillary tangles of which the main component is the microtubule-associated protein tau. Mutations of the presenilin 1 gene (PS1), located on chromosome 14q24.3, account for the majority of autosomal dominant early-onset AD (EOAD) cases. More than 50 PS1 missense mutations (1–4), two in-frame deletions of exon 9 (5,6) and two splicing mutations affecting the donor site of intron 4 (7) have been described in EOAD families. In contrast, only two missense mutations have been identified in the presenilin 2 gene (PS2), the second member of the presenilin family located on chromosome 1q42.1 (8,9).

The PS1 and PS2 proteins, composed of 467 and 448 amino acids, respectively, display 67% identity and are predicted to contain between seven and nine transmembrane (TM) domains, with a large hydrophilic loop between the sixth and the seventh TM domains (10–14). PS1 mutations are widely distributed within the coding region, with clusters at the N-terminal end of TM2 and between TM6 and the N-terminal region of the hydrophilic loop (4).

Presenilins (PSs) are ubiquitously expressed and, in brain, are expressed mostly in neurons (15–17). At the subcellular level, PSs have been located predominantly in the endoplasmic reticulum (ER) and Golgi apparatus (18–23), in the nuclear region (kinetochore, centrosome and nuclear membrane) (20), at the cell surface (22) and in vesicular structures within the somatodendritic compartment (23). PS1 displays ~25 and 50% identity with the Caenorhabditis elegans SPE4 and SEL12 proteins, respectively (24,25). SPE4, during spermatogenesis, is involved in pre-packaging and delivery of macromolecules to spermatids in the fibrous body–membranous organelle complexes. SEL12 facilitates signalling mediated by the lin-12/Notch family of receptors involved in developmental cell fate specification and lateral inhibition.

The mechanisms through which mutations of PSs lead to AD are unknown. Plasma, fibroblasts and brains from patients with PS mutations have been shown to contain increased amounts of the Aβ1-42/43 peptide, the amyloidogenic form proteolytically released from the amyloid precursor protein (APP) by β- and γ-secretases (26–27). Similar increases have been observed in transfected cells lines and transgenic animals that express human mutant PSs (28,29). In neuronal cells derived from PS1/PS1-mouse embryos, there is an inhibition of APP cleavage by γ-secretase (30). Human mutant PS1 alleles, which rescue PS1/PS1-mouse embryos from lethality, also lead to an increase of Aβ1-42 peptide production (31,32). Furthermore, PS1 mutants down-regulate the production of non-amyloidogenic α-secretase-derived product of APP (33). This non-amyloidogenic cleavage occurs in the trans-Golgi network (TGN) and at the cell surface (34,35), whereas the amyloidogenic cleavage mediated by γ-secretase normally occurs in the ER for cleavage at position 42 and in the TGN for cleavage at position 40 (36). A direct interaction between APP and PSs in the ER compartment has been demonstrated recently (37–39). All these data suggest that mutations in PSs linked to familial EOAD alter APP processing through a gain of function, which

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remains to be elucidated. In order to characterize the function of PSs, we have screened for proteins able to interact with PSs.

RESULTS

The small GTPase Rab11 interacts in yeast with hydrophilic loops of PSs

To identify proteins that interact with PSs, we have screened an adult human brain cDNA expression library, using a two-hybrid system in yeast Y190 (40). The bait plasmid expresses, as a fusion protein, the mutant PS1 hydrophilic loop (codons 263–407) carrying the Leu392Val substitution linked to EOAD in a large French family (2). The hydrophilic loop of PS1, whose functional importance had been suggested by complementation performed in C. elegans, is orientated towards the cytoplasm, and is therefore more susceptible to interacting with cytoplasmic proteins (41). We initially cloned the mutant PS1 loop into the pAS2-1 expression vector, but a toxic over-expression of the loop led us to subclone it into the pGBT9 vector which contains an ADH1 truncated promoter with a lower level of expression. The pGBT9 (PS1LH392) construct, which did not lead by itself to the activation of the two reporter genes HIS3 and LacZ, was used to screen ~600 000 transformants, and 23 clones were found to co-activate both reporter genes. cDNAs were isolated and retransformed into yeast to confirm that these cDNAs encode proteins which interact with the PS1 hydrophilic loop region. Sequencing analysis revealed that one of these clones corresponds to the δ-catenin identified by Zhou et al. (42). Two other clones correspond to the C-terminal half of Rab11, codons 100–216 and 106–216, respectively. These proteins did not interact with different irrelevant proteins including human lamin C, murine p53 (codons 72–390) and GAL4 DNA-binding domain. Similarly, no interaction was detected between PS1 and the C-terminal half of Rab5 and Rab6 (codons 106–216) (43). Partial Rab11 (codons 106–216) was also found to interact, in the yeast two-hybrid system, with the wild-type PS1 and PS2 hydrophilic loops (Fig. 1).

