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Presenilin 1 Facilitates the Constitutive Turnover of β-Catenin: Differential Activity of Alzheimer's Disease–Linked PS1 Mutants in the β-Catenin–Signaling Pathway

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Although an association between the product of the familial Alzheimer's disease (FAD) gene, presenilin 1 (PS1), and β-catenin has been reported recently, the cellular consequences of this interaction are unknown. Here, we show that both the full length and the C-terminal fragment of wild-type or FAD mutant PS1 interact with β-catenin from transfected cells and brains of transgenic mice, whereas E-cadherin and adenomatous polyposis coli (APC) are not detected in this complex. Inducible overexpression of PS1 led to increased association of β-catenin with glycogen synthase kinase-3β (GSK-3β), a negative regulator of β-catenin, and accelerated the turnover of endogenous β-catenin. In support of this finding, the β-catenin half-life was dramatically longer in fibroblasts deficient in *PS1*, and this phenotype was completely rescued by replacement of PS1, demonstrating that PS1 normally stimulates the degradation of β-catenin. In contrast, overexpression of FAD-linked PS1 mutants (M146L and ΔX9) failed to enhance the association between GSK-3β and β-catenin and interfered with the constitutive turnover of β-catenin. *In vivo* confirmation was demonstrated in the brains of transgenic mice in which the expression of the M146L mutant PS1 was correlated with increased steady-state levels of endogenous β-catenin. Thus, our results indicate that PS1 normally promotes the turnover of β-catenin, whereas PS1 mutants partially interfere with this process, possibly by failing to recruit GSK-3β into the PS1–β-catenin complex. These findings raise the intriguing possibility that PS1–β-catenin interactions and subsequent activities may be consequential for the pathogenesis of AD.

Key words: presenilin; β-catenin; glycogen synthase kinase-3β; immunoprecipitation; turnover; half-life; Alzheimer's disease

Inherited mutations in the gene coding for presenilin 1 (PS1) cause the most aggressive form of Alzheimer's disease (AD) and account for a large proportion of familial early-onset AD (FAD) (Sherrington et al., 1995). However, neither the physiological nor the aberrant activities associated with FAD-linked presenilin gene products are clearly understood. PS1, a six to eight multipass transmembrane protein (Doan et al., 1996; De Strooper et al., 1997; Lehmann et al., 1997) enriched in nuclear, endoplasmic reticulum (ER), and Golgi membranes (Kovacs et al., 1996; Li et al., 1997), undergoes constitutive endoproteolytic processing into relatively stable N- and C-terminal fragments in the ER (Thinakaran et al., 1996; J. Zhang et al., 1998). *PS1* deficiency in mice results in an embryonic lethal phenotype associated with severe malformations of the axial skeleton and cerebral hemorrhage (Shen et al., 1997; Wong et al., 1997). In cell culture, we have likewise demonstrated that inhibition of PS1 expression in Ntera2 neuronal precursor cells results in the loss of neuronal differentiation and the concomitant increase in cell death (Hong et al., 1999). In *Caenorhabditis elegans*, PS1 facilitates signaling mediated by the Lin-12/Notch family of receptors (Levitan and Greenwald, 1995). However, the molecular processes by which PS1 exerts these functions are unknown.

Recent studies have documented the interaction of PS1 with β-catenin, suggesting that PS1 may be involved in modulating the Wnt–β-catenin signaling pathway (Zhou et al., 1997; Yu et al., 1998). The binding of Wnt ligands to cell-surface receptors initiates a cascade of intracellular signals resulting in the inactivation of glycogen synthase kinase-3β (GSK-3β) and the translocation of β-catenin into the nucleus (Orsulic and Peifer, 1996). Subsequently, the association of β-catenin with the T-cell factor/lymphoid enhancer factor-1 family of transcription factors mediates the expression of downstream genes (Behrens et al., 1996). In the absence of Wnt stimulation, β-catenin is rapidly targeted for degradation via the ubiquitin-proteosome pathway, a step that requires the phosphorylation of β-catenin by GSK-3β and the binding to adenomatous polyposis coli (APC) (Aberle et al., 1997; Morin et al., 1997). It was shown recently that axin negatively regulates β-catenin by bridging β-catenin and GSK-3β together in the same complex and thereby facilitating the phosphorylation of β-catenin by GSK-3β (Hart et al., 1998; Ikeda et al., 1998).

Recent studies have documented apparently conflicting results regarding the effects of PS1 proteins on the "stability" of

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β-catenin. However, of the two recent reports, one examined the turnover of epitope-tagged β-catenin and the generation of putative β-catenin proteolytic fragments (Z. Zhang et al., 1998), whereas the other analyzed steady-state levels of endogenous, cytosolic β-catenin (Murayama et al., 1998). Because β-catenin exists in multiple cellular pools (Ozawa and Kemler, 1992; Papkoff, 1997), it is conceivable that measuring a partial, static, or transfected pool of β-catenin can yield different outcomes. In this study, we examined the turnover rate and steady-state levels of endogenous, full-length β-catenin in cultured cells and transgenic mice. Our results indicate that PS1 constitutively stimulates the turnover of endogenous β-catenin, whereas FAD-linked PS1 mutations interfere with this activity.

MATERIALS AND METHODS

Antibodies. PS1 polyclonal antibodies including J27 (against residues 27–42), 4627 (against residues 457–467), αPS1Loop (against residues 319–442), and the PSN2 monoclonal antibody (against residues 31–56) were used in this study (Thinakaran et al., 1996; J. Zhang et al., 1998). Additional monoclonal antibodies include β-catenin, E-cadherin, and GSK-3β (Transduction Laboratories, Lexington, KY) and APC (Ab-1 and Ab-5; Calbiochem, La Jolla, CA). The β-catenin polyclonal and actin monoclonal antibodies were purchased from Sigma (St. Louis, MO).

Generation of cell lines. EcR293 cells (Invitrogen, San Diego, CA) inducibly expressing PS1 variants [PS1 wild type (WT), M146L, and ΔX9] have been described previously (J. Zhang et al., 1998). At least three independent clones of each construct were generated, and representative results of two clones are shown. Induction was typically performed with 0.1–5 μM muristerone. *PS1* −/− and −/+ fibroblast cultures were prepared from the skin of embryonic day 15.5 fetal mice (Wong et al., 1997). Cells were enzymatically dissociated by the addition of 0.25% trypsin and 20 μg/ml DNase I in HBSS at 37°C for 30 min. After dissociation by repeated trituration, cells were spun at 200 × *g* and resuspended in DMEM containing 10% FCS, and the suspension was plated into 25 cm^2 flasks to yield an initial plating density of $4–5 \times 10^4$ cells/cm^2. Cells (80–90% confluent) were passaged by trypsinization and cultured as above. Immortalization of *PS1* −/− fibroblasts was accomplished by expression of SV40 T antigen. In brief, the retroviral vector plasmid expressing SV40 T antigen was constructed by replacing the chloramphenical acetyltransferase gene in the vector LoCRNL0 with the SV40 T antigen cDNA, and the virus was produced as a VSV-G pseudotype as described previously (Wang et al., 1996). Primary *PS1* −/− fibroblasts were infected by adding the virus in the presence of 4 mg/ml polybrene. Transformed PS1-deficient fibroblasts were then transfected with human PS1 WT or ΔX9 mutant subcloned into pIREShygro (Clontech, Palo Alto, CA) or vector control, and stable transfectants were selected by hygromycin resistance.

