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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

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Abstract

Presenilins 1 (PS1) and 2 (PS2) are multispanning transmembrane proteins associated with familial Alzheimer disease (FAD). They are developmentally regulated, being expressed at highest levels during neuronal differentiation and are sustained at a lower level throughout life. We investigated the distribution and metabolism of endogenous murine PS1 as well as human wild-type (wtPS1) and the familial AD Met146Leu (M146L) mutant presenilins in dissociated cultures of hippocampal neurons derived from control and transgenic mice. We found that the PS1 endoproteolytic fragments and, to a lesser extent, the full-length protein, were expressed as early as day 3 post-plating. Both species increased until the cells were fully differentiated at day 12. Confocal microscopy revealed that presenilin is present in the Golgi and endoplasmic reticulum and, as in punctate, vesicle-like structures within developing neurites and growth cones. Using a human-specific PS1 antibody, we were able to independently examine the distribution of the transgenic protein which, although similar to the endogenous, showed some unique qualities. These included (*i*) some heterogeneity in the proteolytic fragments of human PS1; (*ii*) significantly reduced levels of full-length human PS1, possibly as a result of preferential processing; and (*iii*) a more discrete intracellular distribution of human PS1. Colocalization with organelle-specific proteins revealed that PS1 was located in a diffuse staining pattern in the MAP2-positive dendrites and in a punctate manner in GAP43-positive axons. PS1 showed considerable overlap with GAP43, particularly at the growth cones. Similar patterns of PS1 distribution were detected in cultures derived from transgenic animals expressing human wild-type or mutant presenilins. The studies demonstrate that mutant presenilins are not grossly different in their processing or distribution within cultured neurons, which may represent more physiological models as compared to transfection systems. Our data also suggest that the molecular pathology associated with PS1 mutations results from subtle alterations in presenilin function, which can be further investigated using these transgenic neuronal cell culture models.

Introduction

The most common form of early onset familial Alzheimer's disease (FAD) is associated with missense mutations in the presenilin family of proteins (1–4). Presenilin-1 (PS1) and presenilin-2 (PS2) are integral membrane proteins containing multiple transmembrane domains and sharing ~67% homology, diverging most substantially in the nonmembranous sequences. PS1 and PS2 are expressed in the brain as well as in most peripheral tissues, with PS1 having a wider expression pattern compared to PS2 (1,4). Both presenilins are proteolytically cleaved into 30 kD N- and 18 kD C-terminal fragments (5). Current evidence indicates that these two proteolytic products remain associated with each other after cleavage (7–9). Brain expression of PS1 is highest in the cerebrum, hippocampus, and entorhinal cortices (10–15). There are reports of localization of PS1 to neurofibrillary tangle-bearing neurons (16) and to dystrophic neurites of amyloid plaques (17,18) in Alzheimer's brains. Presenilins in the CNS are predominantly localized in neuronal cell bodies and processes, while relatively lower levels are found in astrocytes and blood vessels (10,11,13,17). In both cultured neurons and transfected cells, PS1 has been found within the nuclear membrane, the endoplasmic reticulum (ER), the Golgi complex and in some small unidentified vesicles (15,19–21).

Mutations of the presenilins do not seem to affect the overall distribution of these proteins in brain (16–18,22). However, PS1 mutations have been associated with abnormalities in amyloid precursor protein (APP) processing, leading to an increase in the longer amyloid-β 1–42 species (Aβ42) in vivo and in vitro (6,23–29). In addition, PS1 is essential for Aβ generation, as shown by a recent study of APP metabolism in neuronal cultures derived from PS1 knock-out animals (30).

Full-length presenilin protein and its fragments have been implicated in a number of functional pathways such as protein transport, apoptosis, intracellular signaling, and/or calcium regulation (1,31–35). These functional aspects are based largely on sequence homologies (e.g., to the *C. elegans* protein sel-12 which is involved in the Notch/lin-12 signaling pathway; 36,37). This relationship has been strengthened by the observation that the sel-12 mutant phenotype can be rescued by either PS1 or PS2 (38,39). Links to Notch signaling pathways have been emphasized by the recent demonstration of PS1-dependent cleavage and activity of Notch (40–42). In the brain, PS1 expression increases in parallel with neuronal differentiation and may therefore be instrumental in establishing and maintaining neurons (12,43–45). Furthermore, PS1 knock-outs produce a lack of somite segmentation, stubby tail, and brain hemorrhage, resulting in fetal lethality (33,46), similar to the phenotype associated with deletion of the important developmental gene *Notch1* (47).