Rab11 interacts with PSs in COS cells

To confirm the validity of the interaction of PSs with Rab11, we performed immunoprecipitation–western blot experiments in COS cells. The partial Rab11 cDNA (codons 106–216), derived from the library screening, and the full-length Rab11 cDNA, derived from human immortalized lymphocytes, were cloned into the pCNW8 expression vector which confers an N-terminal myc tag, and the resulting plasmids were transfected with vectors expressing full-length wild-type PS1 or PS2 (39). Cell lysates were immunoprecipitated with a polyclonal antibody directed against either PS1 or PS2 (37), and immunoprecipitates were analysed by western blot using an anti-myc antibody (Fig. 2). Rab11 was clearly detected (Fig. 2B) in PS2 immunoprecipitates only when both proteins were expressed in COS cells. No Rab11 was detected when immunoprecipitation was performed with a pre-immune serum (data not shown). A similar interaction, although weaker, was also observed in PS1 immunoprecipitates with both partial and full-length Rab11 (Fig. 2A).
We generated a set of wild-type and mutant PS1 deletion mutants fused to the GAL4 DNA-binding domain and a set of Rab11 deletion mutants fused to the GAL4 activation domain in order to identify the binding domains on both proteins. Yeasts were co-transformed with different combinations of plasmids and assayed for their ability to grow on medium without histidine and for β-galactosidase expression. As shown in Figure 3, we found that the essential interaction domain of PS1 was located between amino acids 374 and 400, corresponding to the C-terminal end of the large hydrophilic loop. The essential interaction domain of Rab11 was located between amino acids 106 and 179, which includes the fourth GTP-binding domain.

**DISCUSSION**

Our results indicate that Rab11 interacts with presenilins as: (i) two distinct cDNAs corresponding to the C-terminal half of Rab11 were identified in a two-hybrid screen using mutant Leu392Val PS1 hydrophilic loop as a bait; (ii) Rab11 was shown to interact not only with the hydrophilic loop of PS1, but also with that of PS2, the second member of the PS family; and (iii) the interaction was confirmed in cells transfected with plasmids expressing the full-length PSs and Rab11 as well as Rab11106–216. The hypothesis of a gain of function led us to perform the screening with a mutant form of PS1. In yeast, we were unable to detect any difference in the binding to Rab11 between the wild-type and mutant form of PS1, which does not exclude a subtle difference in the affinity of both proteins. In transfected COS cells, the PS1–Rab11 interaction was more difficult to detect than the PS2–Rab11 interaction (Fig. 2), which might be explained by a difference in the quality of PS1 and PS2 antibodies. The alternative hypothesis is that the interaction between Rab11 and PS1 is either transient or of low affinity. It should be noted that the Rab11-binding domain of PS1 (amino acids 374–400) corresponds to a region of the loop highly conserved between PS1 and PS2 (89% identity), which supports the functional importance of this domain.

Rab11, which is composed of 216 amino acids with a predicted molecular weight of ~25 kDa, belongs to the Rab subgroup of the Ras-related superfamily of small GTPases, which comprises >30 members. Rab proteins have emerged as important regulatory components of the vesicular transport and organelle dynamics in eucaryotic cells, each step of cellular trafficking involving a different set of Rab proteins (44,45). Rab proteins contain four consensus regions (G1–4) known to be involved in GTP/GDP binding and, as is the case for other members of the Ras superfamily, Rab proteins undergo a functional cycle between a GDP-bound inactive and a GTP-bound active conformation. Interestingly, the domain of Rab11 involved in PS1 binding involves the G4 region (46), which might suggest that such an interaction might interfere with the GTP/GDP binding status of Rab11. Rab11 is ubiquitously expressed and, at the subcellular level, has been localized in different cell types within different compartments including the TGN and TGN-derived vesicles (47), the pericentriolar recycling endosome (48) and apical tubulovesicle membranes (49). At the present time, the function of Rab11 has not been elucidated fully. The association of Rab11 with transferrin-containing recycling compartments (50) suggests a role in the trafficking and recycling of internalized proteins. Rab11 has also been implicated in protein transport from the TGN through post-Golgi vesicles to the plasma membrane (51,52). Therefore, considering that PSs have also been localized in the Golgi apparatus and perinuclear vesicles, the interaction that we report between PSs and Rab11 might be relevant in vivo. Furthermore, recent studies have implicated several Rab proteins in trafficking and recycling of APP in specific cell compartments. It has been proposed that Rab1b protein might regulate early steps in exocytic transport and processing of APP (53).
APP has been localized in Rab5-covered vesicles and, in sporadic AD cases, an increased neuronal endocytosis and protease delivery to Rab5-positive early endosomes, which might be a mechanism of increased β amyloidogenesis, has been reported (54,55).

