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Expression of Presenilin 1 and 2 (PS1 and PS2) in Human and Murine Tissues

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Mutations in genes encoding related proteins, termed presenilin 1 (PS1) and presenilin 2 (PS2), are linked to the majority of cases with early-onset familial Alzheimer's disease (FAD). To clarify potential function(s) of presenilins and relationships of presenilin expression to pathogenesis of AD, we examined the expression of PS1 and PS2 mRNA and PS1 protein in human and mouse. Semi-quantitative PCR of reverse-transcribed RNA (RT-PCR) analysis revealed that PS1 and PS2 mRNA are expressed ubiquitously and at comparable levels in most human and mouse tissues, including adult brain. However, PS1 mRNA is expressed at significantly higher levels in developing brain. *In situ* hybridization studies of mouse embryos revealed widespread expression of PS1 mRNA with a neural expression pattern that, in part, overlaps that reported for mRNA encoding specific Notch homologs. *In situ* hybridization analysis in adult mouse brain revealed that PS1 and PS2 mRNAs are enriched in neurons of the hippocampal formation and entorhinal cortex. Although PS1 and PS2 mRNA are expressed most prominently in neurons, lower but significant levels of PS1 and PS2 transcripts are also detected in white matter glial cells. Moreover, cultured neurons and astrocytes express PS1 and PS2 mRNAs. Using PS1-specific antibodies in immunoblot analysis, we demonstrate that PS1 accumulates as ~28 kDa N-terminal and ~18 kDa C-terminal fragments in brain. Immunocytochemical studies of mouse brain reveal that PS1 protein accumulates in a variety of neuronal populations with enrichment in somatodendritic and neuropil compartments.

Key words: Alzheimer's disease; presenilins; development; mRNA expression; protein accumulation; immunocytochemistry

Alzheimer's disease (AD), the most common progressive type of dementia occurring in adult life, is characterized neuropathologically by the presence of numerous senile plaques and neurofibrillary tangles in cerebral cortex and hippocampus (Wisniewski and Terry, 1973). AD is a genetically heterogeneous disorder (St. George-Hyslop et al., 1992; Schellenberg, 1995). Missense mutations in the presenilin 1 (PS1) gene and a highly related presenilin 2 (PS2) segregate with the vast majority (~40-50%) of early-onset cases of familial Alzheimer's disease (FAD; Alzheimer's Disease Collaborative Group, 1995; Campion et al., 1995; Chapman et al., 1995; Levy-Lahad et al., 1995a,b; Pereztur et al., 1995; Rogaev et al., 1995; Sherrington et al., 1995; Wasco et al., 1995; Boteva et al., 1996).

Presenilins 1 and 2 are transmembrane proteins (Rogaev et al., 1995; Sherrington et al., 1995) that exhibit significant homology to the *Caenorhabditis elegans* spe-4 (~25% identity; L'Hernault and Arduengo, 1992) and sel-12 (~50% identity; Levitan and Greenwald, 1995) polypeptides. Neither the normal biological function(s) of presenilins nor the mechanism(s) by which FAD-associated mutations in presenilins cause disease are known. In view of the significant homology between presenilins and sel-12, a molecule that functions in signaling mediated by lin-12 (Levitan and Greenwald, 1995), it has been suggested that presenilins play a role in mammalian cell-fate decisions. Additional functional roles for presenilins in ion gating or membrane organization have been suggested solely on the basis of predicted structures of the molecules (Alzheimer's Disease Collaborative Group, 1995; Levy-Lahad et al., 1995b; Selkoe, 1995; Sherrington et al., 1995; Slunt et al., 1995; Kovacs et al., 1996). Although the structural similarities between PS1 and PS2 suggest that the molecules play highly related functional roles, highly divergent hydrophilic regions at the N-terminal "head" and the central "loop" domains are likely to mediate cell- or PS-specific functions via differential interaction(s) with other ligands.

Previous studies demonstrated that both PS1 and PS2 are expressed ubiquitously in a variety of human tissues and brain regions (Rogaev et al., 1995; Sherrington et al., 1995). More recently, *in situ* hybridization analysis revealed that both PS1 and PS2 are expressed at highest levels in hippocampus and in cerebellum (Kovacs et al., 1996; Suzuki et al., 1996). However, these previous studies did not provide information regarding the quantitative levels of each presenilin transcript in brain or in systemic organs, and there are apparent disagreements in the cellular specificity of presenilin expression in brain (Kovacs et al., 1996; Suzuki et al., 1996).

To clarify potential function(s) of presenilins and relationships of presenilin expression in the pathogenesis of AD, we examined the expression of PS1 and PS2 mRNA and PS1 protein in human and mouse. Our semi-quantitative RT-PCR studies reveal that

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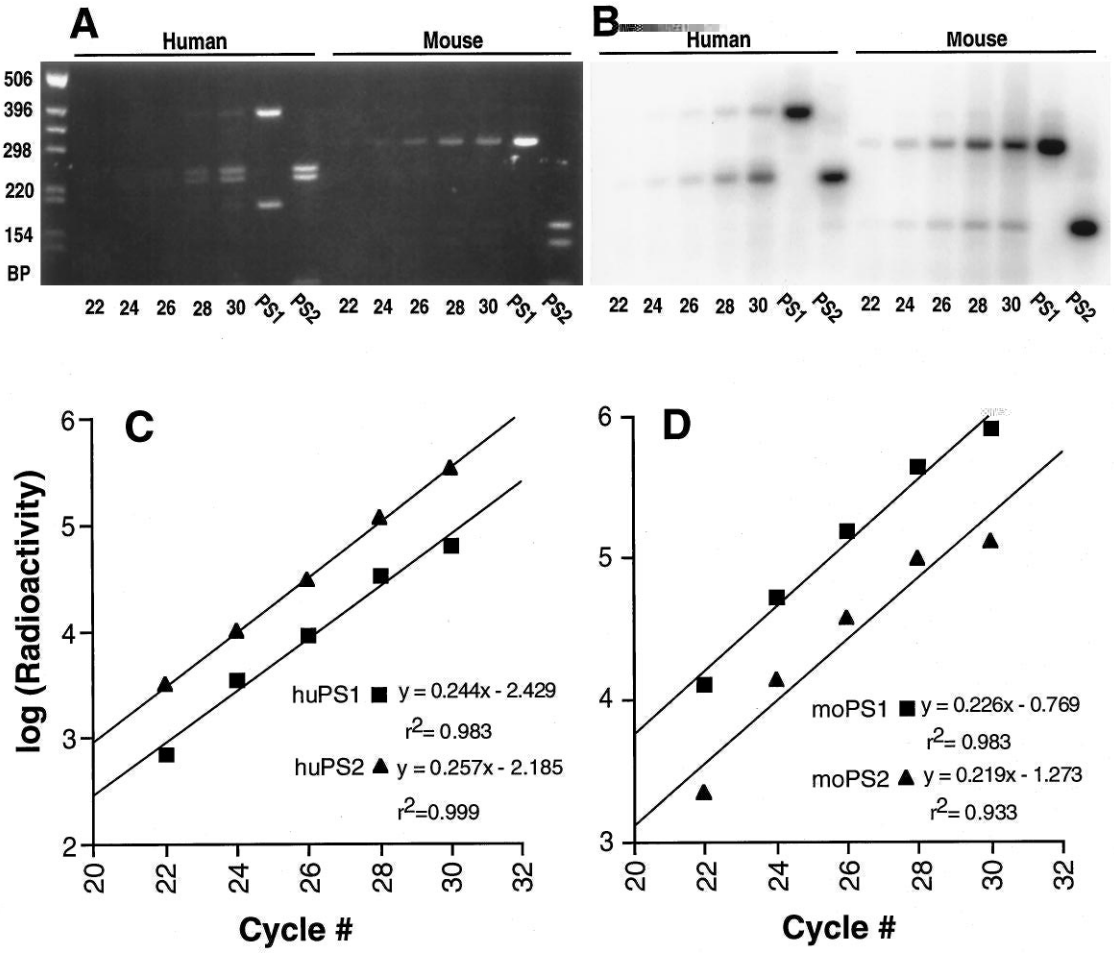