The current study was performed to elucidate the distribution and processing of PS1 in differentiating hippocampal neurons in dissociated culture. For this purpose we examined endogenous murine PS1 and, in cultures from transgenic animals, the wild-type human (wtPS1) and the missense mutant Met146Leu.

Materials and Methods

Transgenic Animals and Neuronal Cultures

Transgenic mice expressing human wtPS1 or Met146Leu (M146L) mutant PS1 were driven by the prion promoter as previously described (26). Mouse hippocampal neurons were cultured using a protocol adapted from Goslin and Banker (48). Briefly, E18 mouse hippocampi were trypsinized, dissociated with fire-polished Pasteur pipets, and plated onto poly-L-lysine (Sigma)–coated culture dishes or glass coverslips. Cells were incubated for the first 4 hr in minimum essential medium (MEM) with 10% horse serum and then maintained in serum-free neurobasal medium with B7 supplement (Gibco). Coverslips were transferred to plates containing a confluent layer of glial cells. All cells were incubated at 37°C with 5% CO_2.

Antibodies

The human specific NT1 monoclonal antibody (MAb) recognizes the unique PS1 sequence contained within residues 41–49. Rabbit polyclonal Ab14 was raised against an N-terminal synthetic peptide (residues 1–25 of PS1) as previously described (5). An additional rabbit polyclonal antibody, designated 17.2, was generated against the putative transmembrane 6 and 7 loop domain

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(1,5) using a synthetic peptide corresponding to residues 299–214 coupled to keyhole limpet hemocyanin (Pierce). Antibody 17.2 was subsequently affinity purified using immobilized peptide.

Anti-BiP mouse MAb was obtained from Stressgen (Victoria, BC, Canada). Monoclonal antibodies to other diagnostic neuronal markers such as p58, MAP2, TGN38, and GAP43 were purchased from Sigma. Secondary antibodies conjugated to horseradish peroxidase were purchased from Bio-Rad and used at a dilution of 1:10,000. Secondary goat anti-rabbit FITC (Sigma) was used at 1:100 and goat anti-mouse Cy3 (Jackson Laboratories) at 1:600.

Western Blotting

Cells were scraped in phosphate-buffered saline (PBS) and sonicated in a lysis buffer containing 0.25 M sucrose, 0.1 mM EDTA, 5 mM Tris, pH 7.3, with Trasylol and pepstatin A. Mouse brain homogenates were obtained by sonicating snap-frozen tissue in sucrose buffer as described above. Protein concentration was determined by the Bradford assay (Bio-Rad). Cell homogenates (4 μg protein) were suspended in Laemmli sample buffer containing 1 mM Cleland's reagent (DTT) and separated by SDS-PAGE using a 4–20% Tris-glycine gradient gel (Novex). Proteins were transferred to a polyvinylidene difluoride (PVDF) membrane (Millipore) using glycine transfer buffer (25 mM Tris, 190 mM glycine, 20% methanol, pH 8.6). Membranes were washed with TBS-T (20 mM Tris, 154 mM NaCl, 10 mM EDTA, 0.1% Tween 20, pH 7.4), blocked in 5% milk, and incubated with primary antibody in TBS-T at room temperature overnight. Blots were washed in TBS-T and immunoreactive proteins were visualized using horseradish peroxidase–conjugated secondary antibodies, using an enhanced chemiluminescence (ECL; Amersham) detection system.

