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The Role of Alzheimer's Disease-Related Presenilin 1 in Intercellular Adhesion

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Most cases of familial early-onset Alzheimer's disease are caused by mutations in the presenilin 1 (PS1) gene. However, the cellular functions of PS1 are unknown. We showed predominant localization of PS1 to cell–cell contacts of the plasma membrane in human prostate epithelial tissue and in a human epithelial cell line HEp2 stably transfected with an inducible PS1 construct. PS1 co-immunoprecipitated with β-catenin from cell lysates of stable transfectants. Conversely, PS1 lacking the PS1–β-catenin interaction site did not co-immunoprecipitate with β-catenin and was not recruited to the cell–cell contacts. L cells, which do not form tight intercellular contacts, formed clusters of adhered cells after stable transfection with GFP-PS1 cDNA and demonstrated a clear preference for independent aggregation in the mixed cultures. However, L cells transfected with mutant GFP-PS1 constructs, which had a truncated N-terminus of PS1 or deleted PS1–β-catenin interaction site, failed to form intercellular contacts. In addition, in primary cultures of mouse cortical neurons PS1 was highly concentrated on the surface of extended growth cones. Taken together, our results suggest an important role of PS1 in intercellular adhesion in epithelial cells and neurons.

Key Words: **Alzheimer's disease; presenilin 1; cell–cell adhesion; epithelial cells; primary neuronal cultures.**

INTRODUCTION

More than 40 missense mutations in the presenilin 1 (PS1) gene co-segregate with early-onset familial Alzheimer's disease (FAD) [64, 48] (reviewed in [29]). PS1 is a six- to eight-transmembrane domain protein with the hydrophilic N terminus and the hydrophobic C terminus. A large hydrophilic loop is located between transmembrane domains 6 and 7 (see review [28]). FAD mutations were found through the entire PS1 protein but "hot spots" were discovered in transmembrane domain 2, and the region adjacent to the hydrophilic loop domain (reviewed in [29, 48]). In addition, splice-site mutations and an in-frame deletion were described for exon 9 [56, 12]. Although numerous PS1 mutations have been well documented, neither the nature of the dysfunction caused by these mutations nor the normal functions of PS1 are clearly understood.

There are a number of clues indicating an important role of PS1 in the regulation of intramembrane proteolysis (reviewed in [8]). PS1 is required for γ-secretase activity in the proteolytic processing of the amyloid precursor protein (APP) [15, 73, 1]. FAD-linked mutations in PS1 alter proteolytic processing of APP and result in an increase in the production of highly amyloidogenic forms of amyloid-β-protein (reviewed in [28]). Another notable example of the PS1 role in intramembrane proteolyis is added by observations that PS1 controls proteolytic processing of Notch receptors [16, 65, 66, 77, 59]. The Notch receptors are cell surface proteins that are involved in the regulation of cell–cell interactions and cell fate choices during development (reviewed in [2]). Therefore, functional interaction between Notch and PS1 may be critical for embryonic development and explains impaired ontogenesis of PS1 null mice [63, 74, 15] and the Notch-deficient-like phenotype of PS1 knockout in Drosophila [66, 77]. Moreover, genetic studies demonstrated that sel-12 protein (PS1 homologue) facilitates Notch/lin-12 signaling in *C. elegans* [41].

Other described roles of PS1 are contribution to neuronal calcium homeostasis (reviewed in [47]) [48], involvement in T-cell receptor-induced apoptosis [71], and caspase activation [70]. Studies of PS1 null mice showed that the lack of PS1 results in neuronal loss in specific brain regions [63] and abnormal migration of

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developing neurons [30]. In summary, although cellular functions of PS1 are not clearly understood, it appears that PS1 has an important role in many physiological cellular processes.

Considerable progress has been made in defining subcellular localization of PS1. Immunocytochemical and biochemical analysis of transfected cells, primary neuronal cultures, and rodent brains revealed predominant localization of PS1 to endoplasmic reticulum (ER)–Golgi compartments and to coated transport vesicles [11, 37, 38, 22].

However, several reports raised the possibility that PS1 may also function in other subcellular compartments. In transfected cells PS1 has been identified in the nuclear membrane, interphase kinetochores and centrosomes [45], and in the plasma membrane [67, 17]. Endogenous PS1 has been detected directly at the plasma membrane in Drosophila [76], human T-lymphocytes [62], rat brain [4], mouse corneal epithelium [25], and mouse fibroblasts [59].

Recently it was shown that PS1 is recruited to intercellular and synaptic contacts, forms complexes with the cadherin/catenin cell–cell adhesion system, and increases cell aggregation in transfected HEK293 cells [25]. These observations clearly indicate the role of PS1 in intercellular adhesion.

PS1 interacts *in vitro* and *in vivo* with actin binding proteins filamin [79, 62] and β-catenin [81, 70, 80], which are known to link cell surface receptors with the cytoskeleton and mediate cell adhesion and cell motility [46]. Accordingly, cell surface labeled PS1 forms complexes with intracellular filamin and, like other proteins that are involved in cell adhesion, redistributes to the surface of lamellipodia in adhered T-lymphocytes (Jurkat cells) [62].

The possibility that PS1 may be involved in cell adhesion is particularly important in view of new findings that cell adhesion molecules play a role in memory formation. The cadherin family of cell adhesion molecules is implicated in regulation of synaptic plasticity and long-term potentiation [69, 75]. A new integrin, Volado, which was identified in Drosophila is required for short-term memory processes [27]. In addition, Parent and colleagues recently reported that the FAD-linked A246E variant of PS1 leads to the changes of induction of long-term potentiation in transgenic mice [54].

Here we report that in human prostate epithelial tissue, PS1 accumulates at cell–cell contacts of plasma membrane. In stably transfected human epithelial cell line HEp2, wild-type PS1 is also localized at cell–cell contacts of plasma membrane and forms complexes with β-catenin. On the contrary, PS1 lacking the PS1–β-catenin interaction site did not co-immunoprecipitate with β-catenin and was not recruited to the cell–cell contacts of plasma membrane. Cell adhesion molecules (CAM)-deficient L cells, which do not form tight intercellular contacts, demonstrated a clear preference for independent aggregation after stable transfection with GFP-PS1 cDNA. However, L cells transfected with mutant GFP-PS1 constructs, which had a truncated N-terminus of PS1 or deleted PS1–β-catenin interaction site, failed to form intercellular contacts. In addition, in primary cultures of mouse cortical neurons, PS1 was highly concentrated on the surface of extended growth cones. Taken together, our results indicate an important role of PS1 in intercellular adhesion in epithelial cells and neurons.

MATERIALS AND METHODS

Antibodies. Monoclonal antibody MKAD 3.4, which recognizes residues 45–48 of human PS1 [50], was a gift of Dr. A.Takashima and Dr. T.Honda (Riken Brain Science Institute, Japan). Affinity-purified polyclonal antibody PS1-Nm raised against synthetic peptide corresponding to the N-terminal fragment 31–46 of mouse PS1 was provided by Dr. T. Koothan (Cold Spring Harbor Laboratory). Rabbit polyclonal antibody Ab14 was generated against residues 3–15 of PS1 (provided by Dr. S.Gandy, Nathan Kline Institute). Other antibodies used included anti-GFP monoclonal antibody (Clontech), rabbit anti-GFP polyclonal antibody (Clontech), rabbit anti-β-catenin polyclonal antibody (Sigma), anti-CD44 monoclonal antibody A3D8 (Sigma), and goat polyclonal anti- (E,P and N) cadherin antibodies (Santa Cruz).

Cell cultures. HEp2 cells (human epithelial cell line) and L cells (murine L-cell fibroblasts) were obtained from the American Type Culture Collection. HEp2 cells were maintained in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. Generation of PS1 (−/−) knockout mice was described previously [63]. Primary cultures of mouse cortical neurons from embryonic 14- to 15-day fetuses from PS1(−/−) were obtained according described procedure [15]. In addition, primary neuronal cultures from PS1(−/−) knockout mice were generously provided by.Dr. B. De Strooper and Dr. P. Saftig (Experimental Genetics Group, Center for Human Genetics, Campus Gasthuisberg, Belgium).

Immunohistochemistry, immunoprecipitation, and immunoblotting. Human prostate specimens were obtained at the time of the surgery, fixed with 10% buffered formalin, and embedded in paraffin. The tissue sections (4 μm) were further processed for light microscopy and DAB-based immunohystohemistry using the DAKO amplification system (DAKO, Denmark). Tissue sections were examined using Nikon inverted Diaphram microscope at magnification 20×. For immunoprecipitation, HEp2 cell or L-cell extracts containing complete protease inhibitor cocktail (Boehringer Mannheim) were preincubated with protein G Sepharose (Pharmacia) for 1 h. Beads were removed, and supernatants were incubated with MKAD 3.4 or monoclonal anti-GFP antibody at 4°C overnight. The immunocomplexes were precipitated with protein G Sepharose at room temperature for 2 h, washed in TBS and 1% Triton X-100, and diluted with 2× Laemmli sample buffer (40% glycerol, 6% SDS, 4% 2-mercaptoethanol). Then all samples were electrophoresed on 10% Tris–glycine gels and transferred to a PVDF membrane (Bio-Rad). Western blotting (using SuperSignal West Dura Extended Duration substrate, Pierce) was performed as per the manufacturer's recommendation. The bands were visualized using Chemi Doc detection system (Bio-Rad). Molecular sizes of the visualized bands were determined with a Kaleidoscope prestained molecular standard (Bio-Rad).

cDNA construction and transfection. The wild-type PS1 cDNA was cloned into *Bam*H 1–*Eco*R I sites in the polylinker of the pET 22b vector (Novogen) as previously described [61]. For generation of

deletion mutant N-288 we introduce a stop codon into position 288 of PS1 cDNA using method of site-directed mutagenesis described by Kunkel [83]. The *Bam*H1–*Eco*R1 fragments of wild-type PS1 cDNA and N-288 PS1 cDNA were then cloned in-frame with the N-terminal GFP into the *Bgl*II–*Eco*R 1 sites of the pEGFP-C1 polylinker (Clontech). For generation of D 1-44 and D 1-77 deletion mutants, we introduced *Bgl*II sites in the PS1 cDNA cloned in the pET 22B vector using the same method of site-directed mutagenesis [83]. The *Bgl*II–*Eco*R 1 fragments of pET 22B-PS1 mutants (D 1-44 and D 1-77) were then cloned into the *Bgl*II–*EcoR* 1 sites of pEGFP-C1 polylinker. Expression of fusion protein was studied in the ecdysone (ponasteron A)-inducible expression system (Invitrogen). To introduce the PS1 cDNA into pind (SP1) expression vector (Invitrogen), the *Nhe* I–*Eco*R I fragments of PEGFP-C1 plasmids, containing GFP-PS1 cDNA (wild-type, N-288, D 1-44, and D 1-77) were cloned into the polylinker of pind (SP1). The resulting plasmids were cotransfected with pVgRXR into HEp2 or L cells using the FuGENE transfection reagent (Boehringer Mannheim). Stable transfectants were identified using dual selection for neomycin (G418) (750 μg/ml) and Zeocin (500 μg/ml) as described by Invitrogen Protocol 160906 (Invitrogen).