Figure 1. Quantitative PCR amplification of PS1 and PS2 cDNAs from human brain and mouse spinal cord. *A*, EtBr-stained gel of *Pfl*MI/*Nco*I double-digested PCR products from human fetal brain and adult mouse spinal cord cDNA after 22, 24, 26, 28, and 30 cycles of amplification. Also shown are PCR products obtained by using PS1 and PS2 cDNA as templates. The marker DNA fragments are indicated in bp. *B*, Autoradiogram of the gel shown in *A*. *C, D*, Semilog plots of radioactivity (density) versus cycle number for the human (*C*) and mouse (*D*) PS1 and PS2 fragments shown in *B*. The linear regression equation for each plot is shown in the *inset*.

PS1 and PS2 mRNAs are expressed at similar levels in most tissues, with minor exceptions. *In situ* hybridization analysis of mouse embryos at various developmental stages and adult mouse brain indicate that PS1 and PS2 transcripts are widely expressed. In brain, PS1 and PS2 mRNAs are expressed at highest levels in neurons, with somewhat lower levels in glial cells. Immunoblot analysis using two anti-PS1 antibodies raised against nonoverlapping epitopes revealed that PS1 accumulates as proteolytically processed fragments in brain and systemic tissues. Finally, we performed immunocytochemical investigations to identify the cellular and subcellular distributions of PS1 in mouse brain. We document that PS1 is expressed in somatodendritic and neuropil compartments of neurons in the neocortex and hippocampal formation.

MATERIALS AND METHODS

PS1 and PS2 mRNA analysis. Total RNA was isolated by homogenization of selected tissue in 4 M guanidium thiocyanate and centrifugation of the lysates over a 5.7 M cesium chloride cushion (Chirgwin et al., 1979).

The levels of PS1 and PS2 mRNA in mouse and human brain, peripheral tissues, and developmental stages were assayed by PCR of reverse-transcribed RNA (RT-PCR; Chelly et al., 1988; Golde et al., 1990; Slunt et al., 1994). Reverse-transcribed RNA was added to a PCR reaction mixture containing degenerate primers (1 μM each) and ^{32}P-end-labeled sense primer (10 pM), reaction buffer, and *Taq* DNA polymerase. For mouse, we used a sense primer, 5′-(A/G)ACGG(G/T)CAGCT(A/C)ATCTACAC-3′, and an antisense primer, 5′-GAT(A/G)AA(C/T)ACCAGGGCCATGAG-3′, to amplify a 386 bp fragment encoding amino acids 110–238 of PS1 and the homologous region of mouse PS2. For human, we used a sense primer, 5′-ATCATGCT(G/C)TTTGT(G/C)CCTG-3′, and an antisense primer, 5′-TTCTCTCCTG(A/G)GC(A/T)GTTTC-3′, to amplify a 588 bp fragment encoding amino acids 83–278 of human PS1 and the homologous region of human PS2. The resulting PCR products were double-digested with *Pfl*MI, which cuts only the PS1 cDNA, and *Nco*I, which cuts only the PS2 cDNA, to generate PS1- and PS2-specific fragments of different length. The fragments were fractionated on 2% Metaphore-agarose gels (FMC Bioproducts, Rockford, ME) and dried. The radioactivity in the PS1 and PS2 cDNAs was quantified by phosphorimaging. To determine the linear range of amplification for both PS1 and PS2 templates, we terminated RT-PCR reactions at 22, 24, 26, 28, and 30 cycles. The linearity of amplification over the cycling range for either PS1 or PS1 cDNAs was analyzed by semilog plots of radioactivity versus cycle numbers (Chelly et al., 1988; Golde et al., 1990; Slunt et al., 1994).

In situ *hybridization.* Mouse PS1 cDNA corresponding to nucleotides 1758–1945 of mouse PS1 mRNA (Sherrington et al., 1995) was generated by RT-PCR and subcloned into pBluescript KS$^+$ plasmid (Stratagene, La Jolla, CA). The cDNA encoding the loop region of mouse PS2 (homologous to codon 275–334 of human PS2) was generated by PCR, using a partial mouse PS2 as template and subcloned into pBluescript KS$^+$ plasmid (Stratagene). Sense and antisense ^{33}P-labeled