Immunoprecipitations, Western blotting, and metabolic labeling. Cultured cells were lysed in 1% NP-40 lysis buffer (50 mM Tris, pH 8.0, 150 mM NaCl, 0.02% sodium azide, 1% NP-40, 100 μg/ml [amino ethyl benzenesulfonyl fluoride (AEBSF), and 10 μg/ml leupeptin] on ice for 20 min. In experiments involving GSK-3β, 1% NP-40 lysis buffer was supplemented with 1 mM sodium vanadate and 20 mM sodium fluoride for inhibition of phosphatase activity. Lysates were precleared with normal rabbit serum and protein A sepharose (for rabbit polyclonal antibodies) or with anti-mouse IgG agarose (for mouse monoclonal antibodies) and then incubated overnight at 4°C with primary antibody in the presence of protein A sepharose or anti-mouse IgG agarose. Immune complexes were washed twice for 15 min each at room temperature in 1 ml of 1% NP-40 lysis buffer and heated to 70°C for 20 min in 2× Laemmli buffer. After separation in SDS-polyacrylamide gels (SDS-PAGE), proteins were transferred onto nitrocellulose membranes. Coimmunoprecipitated proteins were detected by the incubation of the cognate primary antibody followed by HRP-conjugated secondary antibody and enhanced chemiluminescence.

For β-catenin turnover experiments, overnight cultures of fibroblasts or EcR293 cells with or without muristerone induction were incubated in methionine-free medium for 20 min, followed by metabolic labeling with 100 μCi/ml [^{35}S]methionine for 15 min and chasing for 0, 0.5, 1, or 2 hr. At each time point, cells were lysed in 1% NP-40 lysis buffer and immunoprecipitated with a monoclonal antibody directed against the C terminal of β-catenin. Immunoprecipitates were washed twice in radioimmunoprecipitation assay buffer (50 mM Tris, 150 mM NaCl, 0.02% sodium azide, 1% NP-40, 0.5% deoxycholate, and 0.1% SDS), resuspended in 2× Laemmli buffer, and separated in 8% SDS-PAGE.

Dried gels were either exposed to film or quantitated by phosphorimaging (Bio-Rad, Hercules, CA). All cell culture experiments were performed at least three times. The results are presented either from representative experiments or as an average of all experiments as indicated.

Generation of transgenic mice and homogenization of mouse brains. The transgene constructs were assembled using a portion of the human platelet-derived growth factor (PDGF) B-chain promoter (Sasahara et al., 1991) to drive expression of PS1 cDNAs (WT or M146L mutation). A 3.4 kb fragment containing the promoter, cDNA, and other required sequences was then excised and used for pronuclear injection performed in the Transgenic Mouse Facility (Department of Pathology, Brigham and Women's Hospital, Boston, MA). Transgenic lines were expanded via breeding with inbred FVB/N mice. Genomic DNA was isolated from tail snips of 3- to 4-week-old animals using a standard proteinase K digestion procedure. RNA was isolated from brain tissue using TRIzol reagent (Life Technologies, Gaithersburg, MD). For verification of transgenic animals, PCR reactions were run with three primers: a forward PCR primer (FP1320, 5′-GGCCAGAAGAGGAAAGGCT-3′) that anneals equally well to the endogenous mouse and human PDGF B-chain promoter portion of the transgene and two reverse primers specific to either the PS1 cDNA (RP1841, 5′-GTACAGTATTGCTCAGGTGGTTGT-3′) or the mouse genomic PDGF B-chain gene (RP-B2, 5′-AGTCTGCTATCTACCCACTCGCT-3′). The transgene and endogenous mouse gene products of this reaction are 521 and 355 bp, respectively. For reverse transcription (RT)-PCR to examine mRNA expression, first-strand cDNA synthesis was performed with oligo-$dT_{12–18}$ and reverse transcriptase. The primers for the subsequent amplification consist of a common forward primer recognizing both human and mouse PS1 (RT-FP, 5′-GAGCTGCTGTCCAGGAACTTTC-3′) and two reverse primers specific to either the 3′-untranslated region of the transgene (RT-tRP, 5′-TCACTGCATTCTAGTTGTGGTTTGT-3′) or the mouse PS1 3′-untranslated region (RT-mRP, 5′-GAAACATCCATGTTCTAACTGCAGA-3). The transgene (400 bp) and endogenous mouse (360) mRNA gene products are resolved on a 1.9% agarose gel. Two controls were included to ensure that transgene-specific PCR products accurately reflect transcription: (1) DNase I treatment before reverse transcriptase to degrade any possible contaminating genomic DNA and (2) inclusion of RT samples to demonstrate that any signal required the generation of cDNA from mRNA.

Half brains (excluding the cerebellum) from transgenic mice expressing the human wild-type or FAD M146L mutant PS1 and from nontransgenic littermates were homogenized by >60 strokes in a micro-Dounce homogenizer in buffer (0.5% Triton X-100, 50 mM NaCl, 10 mM HEPES, pH 6.8, 3 mM $MgCl_2$, 300 mM sucrose, 100 μg/ml AEBSF, and 10 μg/ml leupeptin). The Triton X-100 insoluble material was precipitated twice by centrifugation and discarded. The supernatant of the second spin was then used for all subsequent procedures. Protein concentrations were measured by the micro-BCA method (Pierce, Rockford, IL).

Affinity precipitation of β-catenin from brain. To generate recombinant glutathione *S*-transferase (GST)–PS1 loop fusion protein, the putative loop region of PS1 (amino acid residues 263–407) was subcloned into pGEX4T-1 plasmid (Pharmacia, Piscataway, NJ), transformed into *Escherichia coli* strain HB101, and induced with isopropyl-β-D-thiogalactopyranoside. GST or the GST–PS1 loop fusion proteins were purified from bacterial lysates using glutathione agarose beads in accordance with the manufacturer's instructions (Pharmacia). For affinity precipitations with recombinant GST fusion proteins, clarified mouse brain lysates were first precleared with GST–agarose beads, and 1 μg of GST–PS1 loop fusion protein was added to the lysates (200 μg) in the presence of glutathione agarose beads. After 1 hr of incubation at 4°C, precipitates were washed three times with 1% NP-40 buffer and subjected to SDS-PAGE and immunoblotting for β-catenin.