Immunofluorescence

Cells were fixed in 4% formaldehyde and 4% glucose in PBS for 10 min at room temperature. Cells were washed with PBS and nonspecific binding was blocked with a combination of 2% bovine serum albumin (BSA), 0.2% cold water fish gelatin (Sigma), and 2% fetal bovine serum (FBS; Gibco) suspended in 0.1% Tween 20. Cells were incubated overnight with primary antibody diluted in blocking solution, and then washed in TBS-T. Immunoreactivity was visualized with FITC/Cy3 secondary antibodies. Immunofluorescence images were obtained using a BioRad 1040 or a Zeiss LSM419-inverted laser-scanning confocal microscope. The digitized images were processed using NIH Image software.

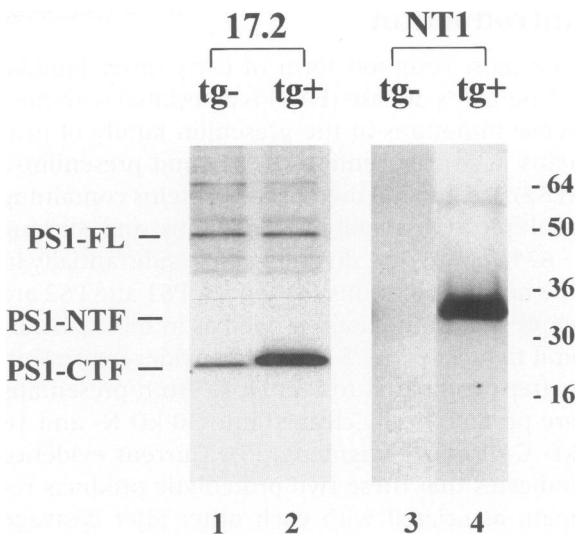

Fig. 1. Characterization of PS1 antibodies. Western blotting of mouse brain homogenates from control nontransgenic (tg−) and transgenic (tg+) animals expressing the wtPS1 human protein. Blots probed with the polyclonal antibody 17.2 raised to the PS1 C-terminal fragment (PS1-CTF; lanes 1, 2) demonstrated an ~18 kD endoproteolytic fragment as well as a less intense PS1 holoprotein at ~50 kD. The human-specific monoclonal NT1 (lanes 3, 4) detected a doublet of PS1 fragments only; little or no full-length human PS1 (PS1-FL) was observed in the transgenic brains. Molecular weight markers are as indicated. PS1-NTF, PS1 N-terminal fragment.

Results

Expression of PS1 in Total Brain Extracts

Specificity of the PS1 antibodies was demonstrated by Western blots of transgenic (tg+) and nontransgenic (tg−) mouse brain homogenates (Fig. 1). The polyclonal 17.2 antibody (Fig. 1, lanes 1, 2) recognizes the full-length PS1 (PS1-FL) that migrates at ~50 kD as well as the 18 kD C-terminal fragment (PS1-CTF) in both transgenic and nontransgenic extracts. The increased level of C-terminal fragment detected in the transgenics without a corresponding increase in PS1-FL in the transgene is consistent with previous findings (49). Using the human-specific NT1

monoclonal antibody, an N-terminal fragment at ~33 kD was observed (Fig. 1, lane 4). In contrast to the endogenous mouse PS1, PS1-FL arising from the human protein was significantly reduced and could only be observed following prolonged exposure (not shown). No NT1 immunoreactive species were observed in the non-transgenic animals (lane 3).

Comparing Endogenous to Transgenic Wild-Type and Mutant PS1

In normal wild-type murine neurons, no PS1-FL was detected at 4 hr post-plating, although in some instances a weak band corresponding to the PS1-CTF was observed (Fig. 2A, lane 1) which was consistent with the NTF fragment (see below; differences in intensity may be antibody related). In addition, a weakly immunoreactive band was observed at ~98 kD (arrow, Fig. 2A) which could represent a dimer of PS1-FL or an aggregate of PS1 fragments. A similar band was observed with PS1-NTF antibodies (see below), which was not observed if the primary antibody was omitted (data not shown), nor was it observed in the total brain extract (cf. Fig. 1). No detectable levels of PS1-FL, and only very low levels of the proteolytic fragments, were present after 1 day of plating (Fig. 2A, lane 2) but successively higher levels of the C-terminal fragment were observed following differentiation at days 3, 7, and 12 (Fig. 2, lanes 3–5). Detectable amounts of PS1-FL were observed beginning at day 3 and increasing to day 12, when the neurons were fully differentiated. An additional but substantially weaker PS1-immunoreactive species was observed at ~55 kD (lanes 4 and 5), perhaps representing a form of PS1-FL that had undergone post-translational modification.