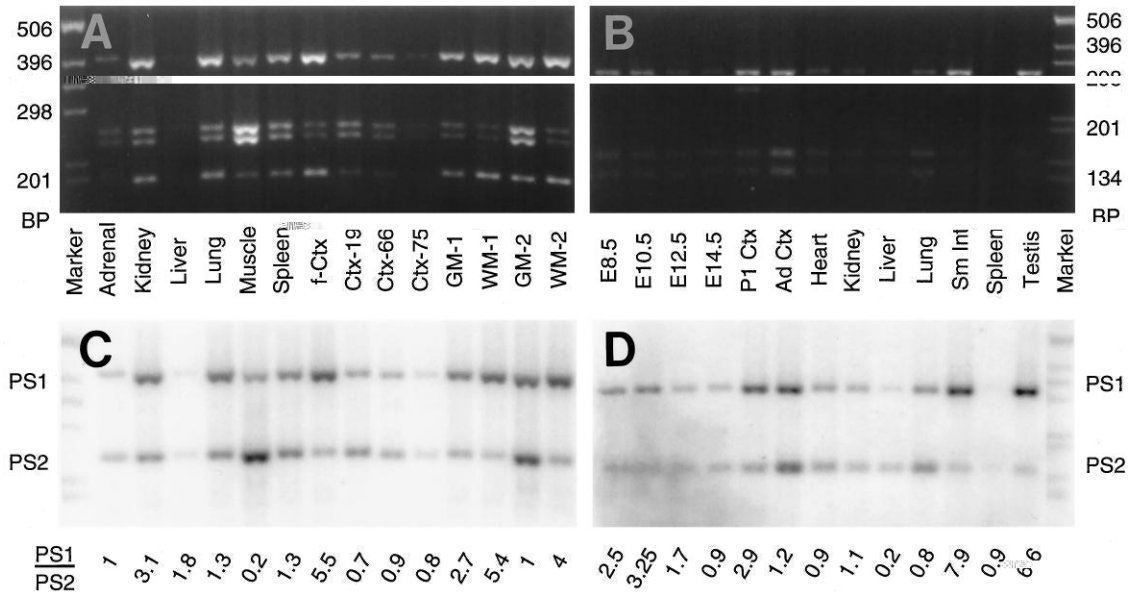

Figure 2. RT-PCR analysis of PS1 and PS2 mRNA in human and mouse tissues. *A*, EtBr-stained gel of *Pfl*MI/*Nco*I double-digested PCR product generated by RT-PCR amplification of RNAs from human fetal tissues (adrenal, kidney, liver, lung, skeletal muscle, and spleen), brains from fetus and adults [cortex from a fetus (*f-Ctx*), a 19 yr old (*Ctx-19*), a 66 yr old (*Ctx-66*), and a 75 yr old (*Ctx-75*)], cortical white matter (*WM-1* and *WM-2*), and cortical gray matter (*GM-1* and *GM-2*). *B*, EtBr-stained gel of *Pfl*MI/*Nco*I double-digested PCR products generated by RT-PCR amplification of RNAs from mouse embryos [embryonic days 8.5 (*E8.5*), 10.5 (*E10.5*), 12.5 (*E12.5*), and 14.5 (*E14.5*)], brain [neonatal cortex (*P1 Ctx*) and adult cortex (*Ad Ctx*)], and adult tissues [heart, kidney, liver, lung, small intestine (*Sm Int*), spleen, and testis]. *C, D*, Autoradiogram of the gels shown in *A* and *B*, respectively. The ratio of PS1 to PS2 cDNA products, averaged from two independent PCR reactions, is shown at the *bottom*. The amount of template (reverse-transcribed RNA) was equated for its actin mRNA content (data not shown).

riboprobes were generated from each template with a riboprobe kit (Promega, Madison, WI). A partial mouse PS2 cDNA was obtained with a sense primer (5′-CTCTGTGCATGATCGTGGTGG-3′) encoding amino acids 96–102 of human PS2 and an antisense (5′-GAAGAGGAGGAAAGGGGCGT-3′) primer complementary to sequences encoding amino acids 334–340 of human PS2.

Sections (10 μm) cut from either fresh-frozen or paraffin-embedded tissues were mounted onto slides and reacted with either sense or antisense probes (1 ng/slide, $\sim 10^6$ cpm/ng). Sections were hybridized overnight at 55°C in a buffer containing 50% formamide, 10% dextran sulfate, 0.3 M NaCl, 20 mM Tris, pH 7.5, 10 mM DTT, 5 mM EDTA, 1× Denhardt's solution and 0.4 mg/ml yeast tRNA, and 1 ng/slide of labeled probe ($\sim 2 \times 10^6$ cpm/ng). Slides were washed as follows: 5× SSC (1× SSC = 0.15 M NaCl and 0.015 M sodium citrate) and 1 mM DTT at 55°C for 20 min; 50% formamide, 2× SSC, and 1 mM DTT at 60°C for 30 min; 10 mM Tris, pH 7.5, 0.5 M NaCl, and 5 mM EDTA (TNE buffer) at 37°C three times for 10 min each; TNE buffer containing 20 μg/ml RNase A at 37°C for 45 min; TNE buffer at 37°C for 15 min; 50% formamide, 2× SSC, and 1 mM DTT at 60°C for 30 min; 2× SSC at 37°C for 20 min; and 0.1× SSC at 37°C for 20 min. After dehydration, slides were dipped into Kodak emulsion NTB-2 from 3 d to 3 weeks and developed in Kodak D-19. The sections were counterstained lightly with hematoxylin and eosin and coverslipped with Permount.

Primary neuronal and glial cultures. Primary neuronal and glial cultures from mouse neocortex were obtained as described by Hertz et al. (1985) with minor modifications. For neuronal cultures, 16 d mouse embryos were removed asceptically from timed pregnant female mice (129/BL6) and rinsed in 70% ethanol; neocortices were dissected into Hank's Buffered Saline Solution (HBSS). The cortices were minced into ~1 mm pieces and treated with 0.2% trypsin in HBSS for 5 min at room temperature. Trypsin was inactivated by addition of Neurobasal medium (Life Technologies, Bethesda, MD) containing 10% heat-inactivated horse serum. The tissue was pelleted by centrifugation, resuspended in B27-Neurobasal medium supplemented with B27 additive (Life Technologies), and cells were dissociated by trituration. Neuronal cells were diluted further in B27-supplemented Neurobasal media and plated at 2×10^5 cells/cm^2 on poly-D-lysine-coated tissue culture plates. The media was changed every 3 d.

The glial culture, enriched in astrocytes, was established from cortices of 2-d-old mouse pups. The cortices were removed asceptically into HBSS, minced into ~1 mm pieces, and treated with trypsin as above. Cells were dissociated and plated on plastic tissue culture dishes at 2×10^5 cells/cm^2 in DMEM containing 10% fetal calf serum. Total cellular RNA from 7 d neuronal and glial cultures was isolated with Trizol reagent (Life Technologies).

Immunoblot analysis of PS1 protein. Tissue and cell homogenates were prepared in TNE buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 5 mM EDTA) containing protease inhibitors (5 mM PMSF, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 10 μg/ml pepstatin) and detergents (1% SDS, 0.25% deoxycholate, and 0.25% NP-40). Protein concentrations of each homogenate were determined by bichichonic acid protein assay (Pierce, Rockford, IL). The homogenates were diluted further with the Laemmli sample buffer (Laemmli, 1970).

Protein in the above homogenates was fractioned by SDS-PAGE (Laemmli, 1970) and transferred to nitrocellulose filter membranes (Towbin et al., 1979). PS1-related peptides were detected using Ab14, a rabbit polyclonal antibody generated against amino acids 1–25 of PS1, or αPS1Loop, a rabbit polyclonal antibody generated against amino acids 263–407 of PS1 (the "loop" region between putative transmembrane domains 6 and 7; Sherrington et al., 1995; Thinakaran et al., 1996). To establish the purity of neuronal and glial cultures, we analyzed the homogenates from primary neuronal and glial cultures by Western blotting with an anti-neuron-specific β-tubulin mouse monoclonal antibody (TuJ1; Lee et al., 1990) or anti-glial fibrillary acidic protein (GFAP) rabbit polyclonal antisera (ICN, Costa Mesa, CA). Bound primary antibodies were visualized via ^{125}I-conjugated protein A, followed by autoradiography or appropriate horseradish peroxidase-conjugated secondary antibodies and then followed by chemiluminescence detection (Pierce).