Laser confocal microscopy. HEp2 cells were fixed for 30 min in 2% paraformaldehyde and 0.1% glutaraldehyde buffered with PBS in eight-chambered coverslips and permeabilized with 0.5% Triton X-100 for 15 min at room temperature. Primary neuronal cultures were fixed for 30 min in 4% paraformaldehyde. All cells were then blocked for 30 min with 5% albumin, fraction V (Sigma). PS1 was stained using anti-PS1 antibodies Ab 14 or PS1-Nm and FITC-conjugated secondary antibodies. Immunofluorescence images were visualized with either a X40 1.4 NA or a X60 1.3 NA oil-coupled objective on a Noran Confocal Odyssey system through a Nikon inverted Diaphram microscope.

Cell attachment, cell aggregation, and cell sorting assays. For the cell attachment assay, 50% confluent L cells were trypsinized and, after 8 h induction with 10 μM ponasteron A in complete MEM media with 5% serum, were plated into 96-well plate precoated with 1% BSA (10^4 cells/well). After 2 h of incubation unattached cells were removed by extensive washing in PBS. Attached cells were fixed with 2% glutaraldehyde, dried at room temperature, and stained with 0.1% crystal violet. Specimens were treated with 10% acetic acid and absorbance at 590 nm was measured using a microplate reader.

Cell aggregation assay was performed as described [51, 53]. Briefly, L cells were dissociated by trypsin–EDTA treatment and gentle pipetting, and were allowed to aggregate in complete MEM media with 5% serum and 100 μm ponasteron A for 16 h. The extent of cell aggregation was represented by the index N_{16}/N_0, where N_{16} was the total "particle" number after 16 h and N_0 was the total particle number at incubation time 0. Single particle represents single cell or single cellular cluster in the field of view. Particle numbers were calculated in a Levy chamber using light microscopy.

For cell sorting (cell segregation), nontransfected and wild-type GFP-PS1 transfectants were mixed in a 1:1 ratio and cultured 16 h in the presence of 50 μM ponastron A. To distinguish between two cell lines, the cells were differentially labeled using a Vybrant DiO and DiI cell-labeling kit (Molecular Probes, Protocol MP22885). Cell cultures were monitoring at different times to follow the progress of cell segregation.

Scanning electron microscopy. Primary cultures of mouse cortical neurons in four-chambered coverslips were fixed in 4% paraformaldehyde. The cells were blocked with 5% BSA for 40 min and incubated with primary antibodies at 4°C for 16 h. The samples were then washed four times with PBS and incubated with 6 nm colloidal gold-conjugated anti-rabbit IgG (Jackson Immunoresearch) at a dilution of 1:40 in PBS for 60 min at room temperature. Each sample was then washed five times with PBS. Silver enhancement was performed following the Goldmark Protocol (Goldmark Biologicals) and then the cells in the chambers were fixed with 2% paraformaldehyde/1.5% glutaraldehyde in 0.1 M cacodylate, pH 7.4, for 1 h. The cells were washed twice in 0.1 M cacodylate, pH 7.4, postfixed with freshly prepared 1% osmium tetroxide (Polysciences, Inc.) in 0.1 M cacodylate, pH 7.4, for 1 h at 22°C, and dehydrated by ethanol. A 1:1 mixture of hexamethyldisilazane and ethanol was then added for 30 min, followed by 100% hexamethyldisilazane for 2 h at room temperature. After complete evaporation of hexamethyldisilazane, specimens were visualized in a scanning electron microscope (Model JSM-5300; Jeol, Tokyo, Japan) at 10 kV with tilt angles of 40–52°. Backscatter electron imaging, which contains information about the difference in average atomic number, was used to detect gold–silver particles.

RESULTS

Distribution of PS1 in Epithelial Cells

Although pathological changes associated with FAD are described mainly in neurons current thinking concerning PS1 is largely based on the study of transfected cells. This is in part due to the technical difficulties of studying neurons. Since neurons are characterized by unique polarized distribution of receptors and integral membrane proteins, selection of correct experimental cell models is a critical step for understanding neuronal PS1 functions. Dotti and Simons [19] proposed that neurons and polarized epithelial cells share common mechanisms of protein targeting. More recent studies demonstrated that many membrane processes and functions of membrane proteins are fundamental to both neurons and epithelia [7, 78]. Therefore, our starting point in understanding PS1 functions was to analyze distribution of PS1 in epithelial tissue and epithelial cell lines.

Immunohistochemical staining of prostate tissue demonstrated that endogenous PS1 is expressed in prostate epithelium but not in stromal cells. In epithelial cells PS1 was distributed along cell borders and concentrated at the cell–cell contacts of lateral plasma membrane (Fig. 1A).

We also analyzed distribution of PS1 in the stably transfected human epithelial cell line HEp2. This line contains a very low level of endogenous PS1 (data not shown) and thus, it is a suitable model for transfection experiments. For stable transfection of HEp2 cells, we generated a GFP-PS1 cDNA construct, in which GFP was fused in-frame with the N terminus of PS1. The fusion protein was expressed using the ecdysone-inducible expression system (Invitrogen) (Fig. 1B).

To obtain HEp2 cells with proper PS1 expression, we performed preliminary selection of stably transfected lines. Recently, Johnston and colleagues showed that overexpression of PS1 leads to the deposition of aggregated misfolded protein in intracellular "aggresomes" [33]. Therefore, we discarded PS1 overexpressing lines in favor of cell lines that exhibited mild or moderate expression of GFP-PS1. The level of expression of inducible GFP-PS1 in these lines was highly regulated by the concentration of ponasteron A and by the time of

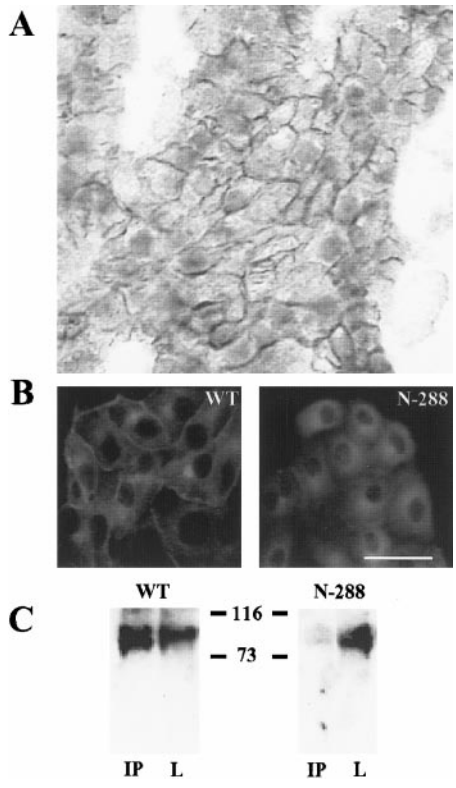

FIG. 1. Expression of PS1 in epithelial cells. (A) PS1 expression in prostate epithelia. Paraffin sections were stained with anti-PS1 monoclonal antibody MKAD 3.4. [50] and DAB-based DAKO kit. Nuclei were stained with hemotoxilin. (B) Laser confocal micrographs of stably transfected epithelial cell HEp2 expressing wild-type GFP-PS1 (WT) and N-288 mutant (N-288). Bar, 50 μm. (C) Western blot analysis of cell lysates and PS1 immunoprecipitates of transfected HEp2 cells. PS1 was immunopreipitated from lysates of HEp2 cells with monoclonal antibody MKAD 3.4. Western blots were were stained with polyclonal anti-β-catenin antibody. WT, wild-type GFP-PS1; N-288, mutant of GFP-PS1with the C terminus of PS1 deleted beyond residue 288 (see details in Fig. 3); L, lysates of HEp cells; IP, MKAD 3.4 immunoprecipitates of GFP- PS1 from HEp2 lysates.

induction. Maximal level of expression of GFP-PS1 in stably transfected cells was observed after 16 h of induction with 50 μM ponasterone A. Laser confocal microscopy showed that inducible wild-type (WT) GFP-PS1 is mainly distributed in the cytoplasm and along the regions of contact sites of lateral plasma membrane (Fig. 1B, WT).