Examination of PS1 expression in transgenic animals indicated a similar pattern for both the human wtPS1 and the M146L mutants (Fig. 2A, lanes 6, 7). With short exposures of the blots, it was revealed that the C-terminal band contained a doublet that included a slightly larger band at ~20 kD (not shown). This heterogeneity may be due to alternate cleavage or to the separation of human and mouse PS1 fragments, as previously reported (50).

Western blots using Ab14 (Fig. 2B) indicated a pattern for the PS1-NTF that was comparable to that observed for the PS1-CTF. A small amount of PS1-NTF was present at 4 hr post-plating and at day 1 (Fig. 2B, lane 1) and increased in intensity at later stages of development (Fig. 2B, lanes 3–5). An N-terminal immunoreactive ~55 kD species was detected at these later stages (Fig. 2B, days 1–12). The origin of this band is unclear and is similar to that observed with the C-terminal antibody (17.2) and may represent a post-translationally modified PS1. As with the C-terminal specific antisera, a 98 kD immunoreactive band was observed when Ab14 immunoblotting was performed at 4 hr post-plating (arrow, Fig. 2B, lane 1). The fact that such a species was observed with two distinct antibodies (Ab14 and 17.2) suggests that this high-molecular species may correspond to an authentic PS1 but the biological relevance of this immunoreactive protein remains to be clarified. As with the human PS1-CTF, there were no appreciable differences in the proteolytic cleavage or expression levels of the NTF in the transgenic neurons expressing either the wild-type or the mutant human proteins (not shown).

Examining the Expression of Transgenic PS1 Using a Human-Specific Monoclonal Antibody

A monoclonal antibody reacting with a unique N-terminal human PS1 sequence (residues 41–49) enabled us to assess the expression of the transgenic protein independent of the endogenous mouse PS1. Western blotting of extracts from hippocampal neurons from both human wtPS1 and M146L mutants during various stages of development indicated that the cleaved species predominated at all stages (Fig. 2C). Unlike the endogenous mouse protein, full-length human transgenic PS1 was present at significantly lower levels even at the final stages of neuronal development (e.g., day 12). This suggests that the full-length protein observed with human–mouse cross-reactive antibodies corresponds primarily to the endogenous murine PS1 (see Fig. 2B). At day 3 post-plating, only the ~33 kD species was observed in the wtPS1 animals (Fig. 2C, lane 1), with a similar pattern being observed in the M146L mutants (Fig. 2C, lane 3). When fully differentiated at day 12, shorter exposures revealed a doublet that contained a slightly lower molecular weight species (not shown). These human-related fragments were observed in both wild-type and mutants and do not represent the separation of the mouse and human proteins as NT1 does not recognize mouse PS1 fragments. Similar patterns of multiple fragments have been reported in differentiating rat cultures (51) and in the developing human brain (45,52). These bands are proba-