RESULTS

Association of the PS1 full length and C-terminal fragment with β-catenin but not with cadherin or APC

To examine the role of PS1 in the β-catenin pathway, we generated stably transfected EcR293 cells capable of inducible overexpression of wild type or FAD mutant PS1 variants (M146L and ΔX9). Figure 1 shows the inducibility profile of EcR293 cells stably transfected with wild-type PS1 or the FAD-linked ΔX9

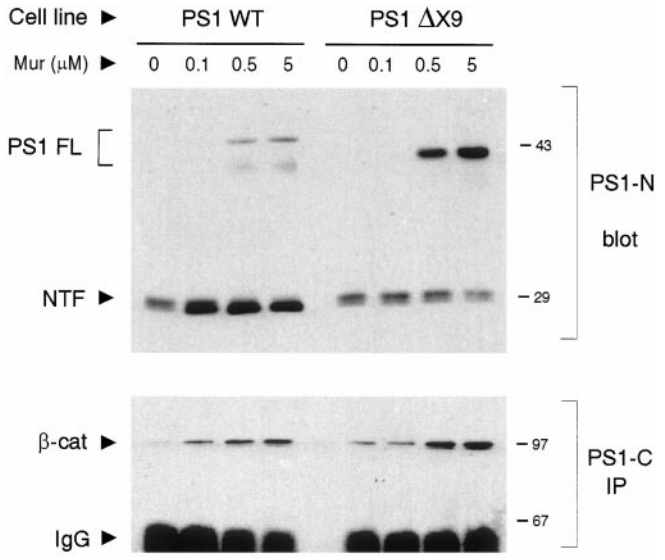


Figure 1. Induction profile of PS1 proteins in stably transfected EcR293 cells and coimmunoprecipitation of β-catenin with PS1 variants. Stably transfected EcR293 cells were treated with increasing amounts of muristerone (*Mur*; 0–5 μM for 20 hr) to induce expression of wild-type PS1 or the ΔX9 mutant, and cells were lysed in 1% NP-40 buffer. *Top*, An immunoblot of PS1 visualized by a monoclonal antibody against the N terminal of PS1 (PSN2; *PS1-N*). The full length (~45 kd) and N-terminal fragment (~29 kd) of wild-type PS1 and the truncated ΔX9 protein (~42 kd) are shown. *Bottom*, The same cell lysates immunoprecipitated with polyclonal antibody against the C terminal of PS1 (4627), followed by immunoblotting for β-catenin (*β-cat*) using a monoclonal antibody directed against the C terminal of β-catenin (*PS1-C*). *IP*, Immunoprecipitation.

mutant. Induction of wild-type PS1 with increasing amounts of muristerone resulted in a dose-dependent increase in both the N-terminal fragment (NTF) and full-length (FL) PS1, whereas the ΔX9 mutant led to a diminution of endogenous NTF concomitant to the appearance of full-length ΔX9 protein (Fig. 1). Consistent with previous reports (Zhou et al., 1997; Yu et al., 1998), our immunoprecipitation studies revealed β-catenin in immune complexes of wild-type PS1 or FAD-linked PS1 mutants (M146L and ΔX9), whereas preimmune serum failed to detect any PS1–β-catenin complexes under identical conditions (see Figs. 1, 2*b*, 3). The amount of β-catenin complexed to wild-type PS1 or ΔX9 mutant was directly correlated with the level of PS1 protein induction by muristerone in EcR293 cells (Fig. 1). Interestingly, in cells expressing wild-type PS1, the amount of PS1–β-catenin complex correlated better with the amount of PS1 fragments than with the appearance of the full-length protein. Extraction of proteins by 1% NP-40 revealed that β-catenin associated with both the full length and the C-terminal fragment (CTF) of PS1 but not with the NTF, demonstrating that the β-catenin–binding domain is contained within the CTF (Fig. 2*a*,*c*). Nevertheless, extraction of proteins in 0.5% 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPS) revealed both the CTF and NTF in β-catenin immune complexes, consistent with previous studies demonstrating the interaction between the CTF and NTF in nondenaturing conditions (data not shown) (Capell et al., 1998). However, E-cadherin, the major transmembranous β-catenin–binding partner, was absent from the PS1–β-catenin complex (Fig. 2*b*), a finding that is consistent with the subcellular localization of PS1 to perinuclear structures and of cadherins to the cytoskeleton and plasma membrane. Moreover, we were unable to detect PS1 in APC immune complexes (Fig. 2*c*). Conversely, APC was not detected in PS1 immune complexes under experimental conditions in which APC was easily detected in β-catenin immune complexes (Fig. 2*d*). Thus, these data indicate that PS1–β-catenin complexes do not contain APC or E-cadherin.

Recruitment of GSK-3β by wild-type PS1 but not by ΔX9 and M146L mutants

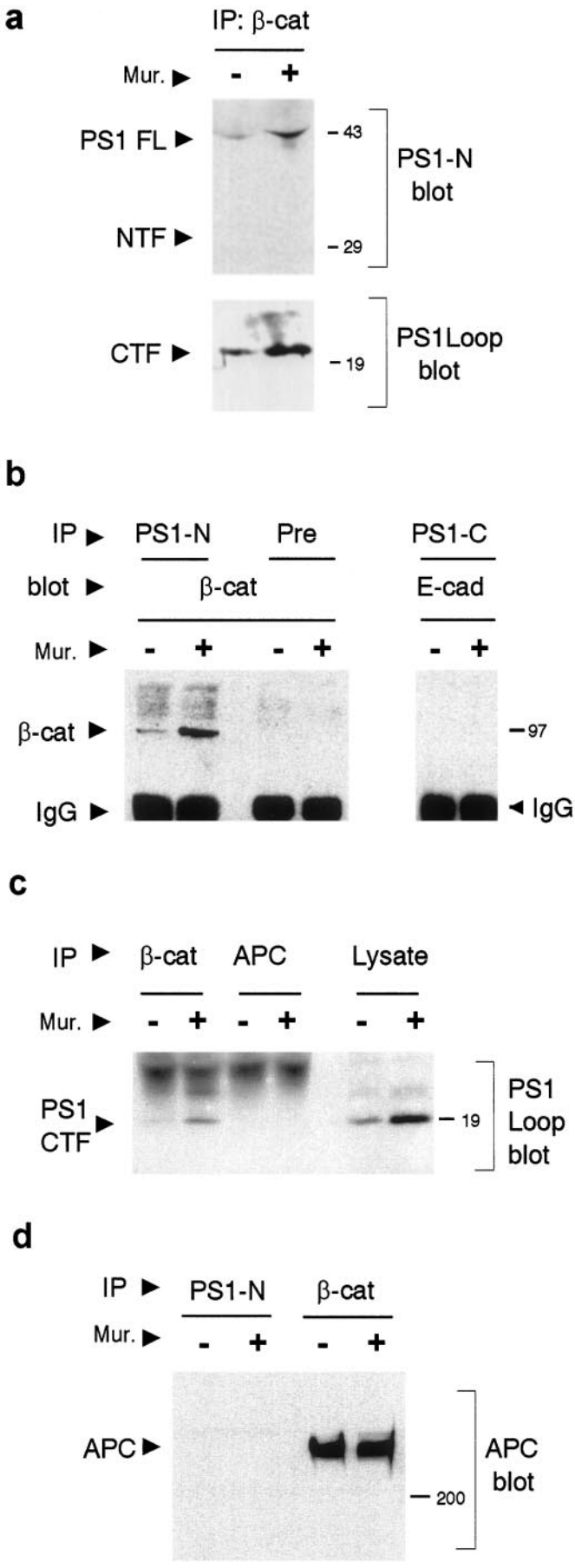
In most cell types, β-catenin–mediated signaling is tightly regulated by rapid and constitutive degradation of β-catenin via the ubiquitin-proteosome pathway, a process that requires the phosphorylation of β-catenin by GSK-3β (Aberle et al., 1997). Thus, we assessed whether GSK-3β might be in complex with PS1 and β-catenin. In rapidly dividing cells, GSK-3β coprecipitated with the full length and the NTF of wild-type PS1 (Fig. 3), in contrast to the interaction of β-catenin with the CTF of PS1 (Fig. 2*a*,*c*). Notably, overexpression of PS1 led to a robust increase in the amount of the β-catenin–GSK-3β complex (Fig. 3), suggesting that PS1 facilitates the β-catenin–GSK-3β interaction, perhaps by serving as a scaffold on which both β-catenin and GSK-3β are recruited into the same complex. In contrast, sequestration of GSK-3β was markedly reduced in cells overexpressing the two different FAD-linked PS1 mutants (M146L and ΔX9), although the binding to β-catenin was unaffected (Fig. 3). Moreover, overexpression of the M146L or ΔX9 mutant did not appreciably increase the amount of β-catenin in GSK-3β immune complexes, suggesting that these PS1 mutants do not enhance complex formation between β-catenin and GSK-3β (Fig. 3).