The observed distribution of PS1 in epithelial cells revealed striking similarity with the described distribution of E-cadherin, the main cell–cell adhesion molecule in epithelial tissue [3]. This similarity is strengthened by the fact that both PS1 and E-cadherin bind β-catenin, which is known to link cell surface cadherin to the cytoskeleton and assemble cadherin–catenin complexes into adherens junctions [68]. Therefore, we examined the role of β-catenin in recruitment of PS1 to the cell–cell contacts of plasma membrane. We generated a GFP-PS1 construct with a deleted C terminus of PS1 beyond residue 288—N-288 (see details in Fig. 3). This construct does not have a PS1–β-catenin interaction site [81]. As expected, WT GFP-PS1, but not GFP-N-288 co-immunoprecipitated with β-catenin from lysates of transfected HEp2 cells (Fig. 1C). The appearance of a very weak band of β-catenin in immunoprecipitates of deletion mutant N-288 was likely a result of co-immunoprecipitation of endogenous PS1 and β-catenin (Fig. 1C). Accordingly, immunoprecipitation of N-288 with anti-GFP antibody did not reveal β-catenin bands in Western blots (data not shown). Most importantly, deletion mutant N-288 lacking a PS1–β-catenin interaction site was not recruited to the intercellular contacts of transfected HEp2 cells (Fig. 1B). These results clearly indicate that the interaction of β-catenin with PS1 is necessary for recruitment of PS1 to the cell–cell contacts.

To avoid possible interfering effects of GFP, we examined subcellular localization of untagged PS1 in transfected HEp2 cells. Confocal images show that WT PS1 is concentrated at intercellular contacts of lateral plasma membrane (Fig. 2, WT-PS1 panels). We also compared distributions of PS1 and CD44, the main cell surface receptor of epithelial cells [40]. As expected, almost all CD44 immunoreactivity was concentrated at the plasma membrane Fig. 2 (WT-CD44 panel). In dual immunostaining, PS1 colocalized with CD44 at the

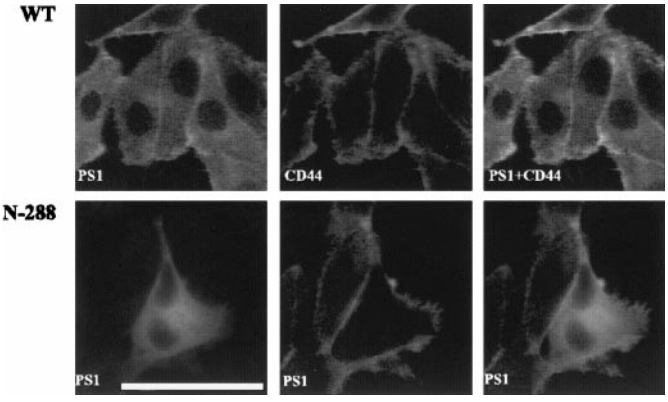

FIG. 2. Immunolocalization of PS1 in transfected HEp2 cells. PS1 panels (green) and CD44 panels (red) represent laser confocal micrographs of transfected epithelial cells HEp2 expressing wild-type PS1 (WT) and N-288 mutant (N-288). N-288 is a GFP-PS1 construct with a deleted C terminus of PS1 beyond residue 288 (see details under Materials and Methods). Expression of PS1 was induced with 25 μM ponasteron A for 24 h and cells then were fixed for 30 min in 2% paraformaldehyde/0.1% glutaraldehyde buffered with PBS. Fixed cells were permeabilized with 0.5%Triton X-100 for 15 min at room temperature. The cells then were blocked with 5% albumin and incubated with anti-PS1 polyclonal antibody Ab14. For dual immunostaining, we used Ab14 and anti-CD44 mAB, followed by FITC- and tetramethylrhodamine B isothiocyanate-conjugated secondary antibodies. Colocalization of PS1 with CD44 is shown on panels labeled PS1 + CD44. Bar, 50 μm.

plasma membrane, mainly at the cell–cell contact sites (Fig. 2, WT-PS1 + CD44 panel). Completely different results were obtained with deletion mutant N-288 (Fig. 2, N-288). Induced PS1 was not concentrated at the intercellular contacts of plasma membrane (Fig. 2, N-288-PS1 panels) and did not colocalized with CD44 (Fig. 2, N-288-PS1 + CD44 panel). These results confirmed the above data that the formation of β-catenin–PS1 complexes is necessary for recruitment of PS1 to the cell–cell contacts.

PS1 Mediates Cell–Cell Interactions in Transfected L Cells

Although localization studies support the hypothesis that PS1 is involved in cell adhesion, they provide only a descriptive view of possible PS1 functions. Therefore, we directly examined cell adhesion functions of PS1 by using a classical approach developed by Edelman [21] and Takeichi [51]. Specifically, these studies demonstrated that in CAM-deficient cell line (murine L-cell fibroblasts), CAM activity and formation of intercellular contacts can be restored by transfecting with a cDNA encoding new CAM.

To examine if PS1 is directly involved in intercellular adhesion, we generated a number of GFP-PS1 cDNA deletion constructs (Fig. 3A). The D 1-44 and D 1-77 contained deletions of the N terminus of PS1 corresponding to positions 1-44 and 1-77, respectively. The N-288 construct lacking the PS1–β catenin interaction site had a stop codon in position 288. L cells were stably transfected with each GFP-PS1 deletion constructs and fusion protein was expressed using the ecdysone-inducible expression system (Invitrogen). Stable transfectants that exhibited mild or moderate expression of GFP-PS1 were selected for analysis. All studied cell lines exhibited very low levels of endogenous PS1 (data not shown). Fusion protein GFP-PS1 was detected in transfected L cells by Western blot analysis after 12 h induction (Fig. 3B). Western blot analysis with monoclonal anti-GFP antibody revealed the N-terminal fragment of fusion protein (approximately 54 kDa [26 kDa GFP + 28 kDa NTF of PS1]) in lysates of all transfected cells. Full-length GFP-PS1 (approximately 72 kDa) in lysates of D 1-44, and D 1-77 transfectants was not detected at this exposure level (Fig. 3B). The C-terminal PS1 fragment of approximately 18 kDa was also detected in lysates of cells transfected with wild-type PS1 cDNA or D 1-44 and D 1-77 constructs (data not shown). These results suggest that GFP does not interfere with normal PS1 processing in transfected L cells. Wild-type GFP-PS1, D 1-44, and D 1-77 co-immunoprecipitated with β-catenin (Fig. 3C, lanes WT, D 1-44, and D 1-77). On the contrary, immunoprecipitates of the deletion mutant N-288 lacking the PS1–β-catenin interaction site did not contain β-catenin (Fig. 3C, lane N-288).

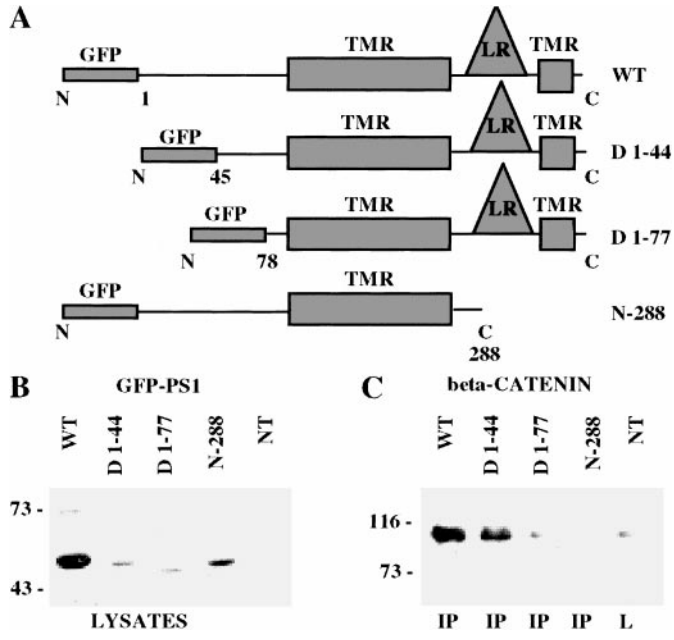

FIG. 3. Analysis of PS1 deletion mutants. (A) Schematic illustration of PS1 deletion mutants: GFP, green fluorescence protein; PS1-presenilin1; TMR, PS1 regions including transmembrane domains; LR, loop region; WT, wild-type GFP-PS1; D 1-44, D 1-77, and N-288 are PS1 deletion mutants. The D 1-44 and D 1-77 constructs contain deletions of the N terminus of PS1 corresponding to positions 1–44 and 1–77, respectively. The N-288 construct lacking the PS1-β catenin interaction site had a stop codon in position 288. (B) Western blot analysis of GFP-PS1 in lysates of transfected (WT, D 1-44, D 1-77, N-288) and nontransfected (NT) L cells after 12 h of induction with ponasteron A. (C) Immunoprecipitation (IP) of GFP-PS1–β-catenin complexes from lysates (L) of transfected L cells with monoclonal anti-GFP antibody. Immunoprecipitated complexes were analyzed by Western blotting with polyclonal anti-β-catenin antibody.

Phase-contrast microscopy shows that plated nontransfected L cells did not form tight intercellular contacts in the presence of ponasteron A (Fig. 4, NT). In contrast, plated L cells expressing GFP-WTPS1 formed small clusters (5–30 cells) after 8–12 h of induction. The cells in these clusters were mainly polygonal in shape, but, unlike untransfected cells, displayed a flattened epithelioid phenotype with a clear interconnection of the lateral domains of neighboring cells. Immunofluorescence studies of living cells showed a striking punctate pattern of GFP-PS1 mainly at cell–cell contact sites (Fig. 4, WT). L cells transfected with GFP-PS1, which had a truncated N terminus of PS1 (D 1-44 or D 1-77), did not form intercellular contacts and GFP-PS1 fluorescence in these cells was detected predominantly in the cytoplasm (Fig. 4, D 1-44, D 1-77).