Immunocytochemistry. Mice were perfused intra-aortically with PBS followed by 4% paraformaldehyde. Brains were cryoprotected in 30% sucrose. Sections were cut (40 μm) on a sliding microtome and processed immunocytochemically by using the peroxidase/antiperoxidase method with diaminobenzidine as chromogen (Martin et al., 1991; Rothstein et al., 1994). To enhance immunocytochemical staining, we pretreated sections by boiling them in distilled water for 3 min before permeabilization in 0.4% Triton-X 100 and blocking them in 4% normal goat serum. PS1 immunoreactivity was visualized with affinity-purified αPS1Loop antibodies or Ab14 antiserum raised against a GST fusion protein containing human PS1 amino acids 263–407 or a synthetic peptide corresponding to

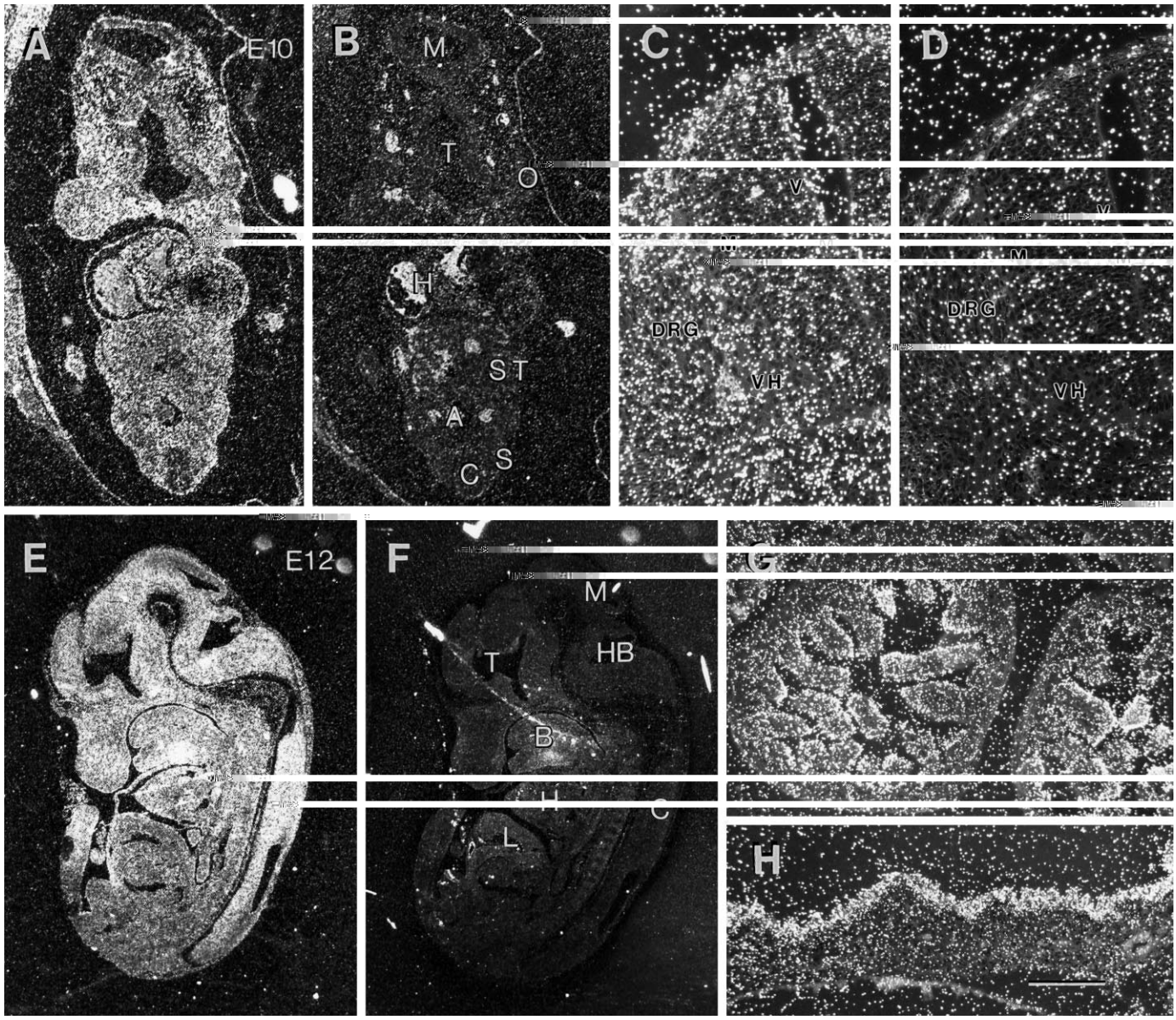

Figure 3. *In situ* localization of PS1 mRNA in mouse embryos. Paraffin-embedded sections of mouse embryos were processed for *in situ* hybridization by PS1-specific riboprobes. The silver grains over the sections, representing specific hybridization, were visualized by dark-field microscopy. The sections hybridized with antisense and control sense probes were photographed and reproduced using identical conditions. *A, B*, PS1 mRNA expression in E10 mouse embryos. Sections from E10 mouse embryos probed with antisense (*A*) and sense (*B*) mouse PS1 riboprobes reveal the presence of PS1 mRNA throughout the embryo. *A*, Aorta; *C*, spinal cord; *H*, heart; *M*, mesencephalon; *O*, optic vesicle; *S*, somite; *ST*, septum transversum; *T*, telencephalon. *C, D*, Detailed view of the spinal neural tube from sections shown in *A* and *B*. The antisense-probed section (*C*) shows higher grain density throughout the neural epithelium, as compared with the section probed with sense probe (*D*). *DRG*, Dorsal root ganglion; *M*, marginal zone; *V*, ventricular zone; *VH*, ventral horn. *E, F*, Sagittal sections of E12.5 mouse embryo hybridized with antisense (*E*) and sense (*F*) mouse PS1 riboprobe show PS1 mRNA expression throughout the nervous system and peripheral tissues. *B*, Brachial arch; *C*, spinal cord; *H*, heart; *HB*, hindbrain; *L*, liver; *M*, mesencephalon; *T*, telencephalon. *G, H*, Sections of E16.5 mouse embryo show high levels of PS1 mRNA expression in epithelial cells of small intestine (*G*) and skin (*H*). Scale bars: *C, D*, 150 μm; *G, H*, 300 μm.

human PS1 amino acids 1–25, respectively (Thinakaran et al., 1996). αPS1Loop antibodies were used at a concentration of 0.82 μg/ml, whereas Ab14 antisera was used at a dilution of 1:1000. For preadsorption controls, αLoopPS1 or Ab14 antibodies were incubated with 25 μg/ml GST fusion proteins or synthetic peptide antigen, respectively, for 16 hr before immunocytochemistry.