ΔX9 and M146L mutants interfere with constitutive turnover of endogenous β-catenin

Because GSK-3β–mediated phosphorylation of β-catenin leads to β-catenin degradation, we examined whether the differential interaction of wild-type and FAD mutant PS1 with GSK-3β alters the turnover of β-catenin. No detectable differences were observed in the steady-state levels of β-catenin in cell lines expressing wild-type and mutant PS1 after 24 hr of induction (data not shown). However, pulse-chase experiments revealed that overexpression of wild-type PS1 promoted the degradation of β-catenin, whereas both FAD mutants (M146L and ΔX9) significantly delayed the constitutive turnover of β-catenin. Figure 4*a* shows a representative experiment in which induction of wild-type PS1 decreased the half-life of β-catenin by ~15 min, whereas induction of the ΔX9 mutant increased the half-life of β-catenin by >1 hr (from ~30 to >90 min) compared with that in uninduced control cells. Because the constitutive half-life of β-catenin appeared to be 20–30 min, we then measured the amount of β-catenin remaining after 30 min of degradation in cells inducibly expressing wild-type or mutant PS1. In multiple experiments (n = 3), levels of β-catenin were reduced by ~40% after induction of wild-type PS1, whereas β-catenin levels were increased by ~50 and ~75% after induction of M146L and ΔX9 mutants, respectively, compared with levels in their uninduced controls (Fig. 4*b*; ANOVA comparison of wild-type, M146L, and ΔX9, $F = 10.593$; $p = 0.0108$; *post hoc* Tukey, mutants compared with wild type, $^*p < 0.05$). These results indicate that overexpression of M146L and ΔX9 mutants partially interferes with the constitutive turnover of endogenous β-catenin.

Association of PS1 with β-catenin in the brains of transgenic mice

To determine whether the PS1–β-catenin complexes are unique to cultured cells, we turned to brain tissue obtained from trans-



genic mice overexpressing human PS1 (WT and M146L mutation). The presence and expression of transgene was established by genotyping and RT-PCR of mRNA from brain homogenate (Fig. 5*a*). In addition, N- and C-terminal PS1 fragments derived from the transgene were detected by Western blotting of brain tissue (Fig. 5*b*,*c*). Specifically, the NTF was readily visualized using PSN2 monoclonal antibody that only recognizes human and not mouse PS1 (Fig. 5*b*). The transgene-derived CTF was recognized by the slower mobility as compared with that of endogenous PS1 on an SDS-PAGE gel (Fig. 5*c*), as reported previously (Thinakaran et al., 1996). In coimmunoprecipitation experiments from brain homogenates of heterozygous transgenic mice and control nontransgenic littermates, antibodies specific for the N and C terminals of PS1 were able to coprecipitate β-catenin (Fig. 6*a*). This observation indicated that the association of PS1 with β-catenin occurs *in vivo.* Both the wild-type and M146L mutant PS1 coprecipitated endogenous β-catenin to a comparable degree in the brains of transgenic animals. Moreover, a recombinant GST–PS1 loop fusion protein affinity precipitated β-catenin from mouse brains, whereas GST alone failed to precipitate β-catenin under identical conditions (Fig. 6*b*), indicating that β-catenin specifically interacts with PS1 within the hydrophilic loop region.

Having established that PS1 can associate with β-catenin *in vivo*, we next asked whether the stabilization of β-catenin by mutant PS1 seen in tissue cultured cells can also be detected *in vivo*. Analysis of three independent transgenic mouse lines expressing the PS1 M146L mutation indicated a trend toward higher amounts of Triton X-100 soluble β-catenin in the brain of transgenic animals as compared with that in nontransgenic littermates (data not shown). However, regression analysis showed that the elevation in steady-state levels of β-catenin was dependent on the level of PS1 M146L mutant expression (Fig. 6*c*). In other words, levels of mutant PS1 protein were positively and significantly correlated with levels of β-catenin in brain and provide *in vivo* support of our investigations *in vitro*. In two different lines of transgenic mice expressing wild-type human PS1, we observed an ~25% reduction in steady-state β-catenin levels compared with that in control littermates; however, limitation of sample size precluded meaningful statistical analysis (data not shown). These *in vivo* results together with β-catenin turnover studies therefore suggest that wild-type PS1 normally promotes the degradation of β-catenin that we hypothesize is caused by the recruitment of GSK-3β into the complex, whereas FAD-linked PS1 mutants perturb the constitutive turnover of β-catenin by failing to sequester GSK-3β.

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Figure 2. The full length and the C-terminal fragment of PS1 interact with β-catenin but not with E-cadherin or APC. *a*, Stably transfected EcR293 cells were treated with or without muristerone (2 μM for 20 hr), and cell lysates were immunoprecipitated with a polyclonal antibody directed against β-catenin and analyzed for the presence of the FL, NTF, and CTF of PS1. The PS1 FL and NTF were detected by the PSN2 monoclonal antibody specific for the N terminal of PS1 (*PS1-N*). The CTF was detected by the αPS1Loop antibody. *b*, *Left*, Cell lysates were immunoprecipitated with a polyclonal antibody against the N terminal of PS1 (J27; *PS1-N*) or J27 preimmune serum (*Pre*) for detection of β-catenin. *Right*, An antibody directed against the C-terminal end of PS1 (4627; *PS1-C*) fails to coimmunoprecipitate E-cadherin (*E-cad*). *C*, Cell lysates were immunoprecipitated with antibody against β-catenin or APC and probed with PS1 loop antibody for the presence of PS1 CTF. *D*, Cell lysates were immunoprecipitated with an antibody against the N terminal of PS1 (J27; *PS1-N*) or β-catenin, and immune complexes were analyzed for the presence of APC. Molecular weights from prestained protein standards are shown in kilodaltons on the *right*.