L-cell-expressing deletion mutants D 1-44 and D 1-77 did not show any changes of parental morphology of L fibroblasts (Fig. 4, D 1-44, D 1-77). In contrast,

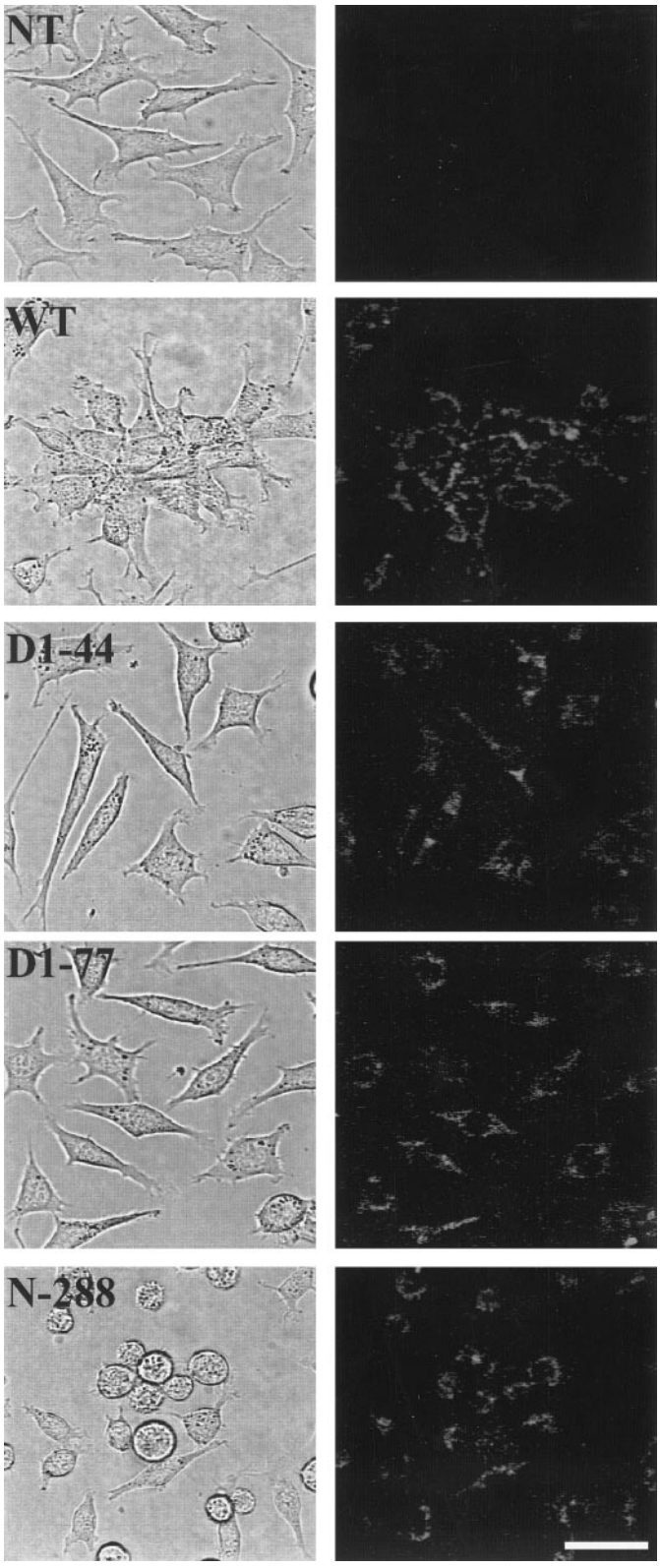

FIG. 4. Cell–cell adhesion properties of deletion GFP-PS1 mutants. Living cells were analyzed after 12 h induction with 50 μM ponasteron A. Left panels represent phase-contrast images and right panels show inducted GFP fluorescence. Bar, 50 μm. NT, nontransfected cells; WT, wild-type GFP-PS1; D 1-44, D 1-77, and N-288 are PS1 deletion mutants. Note the clustering of cells expressing WT but not NT cells or cells expressing mutants D 1-44 or D 1-77. The cells lacking the C terminus of PS1 (N-288) displayed altered morphology.

most cells expressing deletion mutant N-288 resulted in loss of the polygonal phenotype and took on a rounded morphology even after 12 h incubation with ponasteron A. Occasionally, these round cells formed small cellular clusters (three to six cells) but mostly interactions among the cells seemed to be reduced to sporadic contacts (Fig. 4, N-288). This change in morphology was increased proportionally to the GFP-PS1 level and was observed for the entire cell population at the highest (100 μM) concentration of ponasteron A. The effect was reversible, and within 3 days of culture in the absence of ponasteron A in the medium, the cells regained their original morphology.

Interestingly, L cells expressing wild-type PS1 revealed morphological changes related to previously reported morphology for different cadherin transfectants of L cells [51, 49, 24]. Therefore, we tested the possibility that effect of PS1 on the formation of intercellular contacts might be due to the induction of specific cadherins. However, neither parental L cells nor L cells expressing WT GFP-PS1 revealed detectable level of E-, N-, and P-cadherins or changed their behavior after removal of extracellular calcium (data not shown).

To further evaluate the role of PS1 in cell–cell adhesion, the aggregation of stable transfectants was examined by a quantitative assay based on the measuring of number of cellular aggregates and single cells in suspension [21, 51]. As seen in Fig. 5, significant aggregating activity was detected for cells expressing WT GFP-PS1 and untagged WT PS1. Nontransfected cells and cell lines expressing deletion mutants D 1-44, D 1-77, and N-288 showed very weak aggregation under the present assay condition. There was a good correlation between the aggregating activity and the behavior of plated cells in the microscopy experiments described above for each cell line (Fig. 4). Interestingly, D257 mutation, which inhibits PS1 endoproteolysis also leads to inability of mutant PS1 to increase cell aggregation in transfected HEK293 cells [25]. Thus, we can conclude that L cells adhere to each other only when they express full-length functional PS1.

Next, we examined whether L cells expressing wild-type GFP-PS1 (WT) and nontransfected L cells (NT) adhere to each other. Cell lines were differentially labeled with DiO (WT) and DiI (NT). Labeled cells were mixed, and segregation pattern was analyzed for 16 h in the presence of ponasteron A. As seen in Fig. 6 (panel 0 h), in the absence of ponasteron A, cells were randomly intermixed without formation of clusters of either cell type. This pattern did not change after 16 h.

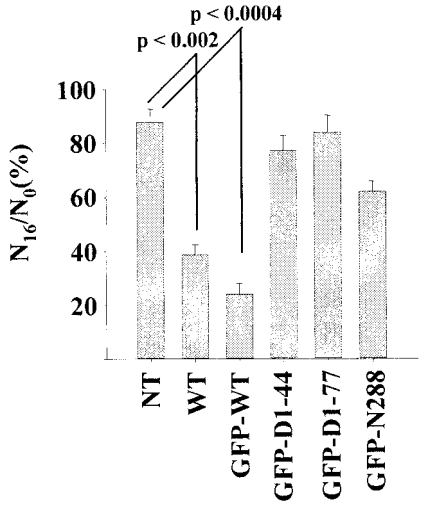

FIG. 5. Aggregation of L cells and PS1 transfectants. Cell aggregation assay was performed as described [51]. L cells (2×10^5/ml) were dissociated by trypsin–EDTA treatment and gentle pipetting, and were allowed to aggregate in complete MEM media with 5% serum and 50 μM ponasteron A for 16 h. The extent of cell aggregation was represented by the index N_{16}/N_0, where N_{16} was the total "particle" number after 16 h and N_0 was the total particle number at the incubation time 0. Single particle represents single cell or single cellular cluster (4–30 cells) in the field of view. Particle numbers have been calculated in Levy chamber using light microscopy. Cell lines: NT, nontransfected L cells; WT, L cells transfected with wild-type untagged PS1; GFP-WT, L cells transfected with wild-type GFP-PS1; GFP-D1-44 and GFP-D1-77, L cells transfected with GFP-PS1 constructs contained deletions of the N terminus of PS1 corresponding to positions 1–44 and 1–77, respectively; GFP-N-288, L cells transfected with GFP-PS1 construct, which had a stop codon in position 288. Each bar represents the average of three independent experiments ± SEM. The statistical significance of the observed differences in aggregation was determined using Student's *t* test (*P*).

We observed another cell behavior after induction of GFP-PS1 expression. By 2 h, we observed the beginning of aggregation of WT cells and nonchanged pattern of NT cells (Fig. 6, panel 2 h). After 16 h, WT cells had segregated from NT cells and formed numerous clusters of different size (Fig. 6, panel WT, 16 h). At this time most NT cells remained as single cells. Although some aggregates contained cells of both types, WT cells demonstrated a clear preference for independent aggregation (Fig. 6, panel NT, 16 h).

It should be noted that we did not find any differences in cell attachment to plastic, collagen-, or laminin-coated plates between nontransfected L cells and cell lines expressing WT GFP-PS1 or deletion mutants D 1-44, D 1-77, and N-288 (data not shown). These results indicate that PS1 is likely not involved in cell–matrix interactions, at least under the experimental conditions used.

Cell Surface Expression of PS1 in Primary Neuronal Cultures

In primary neuronal cultures, neural cell adhesion molecules have a distinct feature—concentration at the surface of extended growth cones [5, 35]. Therefore, as a first step to understand the role of PS1 in neural adhesion, we analyzed cell surface expression of PS1 in primary cultures of mouse cortical neurons. Although direct biotinylation of cell surface demonstrated specific labeling of PS1 fragments in different cell lines [59, 62], topology of PS1 at the plasma membrane is not clear at present. Therefore, we used several anti-PS1 antibodies directed against the N-terminal, C-terminal, or loop PS1 sequences. Positive results were obtained only with antibody PS1-Nm raised against the N-terminal fragment (31–46) of mouse PS1.