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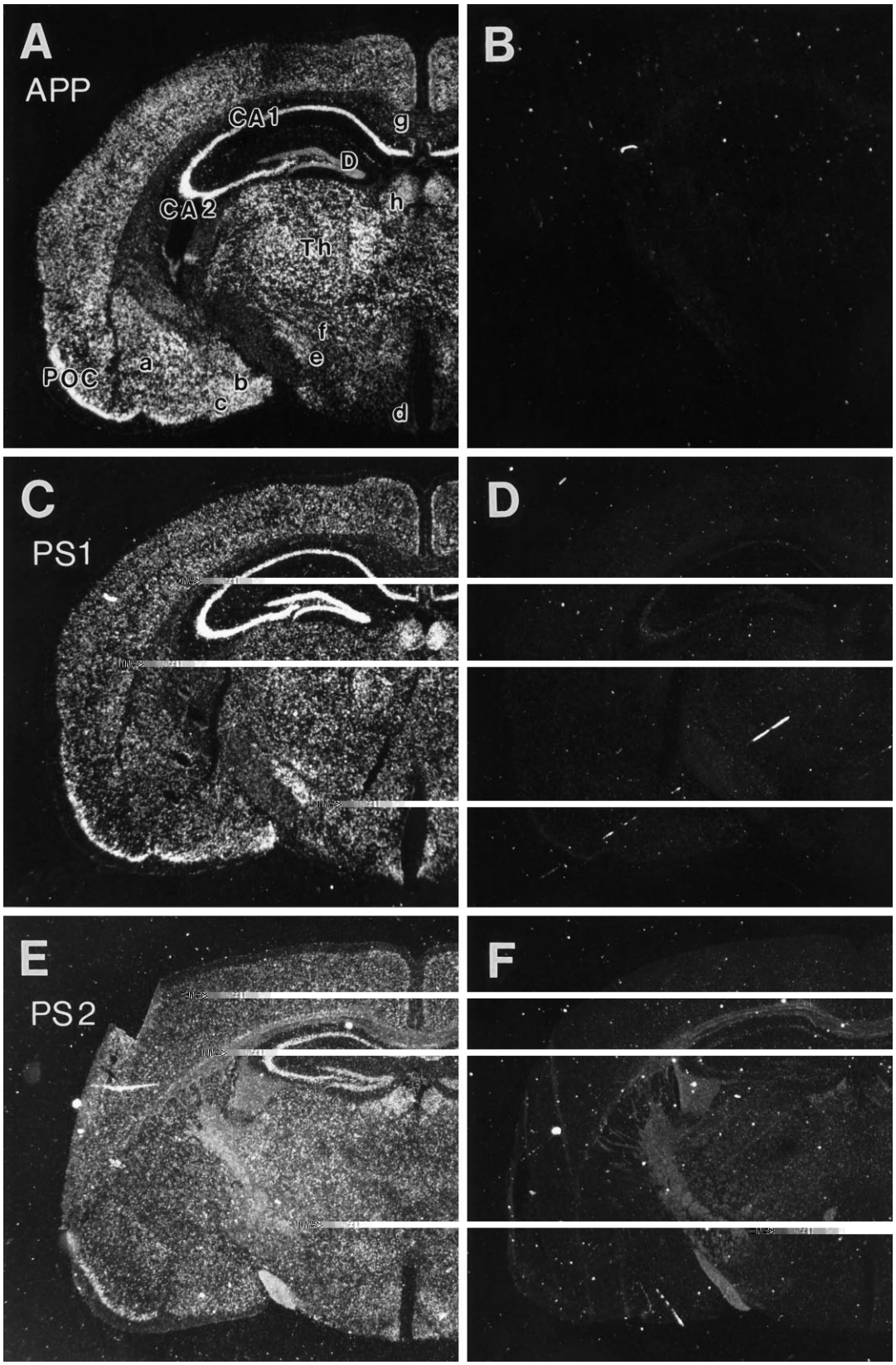
Figure 4. Expression of APP, PS1, and PS2 transcripts in mouse brain. Coronal sections of adult mouse brain at the level of dorsal hippocampus hybridized with antisense riboprobes specific for APP (*A*), PS1 (*C*), and PS2 (*E*). Adjacent sections were hybridized with sense probe (*B, D, F*) to show the specificity of antisense probes. The silver grains associated with specific transcripts were visualized with dark-field microscopy. The antisense and respective sense control sections were photographed and reproduced under identical conditions. *A, B*, APP mRNA is highly expressed in most brain regions, with particularly high levels in hippocampal CA fields (*CA1, CA2*) and primary olfactory cortex (*POC*). The APP sections were exposed to emulsion for 3 d. PS1 (*C, D*) and PS2 (*E, F*) mRNA are widely distributed, but the distribution of cells expressing high (*Figure legend continues)*



levels of each transcript is restricted. The PS1 and PS2 sections were exposed on emulsion for 3 weeks. Longer exposure times were required for photography of sections hybridized with PS2 probes (*E, F*) because of lower signal levels of the section shown in *E*. The longer exposure times under dark-field illumination led to higher levels of nonspecific signal associated with the white matter tracts in both antisense (*E*) and sense (*F*)-probed sections. *CA1, CA2, D,* Hippocampal CA-fields and dentate gyrus; *POC,* primary olfactory cortex; *Th,* thalamus; *a, b, c,* lateral, medial, and cortical amygdala; *d,* arcuate nucleus; *e,* subthalamic nucleus; *f,* zona inserta; *g,* corpus callosum; *h,* habenulae.

old (Fig. 2*A,C*, *lanes GM-1*, *WM-1*) or an 80 yr old (Fig. 2*A,C*, *lanes GM-2*, *WM-2*) revealed that PS1 mRNA is enriched in the white matter, as compared with PS2 mRNA, suggesting that glial cells (mostly oligodendrocytes) selectively express more PS1 mRNA than PS2 mRNA. This observation is consistent with earlier Northern blot studies that showed high levels of PS1 mRNA expression in corpus collosum (Rogaev et al., 1995; Sherrington et al., 1995). In gray matter, PS1 and PS2 mRNAs seem to be expressed at similar levels.

Both PS1 and PS2 mRNAs also are expressed widely in adult mouse tissues (Fig. 2*B,D*). PS1 mRNA levels are significantly higher than PS2 mRNA in small intestine and in testis, whereas PS2 mRNA is four times more abundant than PS1 mRNA in liver. In the remaining adult mouse tissues, PS1 and PS2 mRNAs accumulate to comparable levels. However, during mouse embryonic development, PS1 mRNA expression occurs at earlier developmental stages, with subsequent increases in the relative level of PS2 mRNA (Fig. 2*B,D*). In day 8.5 and 10.5 whole-mouse embryos, PS1 mRNA levels are approximately twice that of PS2 mRNA. By day 12.5, both PS1 and PS2 transcripts are expressed at equivalent levels, and PS2 mRNA may be expressed at slightly higher levels in 14.5 d embryos. In the cortex of newborn mice, PS1 is expressed at approximately three times the level of PS2 mRNA. Consistent with the results obtained using postnatal human brain (Fig. 2*A,C*), the level of PS2 mRNA is similar to the level of PS1 mRNA in adult mouse cortex.

These studies demonstrate that, although both PS1 and PS2 transcripts are expressed in many tissues, the relative levels of PS1/PS2 mRNAs are variable between tissues and during brain development. Specifically, the level of PS2 mRNA increases relative to PS1 mRNA during postnatal development of both human and mouse brain, suggesting that the expression of PS1 and PS2 transcripts is regulated differentially during brain maturation. Clearly, analysis of total RNA samples fails to score differences in mRNA accumulation within specific cell types/substructures; hence, our studies are only a first approximation. Nevertheless, although the structural conservation and relatively ubiquitous expression pattern of PS1 and PS2 mRNAs suggest some degree of functional redundancy, differences in relative levels of expression suggest that the biological roles of PS1 and PS2 may differ during development and in adult tissues.

lin-12, has led to the suggestion that PS1/PS2 may function in

somewhat lower levels (Fig. 5*C*). The expression of PS1 or PS2 mRNAs in glial cells in the neuropil could not be determined conclusively using *in situ* hybridization because of the paucity of the cytoplasm in these cells. Nevertheless, expression of presenilin mRNA and protein has been confirmed in primary cultures of purified astrocytes (see below, Fig. 9).