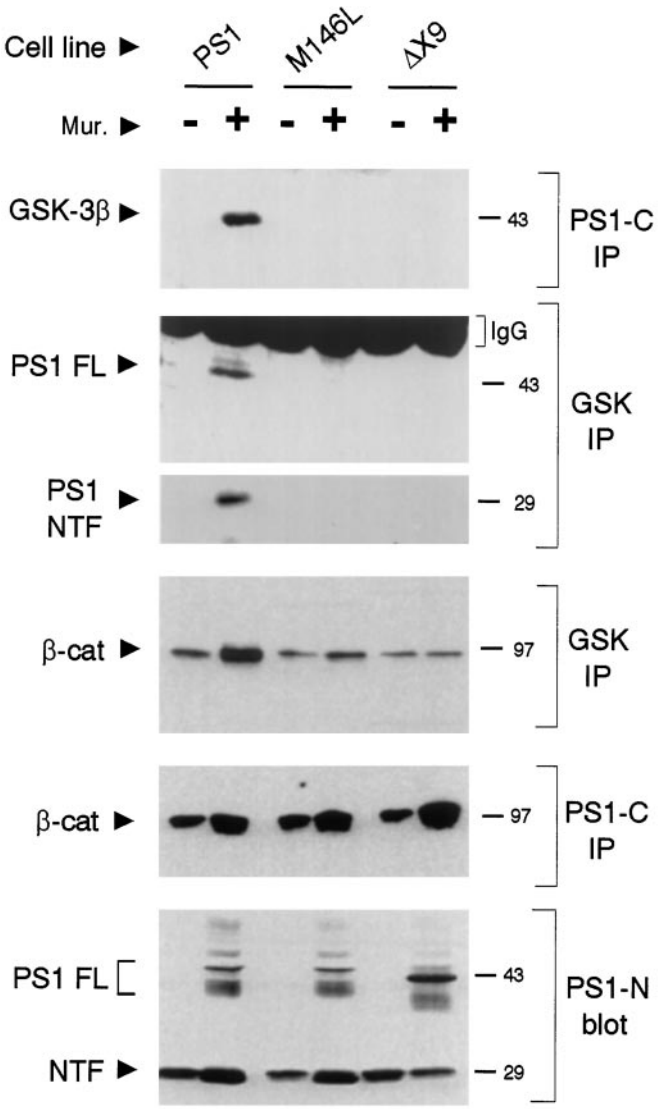

Figure 3. Recruitment of GSK-3β into the PS1–β-catenin complex of wild-type PS1 but not by ΔX9 and M146L mutants. Stably transfected EcR293 cells were treated with or without muristerone (2 μM for 20 hr) to induce the expression of PS1 variants and were lysed in 1% NP-40 buffer. Equal amounts of protein from uninduced and induced parallel cultures were subjected to immunoprecipitation with an antibody against PS1 (4627; *PS1-C*) or GSK-3β. Immune complexes were detected by immunoblotting with antibodies specific for β-catenin, GSK-3β, or PS1 (PSN2) as indicated on the *left*. Note that PS1–GSK-3β complexes are detected only in wild-type PS1–overexpressing cells. Concomitantly, the amount of β-catenin–GSK-3β complex is prominently increased by induction of wild-type PS1 but not by M146L and ΔX9 mutants. The *two bottom panels* show coimmunoprecipitation of β-catenin with wild-type PS1 and mutants (M146L and ΔX9) and the expression level of PS1 variants after induction by muristerone as detected by immunoblotting (PSN2; *PS1-N*). Molecular weights are shown in kilodaltons on the *right* in this and all subsequent figures.

PS1 is necessary for rapid and constitutive degradation of endogenous β-catenin

Our finding that overexpression of wild-type PS1 promoted the turnover of endogenous β-catenin whereas mutant PS1 increased the stability and steady-state levels of endogenous β-catenin prompted us to examine whether the lack of PS1 might differentially alter this activity. Thus, we cultured fibroblasts from homozygous (−/−) and hemizygous (−/+) *PS1*-deficient mouse embryos (Wong et al., 1997). In support of the concept that PS1 facilitates the degradation of β-catenin, *PS1* −/− cells showed dramatically reduced turnover of β-catenin compared with that of *PS1* −/+ cells. Although the half-life of β-catenin was <30 min in *PS1* −/+ cells, it was longer than 2 hr in *PS1*-deficient cells (Fig. 7*a*). This reduced turnover of β-catenin was correlated with more than threefold higher levels at steady state in *PS1*-deficient cells as compared with that in hemizygous cells (data not shown). To test directly whether the prolonged half-life in *PS1* −/− cells is indeed caused by the lack of PS1, we stably transfected *PS1* −/− cells with wild-type PS1 or the PS1 ΔX9 FAD mutation. Pulse-chase analysis showed that wild-type PS1 completely rescued the defective β-catenin turnover phenotype of *PS1* −/− cells, restoring the half-life from >2 hr to <30 min (Fig. 7*b*). On the other hand, expression of the PS1 ΔX9 mutant only partially reversed this phenotype as compared with wild-type PS1 (Fig. 7*b*). This finding, together with corresponding effects of PS1 overexpression, provides compelling evidence that PS1 normally promotes the rapid degradation of cytosolic β-catenin.

DISCUSSION

Although a molecular link between PS1 and β-catenin was recently reported, the functional consequences of these interactions are unknown. Our studies not only confirmed the initial reports of the PS1–β-catenin interaction but also provided a number of novel insights into the molecular and cellular consequences of this interaction. First, we showed that both wild-type and mutant PS1 proteins sequester a pool of β-catenin excluded from E-cadherin and APC. Second, we showed that wild-type PS1 promotes while ΔX9 and M146L mutants interfere with the constitutive turnover of endogenous β-catenin. In support of these findings, the amount of GSK-3β–β-catenin complex was preferentially enhanced by overexpression of wild-type PS1 but not PS1 mutants. Moreover, the finding that wild-type PS1 facilitates β-catenin turnover was confirmed in cells genetically deficient in *PS1*, in which the half-life of β-catenin was dramatically prolonged but corrected to a normal rate after replacement of PS1. Lastly, these *in vitro* results were supported by observations in transgenic mice brain tissue in which PS1–β-catenin association can be detected and in which expression of mutant PS1 augments the steady-state levels of endogenous β-catenin.

In our coprecipitation experiments, β-catenin was detected in immune complexes of endogenous as well as exogenously expressed PS1 in cultured cells and brain tissues. In addition, we showed that this interaction occurs in the C-terminal half of PS1, because the full length and the CTF but not the NTF of PS1 associate with β-catenin. This finding is consistent with the recent observation that residues 322–450 of PS1 interact with β-catenin (Murayama et al., 1998). Under mild detergent conditions (e.g., CHAPS, digitonin), the NTF and CTF of PS1 (Capell et al., 1998; Thinakaran et al., 1998), together with β-catenin (Yu et al., 1998), stay as a complex. Under the NP-40 lysis used in our experiments, however, only the CTF of PS1 was selectively detected in β-catenin immune complexes. In addition, our studies indicated that the two major β-catenin–binding proteins, E-cadherin and APC, were absent from this complex. This observation is consistent with a previous study demonstrating that APC and E-cadherin form independent complexes with β-catenin *in vitro* (Rubinfeld et al., 1995) and the recent finding that PS1 interacts with a domain in β-catenin that binds E-cadherin (Hulsken et al., 1994; Murayama et al., 1998).