Western blot analysis and immunofluorescence were used to verify specificity of PS1-Nm antibody (Fig. 7). As seen in Fig. 7A, PS1-Nm antibody recognized N-terminal fragment and full-length PS1 in the brain extract of PS1 (+/+) mice and did not show any immunoreactivity in brain extracts of PS1 (−/−) knockout mice. Immunofluorescent staining of permeabilized primary neuronal cultures from PS1 (+/+) mice showed intense intracellular staining of PS1, low PS1 immunoreactivity in neuritic processes, and concentration of PS1 in growth cones (Figs. 7C and 7D). In control experiments, immunostaining of neurons from PS1 (−/−) mice was not observed (Fig. 7B). For analysis of cell surface expression of PS1 we used high-resolution scanning electron microscopy. We found that PS1 immunoreactivity was concentrated on the surface of growth cones of longest processes and was almost absent on the surface of neurons and neuritic processes (Fig. 8).

DISCUSSION

In order to understand the role of PS1 in cell–cell adhesion, the cellular localization of PS1 must be primarily clarified. Previous studies demonstrated that PS1 in transfected cell lines predominantly localizes to the ER–Golgi compartments [11, 37]. However, subcellular localization of PS1 *in vivo* may differ significantly from that in transfected cells. Our immunohistochemical analysis of human epithelium showed predominant localization of PS1 to the plasma membrane without enhanced staining of ER–Golgi compartments (Fig. 1A). Similar to our results, PS1 was accumulated at the plasma membrane in Drosophila tissue [76]. Georgakopoulos *et al.* [25] reported concentration of PS1 in the intercellular junctions of rat corneal epithelium and MDCK cells. Beher *et al.* [4] demonstrated a significant enrichment of the PS1 fragments in purified synaptic plasma membrane of rat brain and the lack of coenrichment of the PS1 fragments and the ER marker protein Sec61β during fractionation. These latter findings suggest that accumulation of PS1 in the ER and Golgi may be a transient process and subsequent redistribution of PS1 to the plasma membrane occurs in

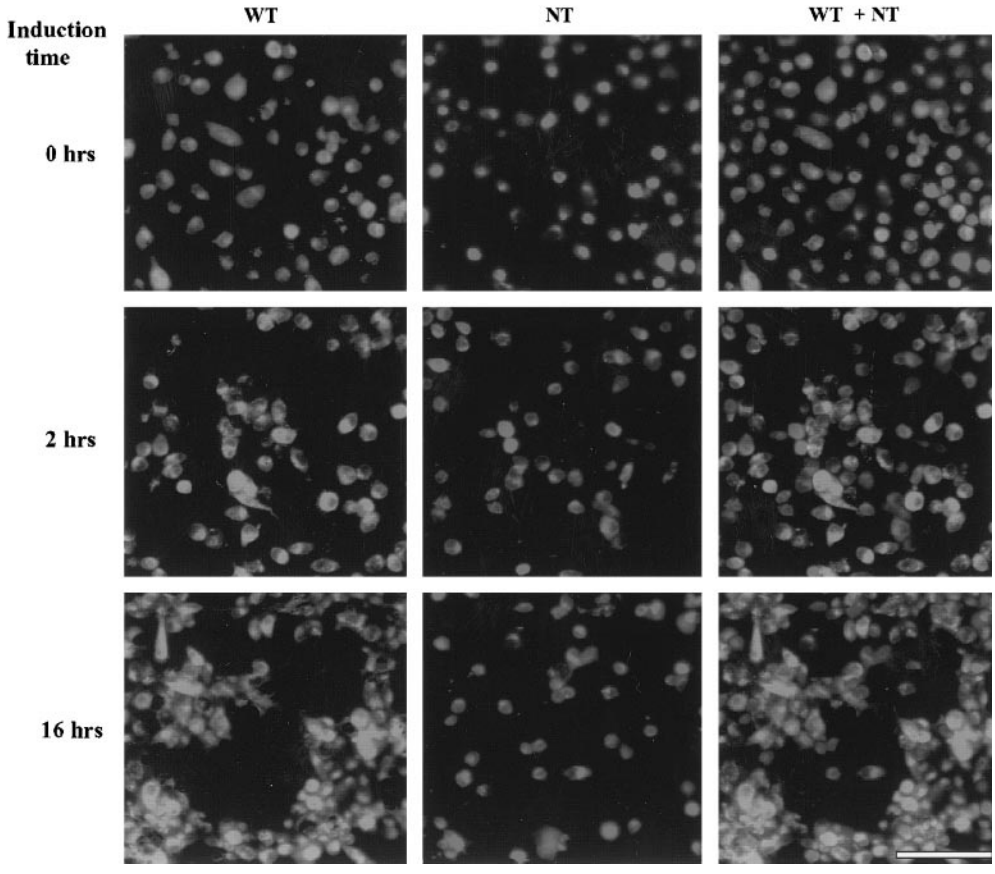

FIG. 6. Segregation pattern of mixed nontransfected L cells and L cells expressing wild-type GFP-PS1. Nontransfected (NT) and wild-type GFP-PS1 transfectants (WT) were mixed in 1:1 ratio and cultured 16 h in the presence of 50 μM ponastron A. To distinguish between the two cell lines, NT and WT cells were labeled with DiI and DiO, respectively. Cell cultures were monitoring at different times to follow the progress of cell segregation. Immunofluorescent images for each time point represent the same field. Bar, 100 μm.

response to specific extracellular signals. Conversely, lack of physiological stimuli may partially or completely abolish redistribution of PS1 in transfected cell lines. In summary, the localization of PS1 at the cell–cell contacts in epithelial cells and neurons is consistent with the suggested adhesion function of this protein in polarized cells.

In addition to localization studies, PS1 demonstrates evident adhesion properties when introduced into L cells that are deficient in intercellular adhesive interactions. Whereas the parental L cells did not form tight intercellular contacts, the GFP-PS1 transfectants formed small clusters of adhered cells. In such clusters GFP-PS1 was located mainly at the cell–cell contact sites of plasma membrane (Fig. 4). Moreover, L cells expressing GFP-PS1 exhibited typical morphological changes and homophilic interactions reported for different cadherin transfectants of L cells [51, 49, 24]. Although these results clearly demonstrate the role of PS1 in cell–cell adhesion, the molecular mechanism behind the observed effects is not quite clear. There are two potential mechanisms that can explain the adhesion properties of PS1. The first one is that PS1 may act as a regulator of expression of new CAMs. Although we did not detect E-, N-, and P-cadherins in transfected L cells and did not observed changing of cell behavior after removal of extracellular calcium, we cannot exclude the possibility that PS1 regulates expression of new Ca-independent CAMs. PS1 may also contribute to stabilization of new adhesion complexes, as was recently suggested for epithelial cells [25]. Another possibility is that the cell surface domain of PS1 is directly involved in homophilic cell–cell interactions. This suggestion, however, needs additional experimental details because topology of PS1 at the plasma membrane remains unclear. The failure of PS1 mutants with the N-terminal truncation to induce intercellular contacts does not automatically mean that the N terminus of PS1 is extracellular. These results rather demonstrate that only full-length PS1 is functional in cell–cell adhesion. Direct biotinylation of the cell surface resulted in specific labeling of the N-terminal and C-terminal PS1 fragments in different cell lines [62, 59] but all attempts to label cell surface PS1 in epithe-

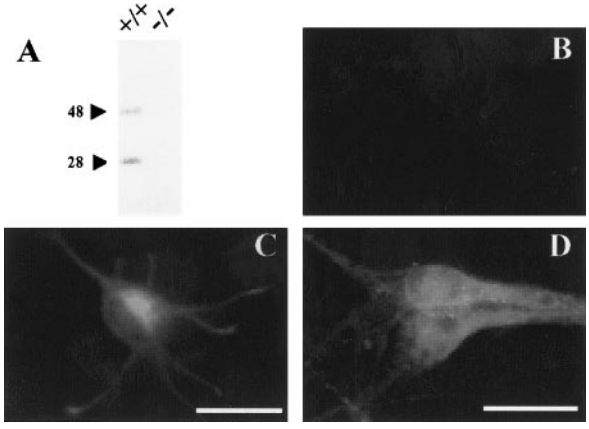

FIG. 7. Expression of PS1 in primary cultures of mouse cortical neurons. (A) Western blot analysis of PS1 in brain extracts from PS1 (+/+) and PS1 (−/−) mice with N-terminal antibody PS1-Nm. Arrows show positions of full-length PS1 (48 kDa) and N-terminal fragment of PS1 (28 kDa). (B) Immunofluorescent staining of PS1 (−/−) primary cultured neurons (Day E14) with PS1-Nm). Permeabilized cells. (C) Immunofluorescent staining of PS1 (+/+) primary cultured neurons (Day E14) with PS1-Nm. Bar, 20 μM. Permeabilized cells. (D) Immunofluorescent localization of PS1 in growth cone in PS1 (+/+) primary cultured neurons (Day E14) with PS1-Nm. Bar, 2 μM. Permeabilized cells.

lial cells using different anti-PS1 antibodies were unsuccessful [25]. It is likely that these contradictions may reflect experimental limitations in the detection of extracellular PS1 as well as the complexity of membrane topology of PS1.

Distinct topological models for PS1 have been proposed [18, 43, 17, 14, 39, 52]. However, topology of PS1 at the plasma membrane is still unknown and results are conflicting. Nakai *et al.* [52] suggested that PS1 has multiple membrane topologies and PS1 molecules expressed on the cell surface may reveal a membrane topological structure distinct from those retained in ER. Moreover, Ota *et al.* [58] demonstrated that hydrophobicity is not, as previously thought, an absolute requirement for the formation of transmembrane segments, but rather hydrophilic domains of integral transmembrane proteins have considerable freedom to move across the membrane. It should be noted that multiple membrane topology has been previously reported for different cellular proteins [84].