Despite the widely distributed expression pattern of PS1, PS2, and APP transcripts, not all neuronal populations express all of these transcripts at high levels. In general, APP mRNA is abundant in most neurons, as previously reported (Bendotti et al., 1988; Neve et al., 1988; Spillantini et al., 1989; Hyman et al., 1993; Sola et al., 1993; Tanzi et al., 1993). Similar to APP, hippocampal pyramidal neurons and the neurons of primary olfactory cortex express both PS1 and PS2 at high levels (Fig. 4*A–F*). However, PS1 and PS2 mRNAs are expressed in dentate granule neurons at levels comparable with neurons in the CA-fields (Fig. 4*C–F*), a pattern distinct from that observed for APP mRNA (Fig. 4*A*). In addition, PS1 mRNA also seems to be expressed (albeit at significantly lower levels than in the CA-fields) in the habenula, the medial and cortical amygdala, subthalamic nucleus, and the arcuate nucleus. In contrast, APP mRNAs seem to be expressed in these latter areas at levels comparable to hippocampal and cortical regions (Fig. 4*A,B*).

Coronal sections through the caudate at the level anterior to hippocampus and sections through caudal hippocampus at the level of entorhinal cortex and substantia nigra show high levels of APP mRNA expression in all cortical and subcortical regions (Fig. 6*A,D*), including neurons in the thalamus, lateral amygdyla, and substantia nigra as well as cortical and hippocampal neurons. Examination of PS1 and PS2 mRNA in adjacent sections shows that, in addition to cells in the hippocampus, presenilin transcripts are expressed prominently in neurons of subiculum and entorhinal cortex (Fig. 6*E,F*). However, unlike APP mRNA, presenilin mRNA expression in nigral neurons and in the lateral amygdala was unremarkable (Fig. 6*D–F*). With the possible exception of the caudate putamen, the general distribution of PS2 mRNA is similar to that observed for PS1 mRNA (Fig. 6*B,C*).

Finally, analysis of PS1 and PS2 transcripts in the cerebellum shows high levels of presenilin expression in granule cells, Purkinje cells, and neurons in the pons (Fig. 7). Grains over smaller nuclei located in the Purkinje cell layer suggest that PS1 and PS2 mRNAs also may be expressed in Bergmann glia or other supporting cells (Fig. 7). In contrast to the general expression pattern seen with PS1/PS2, the highest level of APP expression in cerebellum is limited to the Purkinje cells and the deep cerebellar nuclei (Sola et al., 1993).

Accumulation of proteolytically processed PS1 derivatives in mouse brain and tissues

To complement our analysis of PS1 mRNA expression, we examined the expression of PS1 protein by immunoblot analysis. We detected PS1 protein in whole SDS-soluble extracts of mouse brain regions and selected non-neural tissues by using a rabbit polyclonal antibody, Ab14, generated against the N-terminal 25 amino acids of PS1 and a rabbit polyclonal antibody,

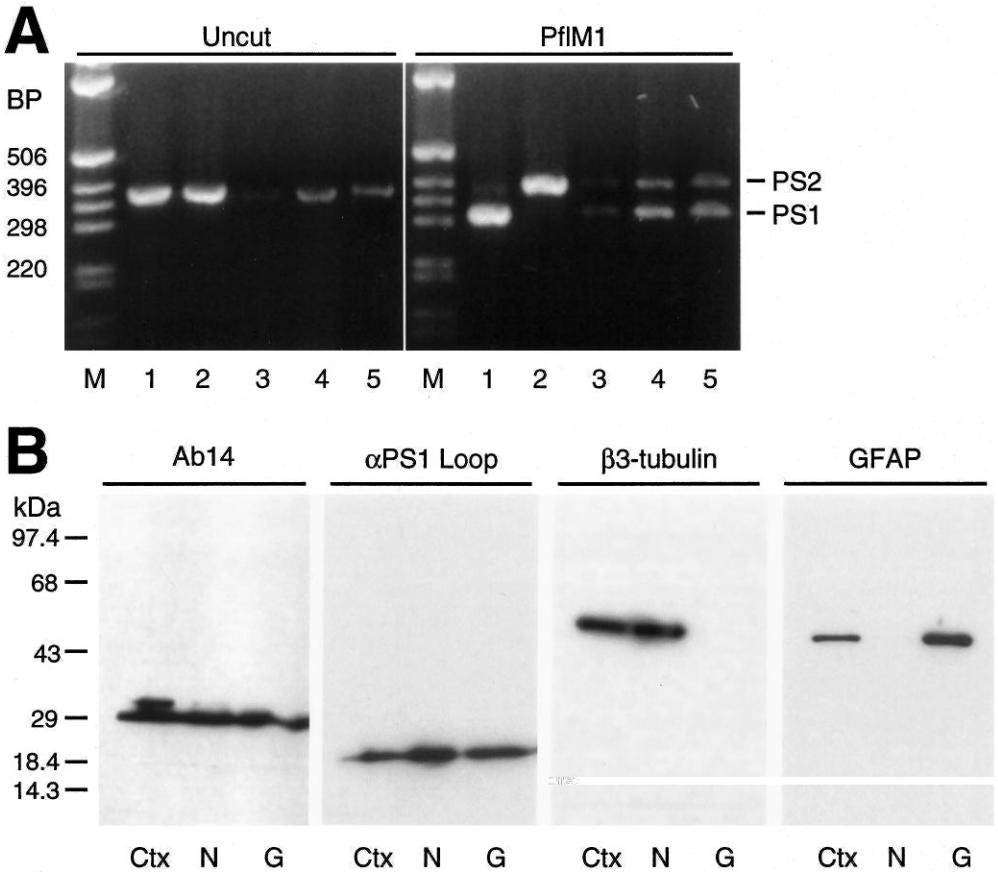


Figure 9. Expression of PS1 and PS2 in mouse neurons and glia in culture. *A*, EtBr-stained gels of PS1 and PS2 products amplified from mouse neuronal or glial RNA. PS1 was distinguished from PS2 by digestion with *Pfl*MI. *Lane 1*, PS1 plasmid; *lane 2*, PS2 plasmid; *lane 3*, mouse neocortex; *lane 4*, primary neuronal culture; *lane 5*, primary glial culture. Marker fragments (*M*) are indicated in bps. *B*, Immunoblot analysis of total SDS-extracts (50 μg) from mouse cortex (*Ctx*), cultured neurons (*N*), and cultured glia (*G*) using Ab14 and αPS1Loop antibodies; for analysis of β3-tubulin and GFAP, 5 μg of total SDS-extracts was assayed. Blots probed with antibodies to PS1 N-terminal fragment (*Ab14*) or PS1 C-terminal fragment (*αPS1Loop*) show that expressed PS1 protein accumulates as processed fragments in cultured neurons and glia. Immunoblot analysis of parallel extracts using antibodies to either neuron-specific β-tubulin (*β3-tubulin*) or GFAP (*GFAP*) demonstrates the purity of each culture. Molecular weight standards are indicated in kDa.

natively spliced human PS1 mRNA has been identified in lymphoblasts, liver, spleen, and kidney (Rogaev et al., 1995; Sahara et al., 1996). In contrast, we detected a single major C-terminal fragment of ~18 kDa in all mouse tissues examined. On longer exposures of Western blots of brain extracts with both Ab14 and αPS1Loop antibodies, we also observed an ~43-45 kDa polypeptide that presumably represents full-length PS1 (data not shown).