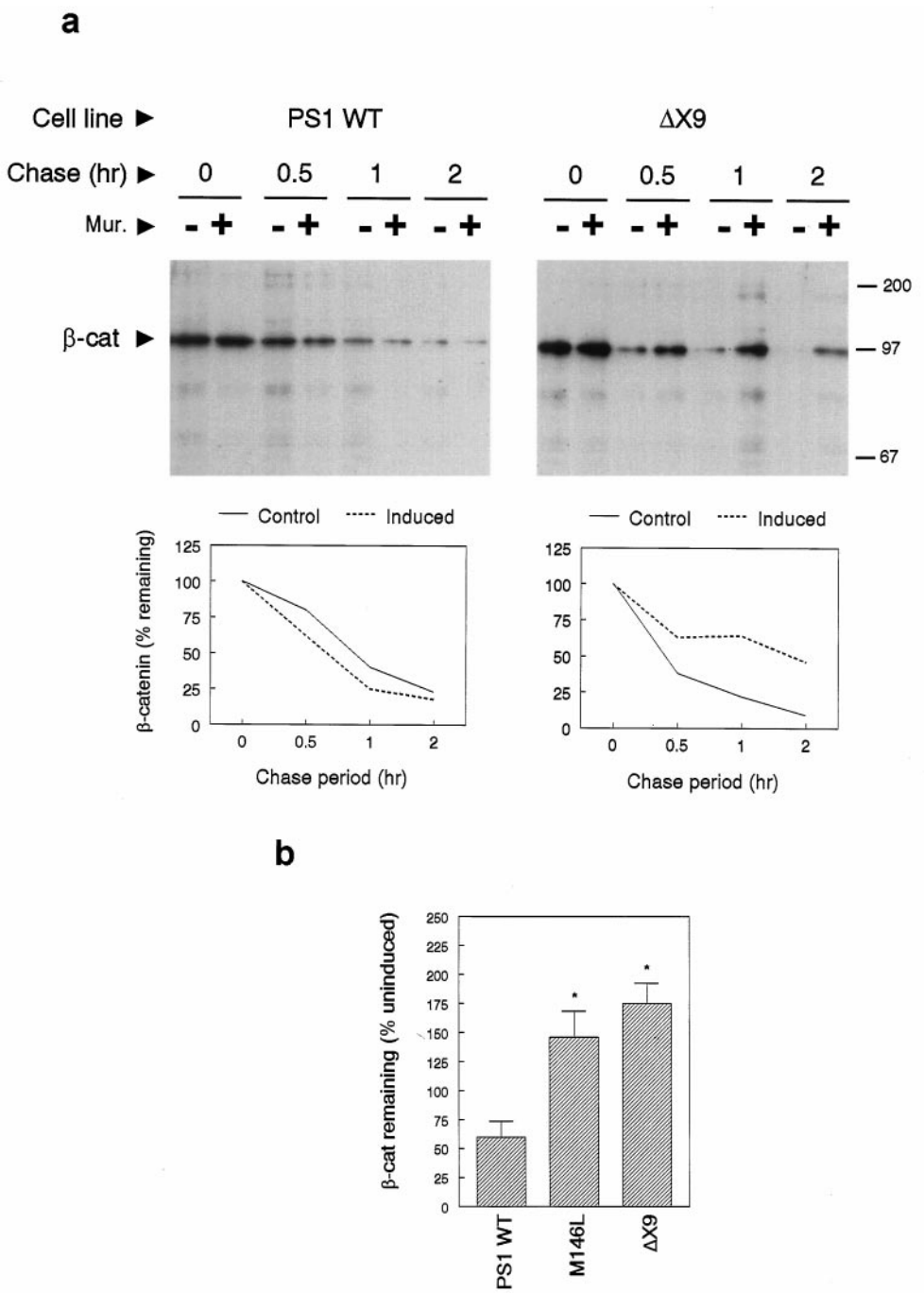

Figure 4. Wild-type PS1 facilitates and FAD-linked PS1 mutants perturb the constitutive turnover of endogenous β-catenin. *A*, Parallel cultures of muristerone-induced and uninduced cells were metabolically labeled with [^{35}S]methionine for 20 min and chased for 0, 0.5, 1, and 2 hr. *Top*, Lysates were immunoprecipitated with a β-catenin monoclonal antibody and analyzed by autoradiography. *Bottom*, The decay rate of β-catenin from the actual autoradiograms was plotted for control and muristerone-induced cells expressing wild-type PS1 or the ΔX9 mutant. *B*, Cells treated with or without muristerone for 20 hr were metabolically labeled with [^{35}S]methionine for 20 min, chased for 0 or 30 min, and immunoprecipitated for β-catenin. The stability of endogenous β-catenin is expressed as the proportion of β-catenin remaining after 30 min of degradation (chase period) in muristerone-induced cells normalized to that in uninduced parallel cultures. The values and SE bars represent the averages of three independent experiments from two different stable clones, each expressing wild-type, M146L, or ΔX9. Statistical analysis is performed by ANOVA ($F = 10.593$; $p = 0.0108$; *post hoc* Tukey, mutants compared with wild type, $^{*}p < 0.05$).

A recent study showed that both GSK-3β and tau associate with PS1 via residues 250–298, a domain within the NTF of PS1, in transient cotransfection experiments (Takashima et al., 1998). In our study using stably transfected cells, we confirmed the association of GSK-3β with PS1 in the N-terminal region of PS1 and extended this finding to show functional changes in the turnover rate of β-catenin. An unexpected finding from our coprecipitation studies was that wild-type PS1 but not FAD-linked PS1 mutants increased the amount of the GSK-3β–β-catenin complex. This result was confirmed by the observation that the association between GSK-3β and two different PS1 mutants (M146L and ΔX9) was virtually undetectable by our coimmunoprecipitation assays. The association of GSK-3β was via the full length and the NTF of wild-type PS1, in contrast to the interaction of β-catenin with the CTF. Thus, the region in PS1 responsible for the binding to GSK-3β is distinct from that of β-catenin. Our results are