In fact, multiple membrane topologies of PS1 may reflect multiple cellular functions of the protein. Latest studies demonstrated many potential binding partners for PS1, which can be implicated in cell signaling or cell adhesion (reviewed in [60]). Although the biological relevance of most these interactions is not currently clear, it appears that *in vivo* PS1 may exist as a part of large dynamic multifunctional complex, which functions in several signal transduction pathways and intercellular interactions. Multiple cellular functions of PS1 are particularly evident when considering the interaction of PS1 with β-catenin. β-Catenin is a multifunctional protein, which independently mediates gene expression and cell adhesion (reviewed in [72]) [82]. Several groups demonstrated that PS1 regulates the intracellular trafficking of β-catenin and, thus, may function in cell signaling [85, 34]. Our results suggest that the PS1–β-catenin interaction is important for assembling PS1 into adherens junctions and PS1-mediated cell–cell adhesion.

Analysis of current literature shows that adhesion

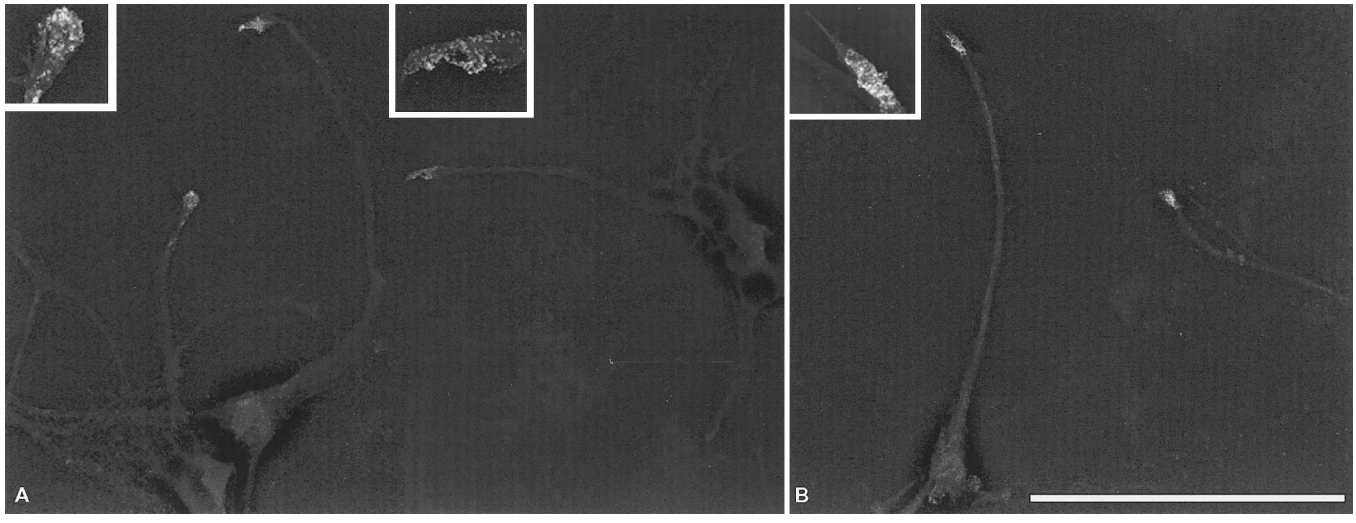

FIG. 8. Scanning immunoelectron micrographs of primary cultured neurons. Primary neuronal culture from PS1 (+/+) mice were stained with N-terminal antibody PS1-Nm. Bound antibody was visualized with 6 nM gold-labeled antibody to rabbit IgG, followed by silver enhancing. Magnification, 350X. Bar, 67. 2 μm. Growth cones, which were analyzed at magnification 10,000X, are shown in the inserts. Note that cell surface PS1 is expressed primarily on growth cones; (A) noncontacting growth cones; (B) contacting growth cones.

properties of PS1 are strikingly similar to those of known neuronal cell adhesion molecules (NCAM). Several studies have demonstrated that NCAM may regulate axonal guidance or outgrowth and synapse formation [26, 31, 32, 75, 36]. Localization studies demonstrate concentration of NCAMs in synaptic junctions of mammalian brain [23, 31] and at the surface of lamellipodia of extended growth cones in primary neuronal cultures [5, 35]. Moreover, morphology of cadherin-based adherens junctions in epithelial cells is known to be remarkably similar to those of synapses [55, 10]. Similar properties were described for PS1. Indeed, PS1 enhances neurite outgrowth *in vitro* [6, 20] and accumulates in growth cones in primary neuronal cultures [9, 86; see also Fig. 8). Furthermore, a significant concentration of PS1 at the cell–cell contacts of epithelial cells (Fig. 1) closely parallels its accumulation in synaptic plasma membrane of rodent brain [4, 25].

Hartmann *et al.* [30] described an abnormal migration of developing neurons in PS1-knockout mice. Interestingly, very similar abnormalities in neuronal migration were detected in mice lacking neural cell adhesion protein L1 (87). Moreover, recently Dowjat *et al.* [20] demonstrated that two FAD-causing PS1 mutations M 146 and P 117 inhibit neurite outgrowth, which is known as an adhesion-dependent process mediated by a number of NCAMs.

Mutations in the PS1 gene cosegregate with autosomal dominant inheritance of FAD [64]. The common classical feature of autosomal dominant disorders is haploid insufficiency of the functional gene product, which leads to developmental or age-associated phenotypes. Consistent with this, Levitan *et al.* [42] reported that wild-type PS1, but not FAD mutants, rescued an egg-laying defect in *C. elegans* lacking the PS1 homolog sel-12. At the same time, it has been shown that a FAD-linked PS1 variant (A246E) rescued the developmental abnormalities of PS1 null embryos [13, 57]. These observations suggest that one FAD allele does not affect normal embryonic development but may not be sufficient for the maintenance of synapses and neuronal survival in the adult brain.

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REFERENCES

1. Annaert, W. G., Levesque, L., Craessaerts, K., Dierinck, I., Snellings, G., Westaway, D., George-Hyslop, P. S., Cordel, B., Fraser, P., and De Strooper, B. (1999). Presenilin 1 controls gamma-secretase processing of amyloid precursor protein in pre-golgi compartments of hippocampal neurons. *JBC* **147,** 277–294.
2. Artavanis-Tsakonas, S., Rand, M. D., and Lake, R. J. (1999). Notch signaling: Cell fate control and signal integration in development. *Science* **284,** 770–776.
3. Bacallao, R., Antony, C., Dotti, C., Karsenti, E., Stelzer, E. H., and Simons, K. (1989). The subcellular organization of Madin–Darby canine kidney cells during the formation of a polarized epithelium. *J. Cell Biol.* **109,** 2817–2832.
4. Beher, D., Elle, C., Underwood, J., Davis, J. B., Ward, R., Karran, E., Masters, C. L., Beyreuther, K., and Multhaup, G. (1999). Proteolytic fragment of Alzheimer's disease-associated presenilin 1 are present in synaptic organells and growth cone membranes of rat brain. *J. Neurochem.* **72,** 1564–1537.
5. Benson, D. L., and Tanaka, H. (1998). N-cadherin redistribution during synaptogenesis in hippocampal neurons. *J. Neurosci.* **18,** 6892–6904.
6. Berezovska, O., Frosch, M., McLean, P., Knowles, R., Koo, E., Kang, D., Shen, J., Lu, F. M., Lux, S. E., Tonegawa, S., and Hyman, B. T. (1999). The Alzheimer-related gene presenilin 1 facilitates notch 1 in primary mammalian neurons. *Brain Res. Mol. Brain Res.* **69,** 273–280.
7. Bradke, F., and Dotti, C. G. (1998). Membrane traffic in polarized neurons. *Biochim. Biophys. Acta* **1404,** 245–258.
8. Brown, M. S., Ye, J., Rawson, R. B., and Goldstein, J. L. (2000). Regulated intramembrane proteolysis: A control mechanism conserved from bacteria to humans. *Cell* **100,** 391–398.
9. Busciglio, J., Hartmann, H., Lorenzo, A., Wong, C., Baumann, K., Sommer, B., Staufenbiel, M., and Yankner, B. A. (1997). Neuronal localization of presenilin-1 and association with amyloid plaques and neurofibrillary tangles in Alzheimer's disease. *J. Neurosci.* **17,** 5101–5107.
10. Colman, D. R. (1997). Neurites, synapses, and cadherin reconciled. *Mol. Cell. Neurosci.* **10,** 1–6.
11. Cook, D. G., Sung, J. C. Golde, T. E., Felsenstein, K. M., Wojczyk, B. S., Tanzi, R. E., Trojanowski, J. Q., Lee, V. M., and Doms, R. W. (1996). Expression and analysis of presenilin 1 in a human neuronal system: Localization in cell bodies and dendrites. *Proc. Natl. Acad. Sci. USA* **93,** 9223–9228.
12. Crook, R., Verkkoniemi, A., Perez-Tur J., Mehta, N., Baker, M., Houlden, H., Farrer, M., Hutton, M., Lincoln, S., Hardy, J., Gwinn, K., Somer, M., Paetau, A., Kalimo, H., Ylikoski, R., Poyhonen, M., Kucera, S., and Haltia, M. (1998). A variant of Alzheimer's disease with spastic paraparesis and unusual plaques due to deletion of exon 9 of presenilin 1. *Nature Med.* **4,** 394–395.
13. Davis, J. A., Naruse, S., Chen, H., Eckman, C., Younkin, S., Price, D. L., Borchelt, D. R., Sisodia, S. S., and Wong, P. C. (1998). An Alzheimer's disease-linked PS1 variant rescues the developmental abnormalities of PS1-deficient embryos. *Neuron* **20,** 603–609.
14. De Strooper, B., Beullens, M, Contreras. B., Levesque, L., Craessaerts. K., Cordell, B., Moechars, D., Bollen, M., Fraser P., George-Hyslop, P. S., and Van Leuven, F. (1997). Phosphorylation, subcellular localization, and membrane orientation of the Alzheimer's disease-associated presenilins. *JBC* **272,** 359–358.
15. De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998). Deficiency of presenilin-1 inhibits the normal cleavage of amyloid precursor protein. *Nature* **391,** 387–390.
16. De Strooper, B., Annaert, W., Cupers, P., Saftig, P., Craessaerts, K., Mumm, J. S., Schroeter, E. H., Schrijvers, V., Wolfe,

M. S., Ray, W. J., Goate, A., and Kopan, R. (1999). A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. *Nature* **398,** 518–522.