Our semi-quantitative analysis of total levels of PS1 in mouse neocortex revealed that the PS1 accumulates to <0.001% of total brain protein (Thinakaran et al., 1996). The present analysis clearly shows that, relative to neocortex, PS1 protein accumulates to even lower levels in most brain regions and non-neural tissues. Moreover, very low levels of PS1 protein accumulate in cranial nerves consisting of myelinated axons and oligodendrocytes (Fig. 8).

Expression of PS1 and PS2 in cultured mouse neurons and glia

To confirm the cellular specificities of PS1 and PS2 expression in neural tissues, we examined the expression of PS1 and PS2 mRNAs in primary cultures of mouse cortical neurons and glia by using optimized RT-PCR conditions (Fig. 9*A*). Our studies clearly show that cultured mouse neurons and glia express similar levels of PS1 and PS2 mRNA (Fig. 9*A*). To examine the relative levels and the nature of the accumulated PS1 protein SDS-extracts prepared from neurons and glia, we performed immunoblotting with the two anti-PS1 antibodies described above (Fig. 8). Immunoblot analysis demonstrated that both neurons and glia accumulate similar levels of N-terminal and C-terminal fragments (Fig. 9B). To establish the purity of neuronal and glial cultures, we subjected extracts from each culture to immunoblot analysis with antibodies specific for neuron-specific β-tubulin isotype (Lee et al., 1991) and glial fibrillary acidic protein (GFAP). Our studies confirm that neuronal culture is free of glial cells, and the glial culture is free of neurons (Fig. 9*B*). Hence, PS1 and PS2 are expressed in neurons and glial cells.

Immunocytochemical localization of PS1 in mouse brain

To date, only a single report has attempted to localize PS1 in brain (Moussaoui et al., 1996). The authors demonstrated that an antibody raised against a synthetic peptide corresponding to a region within the PS1 "loop" showed PS1-immunoreactive (PS1-IR) in a wide variety of CNS neurons. However, we have several concerns regarding the specificity of the antibody: first, successful immunoprecipitation of PS1 from an *in vitro* translation reaction programmed with synthetic PS1 mRNA was the only biochemical assay for antibody specificity; second, immunocytochemical staining was only competible with extremely high levels of peptide (1 mg/2 μl antiserum); and third, Western blot analyses of brain homogenates were not presented. With these latter reservations, we initiated immunocytochemical studies to define the cellular and subcellular distribution of PS1 in rodent brain with Ab14 and αPS1Loop antibodies (see above, Figs. 8, 9). Using affinity-purified αPS1Loop antibodies, we demonstrate that PS1 is widely expressed and enriched in neurons; in neocortex, pyramidal and nonpyramidal neuronal cell bodies are highly PS1-IR. Moreover, dendrites in all layers and the neuropil were diffusely positive for PS1 (Fig. 10*E*). Consistent with our *in situ* hybridization studies (Figs. 4–6), pyramidal neurons in all hippocampal CA subfields and granule cells in the dentate gyrus were strongly PS1-immunoreactive (Fig. 10*D–F*). In addition, the stratum lucidum of CA3 (cor-

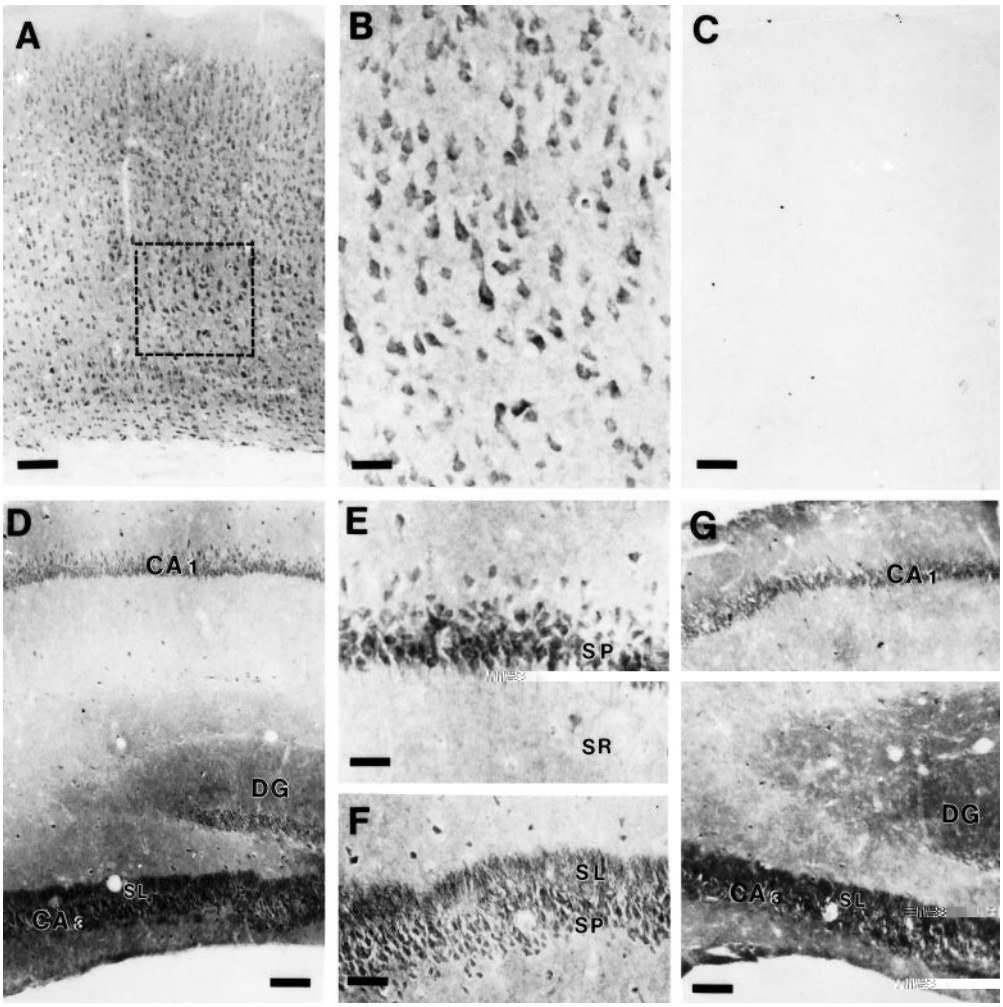


Figure 10. Immunohistochemical localization of PS1 in mouse brain. *A*, αPS1Loop staining in neocortex. PS1-IR is present in all layers of neocortex. *Box* delineates area shown at higher magnification in *B*. Scale bar, 160 μm. *B*, High power view of αPS1Loop staining in neocortex. PS1-IR is enriched in somatodendritic compartments in neurons, with lighter staining in the neuropil. Scale bar, 53 μm. *C*, Somatodendritic and neuropil PS1-IR are fully competed by preadsorption of αPS1Loop antibody with GST-PS1 loop fusion protein. Scale bar, 160 μm. *D*, αPS1Loop staining in the hippocampal formation. PS1-IR is present in *CA1*, *CA3*, and dentate gyrus (*DG*). The cell bodies of pyramidal neurons in *CA1* and *CA3* and those of granule cells of the *DG* are intensely PS1-IR. In addition, the stratum lucidum (*SL*) of *CA3*, corresponding to the terminal fields of mossy fibers originating from granule cells in the *DG*, is also intensely PS1-IR. Scale bar, 320 μm. *E*, Higher magnification of αPS1Loop staining in *CA1*. PS1-IR is prominent in neuronal cell bodies in the stratum pyramidale (*SP*) and to proximal dendrites in the stratum radiatum (*SR*). Scale bar, 64 μm. *F*, Higher magnification of αPS1Loop staining in *CA3*. PS1-IR is present in neuronal cell bodies of the *SP* and prominent in the *SL*. Scale bar, 160 μm. *G*, Ab14 staining in the hippocampal formation. PS1-IR is similar to that using αPS1Loop antibody. Scale bar, 280 μm.