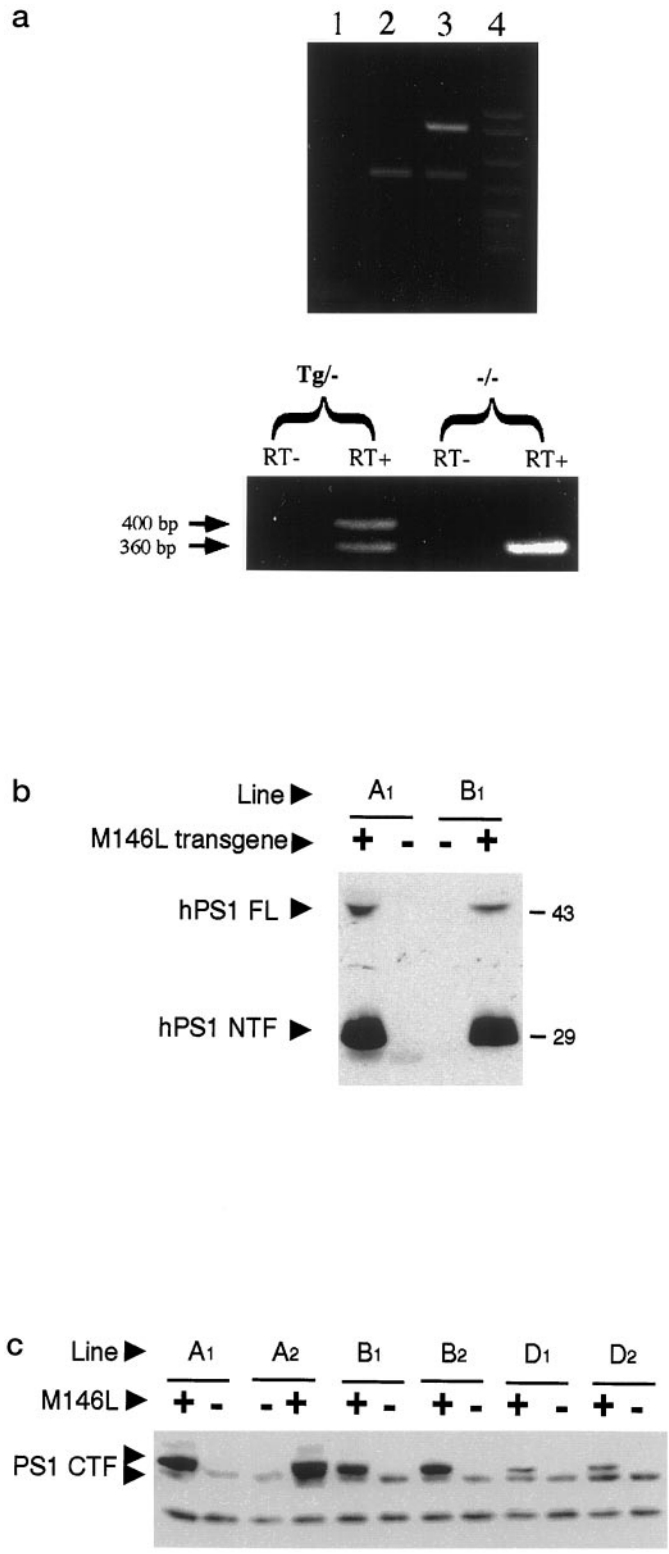

Figure 5. Expression of the human PS1 M146L mutation in transgenic mice. *A*, Confirmation of the transgene in offsprings is made by PCR of genomic DNA obtained from tailsnips. *Top*, Transgene-positive animals give two bands at 355 and 521 bp (*lane 3*), whereas animals without the transgene give only the smaller band (*lane 2*). A negative control is included in each set of PCR reactions (*lane 1*). *Bottom*, mRNA expression from the human PS1 transgene in brain tissue is shown by RT-PCR. Heterozygous transgenic animals (Tg/−) show two bands after RT-PCR. Only one band is seen in nontransgenic littermates (−/−). The *RT− lanes* are negative controls for the reverse-transcriptase reaction to ensure complete digestion of the DNA template. *B*, The full length and the N-terminal fragment of human PS1 (*hPS1*) in brains of transgenic mice expressing the M146L mutation (*lines A*, *B*) are visualized by immunoblotting for the human-specific antibody against the N terminal of PS1 (PSN2). *C*, The CTF from both human and endogenous PS1 can be seen using an αPS1Loop antibody. As reported, the transgenic human PS1 CTF (*upper arrowhead* in *doublet*) migrates higher than the endogenous (*lower arrowhead*) species. Three different lines of transgenic animals expressing M146L are illustrated (*lines A*, *B*, *D*) to show differences in expression level.

somewhat different from those in a recent report demonstrating that binding to GSK-3β was increased in two PS1 mutations (C263R and P264L) (Takashima et al., 1998). However, because both C263R and P264L mutations lie within the putative GSK-3β–binding site, it is possible that the two different sets of mutations used in these two studies may have differential activity with regard to GSK-3β interaction. Nonetheless, these observations taken together indicate that PS1 might function as a membrane-associated scaffold on which both β-catenin and GSK-3β are assembled. This activity is similar to that seen in axin, a cytosolic molecule that assembles β-catenin, GSK-3β, and APC into a multiprotein complex and negatively regulates β-catenin stability (Hart et al., 1998; Ikeda et al., 1998).

Although APC was not detected in the PS1–β-catenin complex in our study, overexpression of wild-type PS1 nevertheless enhanced the degradation of endogenous β-catenin. This observation was confirmed by the striking differences in turnover rates of endogenous β-catenin in rodent *PS1* −/− cells with and without transfection by wild-type human PS1. Furthermore, in EcR293 cells, overexpression of two different PS1 mutants (M146L and ΔX9) delayed the turnover of β-catenin. This finding from cultured cells was confirmed *in vivo* in transgenic mice expressing the M146L mutant PS1, in which the amount of Triton X-100 soluble β-catenin is directly correlated with the mutant transgene expression. Taken together, these data conclusively demonstrate that PS1 is required for rapid and constitutive turnover of the cytosolic, and presumably signaling, pool of β-catenin. Furthermore, we hypothesize that PS1 mutants (M146L and ΔX9) contain only partial activity, and in cells previously expressing wild-type PS1, the mutant proteins exert a dominant–negative activity on β-catenin turnover. We postulate that PS1 mutants interfere with the normal activity of endogenous PS1 to mediate the rapid turnover of β-catenin perhaps by delaying the phosphorylation of β-catenin by GSK-3β. This latter activity is believed to be required for efficient degradation of β-catenin via the ubiquitin-proteosome pathway (Aberle et al., 1997). It remains to be seen whether all FAD-linked PS1 mutations exert a similar activity on the turnover of endogenous β-catenin, an issue highlighted by the GSK-3β results reported recently (Takashima et al., 1998).

Finally, it is important to note that our results are in apparent disagreement with a recent report demonstrating increased β-catenin stability secondary to wild-type PS1 expression, an effect that was partially lost with PS1 mutations (Z. Zhang et al., 1998). However, different experimental approaches between the studies may account for the divergent results. For example, Yankner and colleagues (Z. Zhang et al., 1998) analyzed the turnover rate of myc-tagged β-catenin that was transiently co-transfected with PS1 plasmids, whereas our study examined the turnover of endogenous β-catenin after inducible expression of PS1 proteins in stably transfected cell lines. Moreover, our study determined the turnover of full-length β-catenin in PS1-deficient