17. Dewji, N. N., and Singer, S. J. (1997). The seven-transmembrane spanning topography of the Alzheimer disease-related presenilin proteins in the plasma membranes of cultured cells. *Proc. Natl. Acad. Sci. USA* **94,** 14025–14030.
18. Doan, A., Thinakaran, G., Borchelt, D. R., Slunt, H. H., Ratovitsky, T., Podlisny, M., Selkoe, D. J., Seeger, M., Gandy, S. E., Price, D. L., and Sisodia, S. S. (1996). Protein topology of presenilin 1. *Neuron* **17,** 1023–1030.
19. Dotti, C. G., and Simons, K. (1990). Polarized sorting of viral glycoproteins to the exon and dendrites of hippocampal neurons in culture. *Cell* **62,** 63–72.
20. Dowjat, W. K., Wisniewski, T., Efthimiopoulos, S., and Wisniewski, H. M. (1999). Inhibition of neurite outgrowth by familial Alzheimer's disease-linked presenilin-1 mutations. *Neurosci. Let.* **267,** 141–144.
21. Edelman, G. M., Murray, B. A., Mege, E-M., Cunningham, B. A., and Gallin, W. J. (1987). Cellular expression of liver and neural cell adhesion molecules after transfection with their cDNAs results in specific cell-cell binding. *Proc. Natl. Acad. Sci. USA* **84,** 8502–8506.
22. Efthimiopoulos, S., Floor, E., Georgakopoulos, A., Shioi, J., Cui, W., Yasothornsrikul, S., Hook, V. Y., Wisniewski, T., Buee, L., and Robakis, N. K. (1998). Enrichment of presenilin 1 peptides in neuronal large dense-core and somatodendritic clathrin-coated vesicles. *J. Neurochem.* **71,** 2365–2372.
23. Fannon, A. M., and Colman, D. R. (1996). A model for central synaptic junction complex formation based on the differential adhesive specificity of the cadherins. *Neuron* **17,** 423–434.
24. Friedlander, D. R., Mege, R-M., Cunningham, B. A., and Edelman, G. (1989). Cell sorting-out is modulated by both specificity and amount of different cell adhesion molecules (CAMs) expressed on cell surface. *Proc. Natl. Acad. Sci. USA* **86,** 7043–7047.
25. Georgakopoulos, A., Marambaud, P., Efthimiopoulos, S., Shio, J., Cui, W., Li, H-C., Schutte, M., Gordon, R., Holstein, G. R., Martinelli, G., Mehta, P., Friedrich, V. L., Jr., and Robakis, N. K. (1999). Presenilin-1 forms complexes with the cadherin/catenin cell-cell adhesion system and is recruited to intercellular and synaptic contacts. *Mol. Cell* **4,** 1–20.
26. Goodman, C. S. (1996). Mechanism and molecules that control growth cone guidance. *Annu. Rev. Neurosci.* **19,** 341–377.
27. Grotewiel, M. S., Beck, C. D. O, Wu, K. H., Zhu, X-R., and Davis, R. L. (1998). Integrin-mediated short-term memory in Drosophila. *Nature* **391,** 455–460.
28. Haass, C., and De Strooper, B. (1999). The presenilins in Alzheimer's disease—Proteolysis holds the key. *Science* **286,** 916–919.
29. Hardy, J. (1997). Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci.* **20,** 154–159.
30. Hartmann, D., De Strooper, B., and Saftig, P. (1999). Presenilin-1 deficiency leads to loss of Cajal-Retzius neurons and cortical dysplasia similar to human type 2 lissencephaly. *Curr. Biol.* **9,** 719–727.
31. Inoue, A., and Sanes, J. R. (1997). Lamina-specific connectivity in the brain: Regulation by N-cadherin, neurotrophins, and glycoconjugates. *Science* **276,** 1428–1431.
32. Iwai, Y., Usui, T., Hirano, S., Steward, R., Takeichi, M., and Uemura, T. (1997). Axon patterning requires DN-cadherin, a novel neuronal adhesion receptor, in the Drosophila embryonic CNS. *Neuron* **19,** 77–89.
33. Johnston, J., Ward, C. L., and Kopito, R. R. (1998). Aggresomes: A cellular response to misfolded proteins. *J. Cell. Biol.* **143,** 1883–1898.
34. Kang, D. E., Soriano, S., Frosch, M. P., Collins T., Naruse, S., Sisodia S. S., Leibowitz, G., Levine F., and Koo, E. H. (1999). Presenilin 1 facilitates the constitutive turnover of beta-catenin: Differential activity of Alzheimer's disease-linked PS1 mutants in the beta-catenin-signaling pathway. *J. Neurosci.* **19,** 4229–4237.
35. Kenwrick, S., and Doherty, P. (1998). Neural cell adhesion molecules L1: Relating disease to function. *BioEssays* **20,** 668–675.
36. Kohmura, N., Senzaki, K., Hamada, S., Kai, N., Yasuda, M., Mishina, M., and Yagi, T. (1998). Diversity revealed by a novel family of cadherins expressed in neurons at synaptic complex. *Neuron* **20,** 1137–1151.
37. Kovacs, D. M., Fausett, H. J., Page, K. J., Kim, T. W., Moir, R. D., Merriam, D. E., Hollister, R. D., Hallmark, O. G., Mancini, R., Felsenstein, K. M., Hyman, B. T., Tanzi, R. E., and Wasco W. (1996). Alzheimer-associated presenilins 1 and 2: Neuronal expression in brain and localization to intracellular membranes in mammalian cells. *Nature Med.* **2,** 224–229.
38. Lah, J. J., Heilman, C. J., Nash, N. R., Rees, H. D., Yi, H., Counts, S. E., and Levey, A. (1997). Light and electron microscopic localization of presenilin-1 in primate brain. *J. Neurosci.* **17,** 1971–1980.
39. Lehmann, S., Chiesa, R., and Harris, D. A. (1997). Evidence for a six-transmembrane domain structure of presenilin 1. *JBC* **272,** 12047–12051.
40. Lesley J., Hyman, R., and Kincade, P. W. (1993). CD44 and its interaction with extracellular matrix. *Adv. Immunol.* **54,** 271–335.
41. Levitan, D., and Greenwald, I. (1995). Facilitation of lin-12-mediated signalling by sel-12, a Caenorhabditis elegans S182 Alzheimer's disease gene. *Nature* **377,** 351–355.
42. Levitan, D., Doyle, T. G., Brousseau, D., Lee, M. K., Thinakaran, G., Slunt, H. H., Sisodia, S. S., and Greenwald, I. (1996). Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans*. *Proc. Natl. Acad. Sci. USA* **93,** 14940–14944.
43. Li, X., and Greenwald, I. (1996). Membrane topology of the *C. elegans* SEL-12 presenilin. *Neuron,* **17,** 1015–1021.
44. Li, X., and Greenwald, I. (1998). Additional evidence for an eight-transmembrane-domain topology for Caenorhabditis elegans and human presenilins. *Proc. Natl. Acad. Sci. USA* **95,** 7109–7114.
45. Li, J., Xu, M., Zhou, H., Ma, J., and Potter, H. (1997). Alzheimer presenilins in the nuclear membrane, interphase kinetochores, and centrosomes suggest a role in chromosomal segregation. *Cell* **90,** 917–927.
46. Matsudaira, P. (1994). Actin crosslinking proteins at the leading edge. *Semin. Cell Biol.* **5,** 165–174.
47. Mattson, M. P., and Guo, Q. (1997). Cell and molecular neurobiology of presenilins: A role for the endoplasmic reticulum in the pathogenesis of Alzheimer's disease? *J. Neurosci. Res.* **50,** 505–513.
48. Mattson, M. P., Guo, Q., Furukawa, K., and Pedersen, W. A. (1998). Presenilins, the endoplasmic reticulum, and neuronal apoptosis in Alzheimer's disease. *J. Neurochem.* **70,** 1–14.
49. Mege, R-M., Matsuzaki, F., Gallin, W. J., Goldberg, J. I., Cunningham, B. A., and Edelman, G. M. (1988). Construction of epithelioid sheets by transfection of mouse sarcoma cells with c

DNAs for chicken cell adhesion molecules. *Proc. Natl. Acad. Sci. USA* **85,** 7274–7278.