PS mutations linked to familial AD lead to an increased production of Aβ1-42/43 peptide. Although the biological relevance of the PS–Rab11 interaction that we describe in this study remains to be confirmed, our results led us to speculate that this altered processing of APP might be tightly linked to an abnormal vesicular routing since amyloidergic and non-amyloidergic cleavages occur in different cells compartments.

**MATERIALS AND METHODS**

**Strains and plasmids**

*Saccharomyces cerevisiae* strain Y190, pAS2-1 and pGBT9 expression vectors, containing the GAL4 DNA-binding domain, pACT2-1 containing the GAL4 activation domain and control plasmids pVA3-1, pTD1-1 and pLAM 5'-1 were purchased from Clontech (Palo Alto, CA).

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generated by PCR from pBSK (Rab11\textsubscript{q}) and cloned into pACT2-1. Canine Rab5 and human Rab6 cDNAs (codons 106–216) were PCR-amplified from plex (Rab5) and plex (Rab6) expression vectors (43) and cloned into pACT2-1. Full-length and partial Rab11 cDNAs (codons 106–216) were PCR-amplified from pBSK (Rab11\textsubscript{q}) and cloned into a modified version of the mammalian pcDNA3 expression vector (Invitrogen, CH-Groningen, The Netherlands), pcNW8, containing an N-terminal tag corresponding to the myc epitope (codons 409–418). Full-length wild-type PS1 and PS2 cDNAs were cloned into pcDNA3 (39). All constructs were validated by DNA sequencing using the PRISM AmpliTaqFS Ready Reaction Dye Terminators sequencing kit (Applied Biosystems, Perkin Elmer-Cetus, Foster City, CA) and an Applied Biosystems model 373A automated sequencer.

**Library screening**

A single colony of Y190 harbouring the bait plasmid pGBT9 (PS1LIH392) was grown overnight in SD-Trp and transformed with a human adult brain Matchmaker expression library cloned in pACT2-1 (Clontech). A total of 600 000 independent transformants were plated on 22.5 × 22.5 cm plates containing SD-His-Trap-Leu medium with 40 mM 3-amino-1,2,4-triazole (Sigma, St Louis, MO) to overcome leaky HIs3 reporter expression. After incubation at 30°C for 8–10 days, His\textsuperscript{+} clones were assayed for β-galactosidase activity by filter assay. Replica filters were frozen in liquid nitrogen for 20 s and thawed to room temperature to permeabilize the cells. Filters were then incubated in an X-gal solution at 30°C for a maximum of 8 h. LacZ\textsuperscript{+} clones were streaked onto SD-His-Trap-Leu medium and re-assayed to confirm the His\textsuperscript{+}LacZ\textsuperscript{+} phenotype. Plasmid DNA was extracted, as previously described (56), from positive clones grown overnight in 2 ml of SD-Leu, and was used to transform C600 competent bacteria (Clontech), which were then plated on M9-Leu medium containing 50 µg/ml ampicillin. Single colonies were incubated in 2 ml of LB medium, and plasmid DNA was extracted using an RPM kit (BIO101, La Jolla, CA). Inserts were sequenced as indicated above and DNA searches were performed using the BLAST 2.0 program at the NCBI server (http://www.ncbi.nlm.nih.gov/cgi-bin/BLAST/nph-newblast?Jform=1).

**Cell culture, transfection and immunoprecipitation**

Rab11 expression vectors (4 µg) were transfected with PS expression vectors or empty pcDNA3 (4 µg) using lipofectamine (Boehringer Mannheim, Mannheim, Germany), according to the manufacturer’s instructions, into COS cells at 70% confluence on 10 cm plates. Two days after transfection, cells were lysed in 800 µl of 50 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, 1% NP-40, 10% glycerol, in the presence of a protease inhibitor cocktail (Complete; Boehringer Mannheim). To solubilize membrane proteins, cell lysates were sonicated and gently agitated overnight at 4°C. After centrifugation for 20 min at 15 000 g, supernatants were recovered as detergent-soluble fractions. The detergent-soluble lysates (150–500 µg) were incubated for 1 h at room temperature in 400 µl of 150 mM NaCl, 50 mM Tris, pH 7.5, 0.5% deoxycholic acid and 1% NP-40 with protein A–Sepharose (Amersham Pharmacia Biotech, Little Chalfont, UK), and immunoprecipitations were performed using polyclonal antibodies B1-03 or 95-041 (37) raised against PS1 and PS2 peptides, respectively. Proteins were separated on a 2.8 acryl/ bis-acrylamide 8–16% Tris–glycine gel (Novex, San Diego, CA) and transferred to poly(vinylidene difluoride) membranes. Western blot analysis was performed with anti-myc antibody using an ECL detection kit (Amersham Pharmacia Biotech), according to the manufacturer’s instructions.

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