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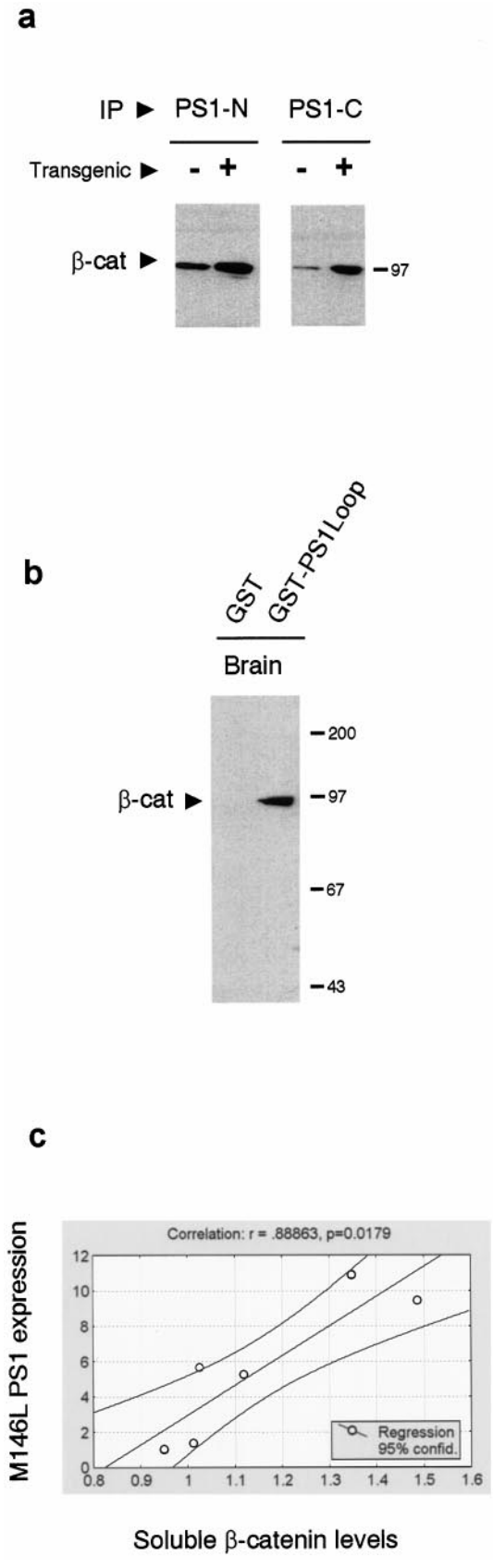

Figure 6. Association of PS1 with β-catenin *in vivo* and correlation of soluble β-catenin levels with PS1 M146L FAD-linked mutant protein expression in the brains of transgenic mice. *A*, Triton X-100 soluble brain extracts from M146L transgenic and nontransgenic littermates were immunoprecipitated with an antibody against the N terminal (J27; *PS1-N*) or C terminal (4627; *PS1-C*) of PS1 and immunoblotted for β-catenin. *B*, Triton X-100 soluble mouse brain extract (200 μg) was affinity precipitated with recombinant GST or GST–PS1Loop fusion protein and immunoblotted for β-catenin. *C*, Triton X-100 soluble brain lysates (30 μg) were subjected to SDS-PAGE, immunoblotted using β-catenin monoclonal antibody, and quantitated by phosphorimaging. The amount of β-catenin in each *lane* was normalized to the amount of actin present in the *same lane*. The amount of soluble β-catenin, expressed as the fold increase in β-catenin in transgenic mice with respect to that in their nontransgenic littermates, was plotted against the amount of human M146L PS1 mutant CTF. Linear regression analysis shows a significant positive correlation between the amount of human M146L mutant PS1 CTF and the amount of β-catenin ($r = 0.889$; $p = 0.0179$).

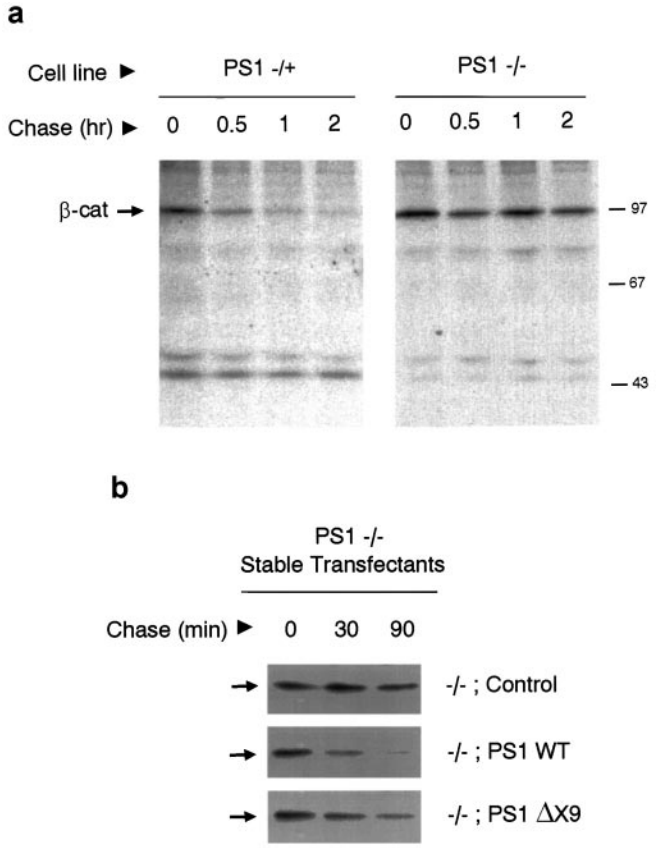

Figure 7. Defective turnover of β-catenin in cells genetically deficient in mouse PS1 and rescue by human PS1. *A*, Immortalized fibroblasts derived from PS1 −/+ and PS1 −/− embryonic day 15.5 embryos were pulse labeled with [^{35}S]methionine for 15 min and chased for 0, 0.5, 1, and 2 hr. Lysates were immunoprecipitated with a β-catenin monoclonal antibody and analyzed by autoradiography. *B*, PS1 −/− fibroblasts stably transfected with vector control, wild-type PS1, or PS1 ΔX9 mutant were pulse labeled with [^{35}S]methionine for 15 min and chased for 0, 30, and 90 min. Lysates were immunoprecipitated with a β-catenin monoclonal antibody and analyzed by autoradiography. *Arrows* indicate immunoprecipitated β-catenin.

cells by pulse-chase paradigm, whereas Z. Zhang et al. (1998) focused on apparent β-catenin proteolytic products from steady-state pools by Western blotting. Although such β-catenin–related proteolytic fragments require further characterization, the appearance of these species indicates processes that are clearly distinct from conventional degradation and turnover of β-catenin per se. On the other hand, a recent study by Takashima and colleagues (Murayama et al., 1998) showed that transient trans-

fection of PS1 in COS cells results in reduced levels of endogenous cytosolic β-catenin that were further reduced by transient transfection of PS1 mutants, findings that are in partial agreement with our studies in stably transfected inducible cells. Thus, apparent discrepancies between different studies highlight the importance of defining the pools of β-catenin under examination and interpreting the findings accordingly. In this study, as an initial step in studying the role of PS1 in the β-catenin pathway, we have analyzed the turnover rate and steady-state levels of endogenous, full-length β-catenin.

Our studies have shown that the PS1–β-catenin interactions lead to corresponding changes in the processing of proteins known to function within the β-catenin–signaling pathway. Specifically, alterations in turnover of endogenous β-catenin resulting from expression of two different PS1 mutations were seen in stably transfected cells, in *PS1* knock-out cells, and in transgenic animals. These findings, therefore, provide compelling evidence that PS1 normally modulates signaling within the β-catenin pathway. Moreover, the functional changes in β-catenin turnover induced by PS1 mutations suggest the intriguing possibility that this pathway may be involved in the pathobiology of AD. From studies of sel-12 in *C. elegans* and *PS1*-deficient animals, it has been proposed that PS1 might function as a mediator of the Notch/Lin-12–signaling pathway (Levitan and Greenwald, 1995), although evidence of a direct molecular link has not been presented. On the other hand, it has been reported that Wingless/Wnt and Notch pathways may interact at the level of disheveled (Axelrod et al., 1996), a negative regulator of GSK-3β activity. Taken together, these observations lead us to hypothesize that the loss of PS1–β-catenin interaction might underlie the embryonic lethal phenotype of *PS1* null mutants.

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