50. Mercken, M., Takahashi, H., Honda, T., Sato, K., Murayama, M., Nakazato, Y., Noguchi, K., Imahori, K., and Takashima A. (1996). Characterization of human presenilin 1 using N-terminal specific monoclonal antibodies: Evidence that Alzheimer mutations affect proteolytic processing. *FEBS Lett.* **389,** 297–303.
51. Nagafuchi, A., Shirayoshi, Y., Okazaki, K., Yasuda, K., and Takeichi, M. (1987). Transformation of cell adhesion properties by exogenously introduced E-cadherin c DNA. *Nature* **329,** 341–343.
52. Nakai, T., Yamasaki, A., Sakaguchi, M., Kosaka, K., Mihara, K., Amaya, Y., and Miura, S. (1999). Membrane topology of Alzheimer's disease-related presenilin 1. Evidence for the existence of a molecular species with a seven membrane-spanning and one membrane-embedded structure. *J. Biol. Chem*. **274,** 23647–23658.
53. Nose, A., Nagafuchi, A., and Takeichi, M. (1988). Expressed recombinant cadherins mediate cell sorting in model systems. *Cell* **54,** 993–1001.
54. Parent, A., Linden, D. J., Sisodia, S. S., and Bochelt, D. R. (1999). Synaptic transmission and hippocampal long-term potentiatin in transgenic mice expressing FAD-linked presenilin 1. *Neurobiol. Dis.* **6,** 56–62.
55. Peters, A., and Palay, S. L. (1996). The morphology of synapses. *J. Neurocytol.* **25,** 687–700.
56. Perez-Tur, J., Froelich, S., Prihar, G., Crook, R., Baker, M., Duff, K., Wragg, M., Busfield, F., Lendon C., Clark, R. F., Roques, P., Fuldner, R. A., Johnston, J., Cowburn, R., Forsell, C., Axelman, K., Lilius, L., Houlden, H., Karran, E., Roberts, G. V., Rossor, M., Adams, M. D., Hardy, J., Goate, A., Lannfelt, L., and Hutton, M. (1995). A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene. *Neuroreport* **7,** 297–301.
57. Qian, S., Jiang, P., Guan, X. M., Singh, G., Trumbauer, M. E., Yu, H., Chen, H. Y., Van de Ploeg, L. H., and Zheng, H. (1998). Mutant human presenilin 1 protects presenilin 1 null mouse against embryonic lethality and elevates Abeta1-42/43 expression. *Neuron* **20,** 611–617.
58. Ota, K., Sakaguchi, N., vonHeijne, G., Hamasaki, N., and Mihara, K. (1998). Forced transmembrane orientation of hydrophilic polypeptide segments in multispanning membrane proteins. *Mol. Cell* **2,** 495–503.
59. Ray, W. J., Yao, M., Mumm, J., Schroeter, E. H., Saftig, P., Wolfe, M., Selkoe, D. J., Kopan, R., and Goate, A. M. (1999). Cell surface presenilin-1 participates in the gamma-secretase-like proteolysis of Notch. *JBC* **274,** 36801–36807.
60. Saftig, P., Hartman, D., and De Strooper, B. (1999). The function of presenilin 1 in amyloid beta-peptide generation and brain development. *Eur. Arch. Psychiatry Clin. Neurosci.* **249,** 271–279.
61. Schwarzman, A., Tsiper, M., Vitek, A., St. George-Hyslop, P., and Goldgaber, D. (1998). Identification of peptides binding to presenilin 1 by screening of random peptide display libraries. *In* "Progress in Alzheimer's and Parkinson's Disease" (A. Fisher, M. Yorshida, and I. Hanin, Eds.), pp. 141–148. Plenum Press, New York.
62. Schwarzman, A. L., Singh, N., Tsiper, M., Gregori, L., Dranovsky, A., Vitek, M., Glabe, C., St. George-Hyslop, P., and Goldgaber, D. (1999). Endogenous presenilin 1 redistributes to the lamellipodia upon adhesion of Jurkat cells to a collagen matrix. *Proc. Natl. Acad. Sci. USA* **96,** 7932–7937.
63. Shen, J., Bronson, R. T., Chen, D. F., Xia, W., Selkoe D. J., and Tonegawa, S. (1997). Skeletal and CNS defects in presenilin-1-deficient mice. *Cell* **89,** 629–639.
64. Sherington, R., Rogaev, E. I., Liang, Y., Rogaeva, E. A., Levesque, G., Ikeda, M., Chi, H., Lin, C., Li, G., Holman, K., Tsuda, T., Mar, L., Foncln, J-F., Bruni, A. C., Montesi, M. P., Sorbi, S., Rainero, I., Pinessi, L., Nee, L., Chumakov, I., Pollen, D., Brookes, A., Sanseau, P., Polinsky, R. J., Wasco, W., Da Silva, H. A. R., Hainess, J. L., Pericak-Vance, M. A., Tanzi, R. E., Roses, A. D., Fraser, P. E., Rommens, J. M., and St. George-Hyslop, P. H. (1995). Cloning of gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375,** 754–759.
65. Song, W., Nadeau, P., Yuan, M., Yang, X., Shen, J., and Yankner, B. A. (1999). Proteolytic release and nuclear translocation of Notch-1 are induced by presenilin-1 and impaired by pathogenic presenilin-1 mutations. *Proc. Natl. Acad. Sci. USA* **96,** 6959–6963.
66. Struhl, G., and Greenwald, I. (1999). Presenilin is required for activity and nuclear access of Notch in Drosophila. *Nature* **398,** 522–525.
67. Takashima, A., Sato, M., Mercken, M., Tanaka, S., Kondo, S., Honda, T., Sato, K., Murayama, M., Noguchi, K., Nakazato, Y., and Takahashi, H. (1996). Localization of Alzheimer-associated presenilin 1 in transfected COS-7 cells. *Biochem. Biophys. Res. Commun.* **227,** 423–426.
68. Takeichi, M. (1995). Morphogenetic roles of classic cadherins. *Curr. Opinion Cell. Biol.* **7,** 619–627.
69. Tang, L., Hung, C. P., and Schuman, E. M. (1998). A role for the cadherin family of cell adhesion molecules in hippocampal long term potentiation. *Neuron* **20,** 1165–1175.
70. Tesco, G., Kim, T-W., Diehlmann, A., Beyreuther, K., and Tanzi, R. E. (1998). Abrogation of the presenilin 1/β-catenin interaction and preservation of the heterodimeric presenilin 1 complex following caspase activation. (1998). *J. Biol. Chem.* **273,** 33909–33914.
71. Vito, P., Lacana, E., and D'Adamio, L. (1996). Interfering with apoptosis: Ca(2+)-binding protein ALG-2 and Alzheimer's disease gene ALG-3. *Science* **271,** 521–525.
72. Willert, K., and Nusse, R. (1998). Beta-catenin: A key mediator of Wnt signaling. *Curr. Opin. Genet. Develop.* **8,** 95–102.
73. Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehi, T. S., Kimberly, W. T., and Selkoe, D. J. (1999). Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature* **398,** 513–517.
74. Wong, P. C., Zheng, H., Chen, H., Becher, M. W., Sirinathsinghji, D. J. S., Trumbauer, M. E., Chen, H. Y., Price, D. L., Van der Ploeg, L. H. T., and Sisodia, S. S. (1997). Presenilin 1 required for Notch 1 and DLL1 expression in the paraxial mesoderm. *Nature* **387,** 288–292.
75. Uemura, T. (1998). The cadherin superfamily at the synapse: More members, more missions. *Cell* **93,** 1095–1098.
76. Ye, Y., and Fortini, M. E. (1998). Characterization of Drosophila presenilin and its co-localization with Notch during development. *Mech. Dev.* **79,** 199–211.
77. Ye, Y., Lukinova, N., and Fortini, M. E. (1999). Neurogenic phenotypes and altered Notch processing in Drosophila Presenilin mutants. *Nature* **398,** 525–529.
78. Yeaman, C., Grindstaff, K. K., and Nelson, W. J. (1999). New perspectives on mechanisms involved in generating epithelial cell polarity. *Physiol. Rev.* **79,** 73–98.
79. Zhang, V., Han, S-V., McKeel, D., Goate, A., and Wu, J. Y. (1998a). Interaction of presenilins with the filamin family of actin-binding proteins *J. Neurosci.* **18,** 914–922.

80. Zhang, Z., Hartmann, H., Do, V. M., Abramowski, D., Sturchler-Pierrat, Staufenbiel, M., Sommer, B., Van de Wetering, M., Clevers, H., Saftig, P., De Strooper, B., He, X., and Yankner, B. A. (1998b). *Nature* **395,** 698–702.

81. Zhou, J., Liyanage, U., Medina, M., Ho, C., Simmons, A. D., Lovett, M., and Kosik, K. S. (1997). Presenilin 1 interaction in the brain with a novel member of the Armadillo. *NeuroReport* **8,** 1489–1494.

82. Zhu, A. J., and Watt, F. M. (1999). Beta-catenin signalling modulates proliferative potential of human epidermal keratinocytes independently of intercellular adhesion. *Development* **126,** 2285–2298.

83. Kunkel, T. A. (1985). Rapid and efficient site-specific mutagenesis without phenotypic selection. *Proc. Natl. Acad. Sci. USA.* **82,** 488–492.

84. Levy, D. (1996). Membrane proteins which exhibit multiple topological orientation. *Essays Biochem*. **31,** 49–60.

85. Nishimura, M., Yu, G., Levesque, G., Zhang, D. M., Ruel, L., Chen, F., Milman, P., Holmes, E., Liang, Y., Kawarai, T., Jo, E., Supala, A., Rogaeva, E., Xu, D. M., Janus, C., Levesque, L., Bi, Q., Duthie, M., Rozmahel, R., Mattila, K., Lannfelt, L., Westaway, D., Mount, H. T., Woodgett, J., and St George-Hyslop, P. (1999). Presenilin mutations associated with Alzheimer disease cause defective intracellular trafficking of beta-catenin, a component of the presenilin protein complex. *Nature Med.* **5,** 164–169.

86. Levesque, L., Annaert, W., Craessaerts, K., Mathews, P. M., Seeger, M., Nixon, R. A., Van Leuven, F., Gandy, S., Westaway, D., St George-Hyslop, P., De Strooper, B., and Fraser, P. E. (1999). Developmental expression of wild-type and mutant presenilin-1 in hippocampal neurons from transgenic mice: Evidence for novel species-specific properties of human presenilin-1. *Mol. Med.* **5,** 542–554.

87. Cohen, N. R., Taylor, J. S., Scott, L. B., Guillery, R. W., Soriano, P., and Furley, A. J. (1998). Errors in corticospinal axon guidance in mice lacking the neural cell adhesion molecule L1. *Current Biol.* **8,** 26–33.

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