responding to the distribution of mossy fiber terminals) was intensely immunoreactive (Fig. 10*D,F*), consistent with the localization of PS1 in granule cells of the dentate gyrus. Preadsorption of αPS1Loop antibody with a GST fusion protein containing the PS1 "loop" region fully competed neuronal, somatodendritic, and neuropil PS-IR (Fig. 1*C*). Using Ab14 antibodies, we observed a pattern of staining nearly indistinguishable to that observed with αLoopPS1 antibody (Fig. 10*G*). Hence, we conclude that the PS1 N- and C-terminal fragments accumulate in similar, if not identical, subcellular compartments in CNS neurons.

DISCUSSION

To address the potential functional role(s) of presenilins during development and to provide a conceptual framework that relates presenilin expression and selective neuronal vulnerability in AD, we examined the expression of presenilins 1 and 2 during embryonic development and in mature brain. The present studies provide new information concerning the expression of presenilin transcripts and protein, the potential biological function(s) of PS, and the roles of PS in the pathogenesis of AD.

First, and in contrast with earlier Northern blotting studies concluding that PS2 mRNA is expressed at low levels relative to PS1 mRNA, our quantitative RT-PCR studies revealed that, although both PS1 and PS2 transcripts are expressed in many tissues, the relative levels of PS1/PS2 expression can vary significantly between tissues and during brain development. Thus, although the structural conservation and relatively ubiquitous expression pattern of PS1 and PS2 mRNAs suggest some degree of functional redundancy, differences in relative levels of expression suggest that PS1 and PS2 may play different biological roles in tissue- or development-specific processes.

Second, in view of the demonstration that sel-12, a *C. elegans* homolog of presenilins, mediates developmental cell-fate decisions elicited by lin-12 (Levitan and Greenwald, 1995), we examined the expression of presenilins during embryonic development. Although elevated levels of PS1 mRNA in intestine and in skin of E16 mouse embryos overlap the expression pattern reported for Notch 1 mRNA (Weinmaster et al., 1992), the general spatial and temporal expression patterns of presenilins mRNAs do not coincide directly with the expression patterns of any specific member of the known mammalian Notch homologs (Reaume et al., 1992; Weinmaster et al., 1992; Lardelli et al., 1994; Lindsell et al., 1995; Williams et al., 1995). For example, although Notch mRNA expression in the developing neural tube is limited to cells near the ventricular zone, PS1 mRNA is expressed in neuroblasts in the ventricular zones as well as in terminally differentiated neurons. Our demonstration that PS1 mRNA is expressed in neuroblasts suggests that PS1 can play a role in mammalian Notch signaling. In support of this view, we have demonstrated that human PS1 can substitute functionally for *C. elegans* sel-12 protein *in vivo* (D. Levitan, S. Sisodia, and I. Greenwald, unpublished observation).

Nevertheless, the ubiquitous expression of PS1 in terminally differentiated populations of cells indicates that PS1 function is not limited to Notch signaling alone.

Third, our *in situ* hybridization analysis of adult mouse brain reveals that both PS1 and PS2 transcripts are expressed in a variety of neuronal populations. Moreover, prominent expression of presenilin transcripts in neurons supports the view that mutations in presenilin genes cause FAD by directly compromising neuronal function. However, in view of significant levels of presenilin mRNA expression in glial cells, pathogenic mechanisms involving glial cells cannot be excluded. Most notable is our finding that neuronal populations known to be at risk in AD coexpress high levels of APP and presenilin transcripts. For example, neurons in the hippocampal CA-fields, neurons of the medial and cortical amygdala, and neocortical neurons express presenilins and APP transcripts at high levels. In contrast, neurons in regions less prone to AD-associated pathology express these transcripts at more variable levels. For example, granule cells in the dentate gyrus and cerebellum express high levels of presenilins mRNA but lower levels of APP mRNA; in contrast, neurons in lateral amygdala and substantia nigra express high levels of APP mRNA but low levels of presenilin transcripts. Thus, our demonstration of high-level presenilin mRNA expression in neurons known to be at risk in AD further supports the view, proposed earlier by Kovacs et al. (1996), that mutations in PS compromise neuronal function and contribute to the pathology of presenilin-linked FAD. At a conceptual level, coexpression of APP and presenilins at high levels in selected neuronal populations raises the intriguing possibility that mutations in presenilins enhance pathogenic processing of APP, hence rendering specific neurons to be more vulnerable in AD. In this regard, elevated levels of highly amyloidogenic β-amyloid 1-42(43) have been reported in the plasma and conditioned medium of fibroblasts from individuals harboring PS1 or PS2 mutations (Scheuner et al., 1996). Notably, recent immunocytochemical studies documented overwhelming amyloid deposition in the brain parenchyma and blood vessels of patients with an FAD-linked PS1 E280A mutation and revealed, for the first time, the presence of ubiquitin-positive neurites surrounding compact amyloid plaques in the cerebellum, particularly in the Purkinje cell layer (Lemere et al., 1996).

Fourth, our studies are the first to examine the expression and distribution of PS1 by using two highly specific antibodies generated against independent epitopes of PS1. We document that PS1 protein accumulates as two proteolytically processed fragments in all tissues examined, similar to our earlier description of PS1 derivatives in human, primate, and rodent brain and brains of transgenic mice expressing human PS1 (Thinakaran et al., 1996). At present, we do not understand the functional significance of proteolytic processing of PS1. Nevertheless, and in view of the paucity of accumulated full-length PS1 and the generality of PS1 processing across tissues, we suggest that PS1 fragments are the relevant functional units.

Finally, our immunocytochemical studies of mouse brain provide strong evidence for the ubiquitous and prominent accumulation of PS1 polypeptides in neurons. PS1-IR in neurons is localized primarily to somatodendritic compartments, but strong neuropil PS1-IR is also evident. The neuropil-specific PS1-IR may correspond to PS1 within axon terminals, a view supported by our demonstration of prominent PS1-IR in the stratum lucidum of CA3, an area enriched in mossy fiber terminals. Alternatively, neuropil-IR may correspond to distal dendrites or to glial processes. In this regard, and despite our demonstration that low levels of PS1 protein accumulate in cranial nerves (Fig. 8*A*,*B*), PS1 mRNA expression in glial cells (Figs. 5*B*, 9*B*), and PS1 protein accumulation in cultured glial cells (Fig. 9*B*), we rarely detected PS1-IR in glial cell bodies in the neuropil. However, in view of findings that certain glial proteins (e.g., glial glutamate transporters; Rothstein et al., 1994) are enriched in the neuropil and do not show appreciable accumulation in cell bodies, it is not unreasonable that PS1 is localized to glial processes. Future immunoelectron microscopy studies are warranted to identify unambiguously the localization of PS1 within neuropil compartments in brain.

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