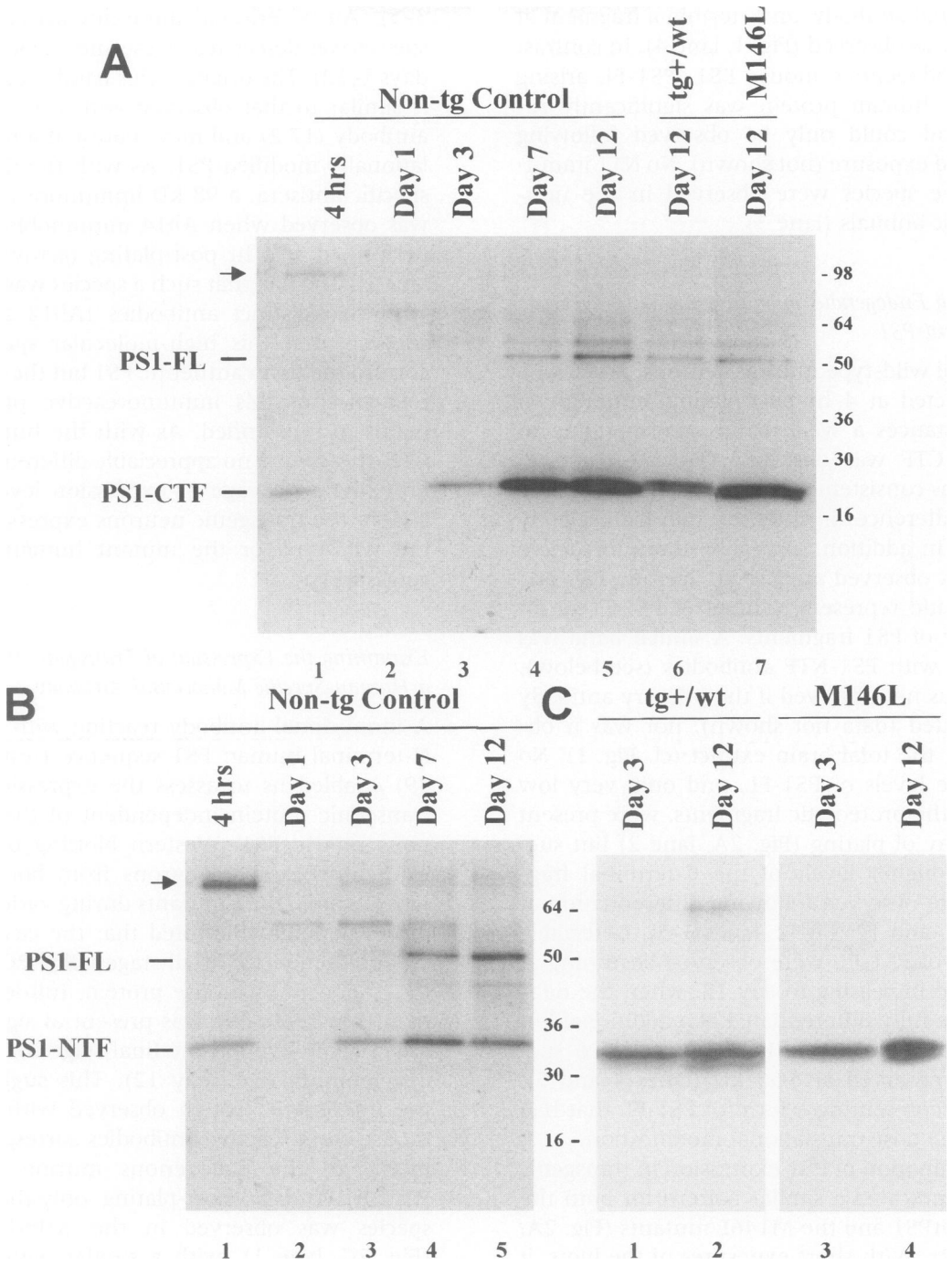

Fig. 2. Developmental expression of PS1 fragments in transgenic and nontransgenic hippocampal neurons. (A) Extracts from normal nontransgenic neuronal cultures were probed with the anti-PS1 C-terminal antibody 17.2 at different stages of development ranging from 4 hr to 12 days postplating (lanes 1–5) to demonstrate the initial appearance of the proteolytic fragment and the later appearance of the full-length protein at ~50 kD. An additional PS1 cross-reactive ~98 kD band was observed at 4 hr (lane 1). Western blot analysis of cultures from transgenic animals expressing wtPS1 (lane 6) or M146L PS1 (lane 7) following differentiation for 12 days is also shown. (B) Homogenates of control nontransgenic neurons were probed with the N-terminal Ab14 antiserum at various stages of development, from 4 hr to 12 days (lanes 1–5). (C) PS1 immunoreactivity was examined in cultured transgenic neurons using the specific NT1 antibody which revealed that the human protein existed primarily in its cleaved form in wild-type (lanes 1, 2) and M146L mutants (lanes 3, 4). An additional and possibly nonspecific band at ~64 kD was observed in the wtPS1 at day 12 (lane 2) which was not detected in the M146L mutant neurons.

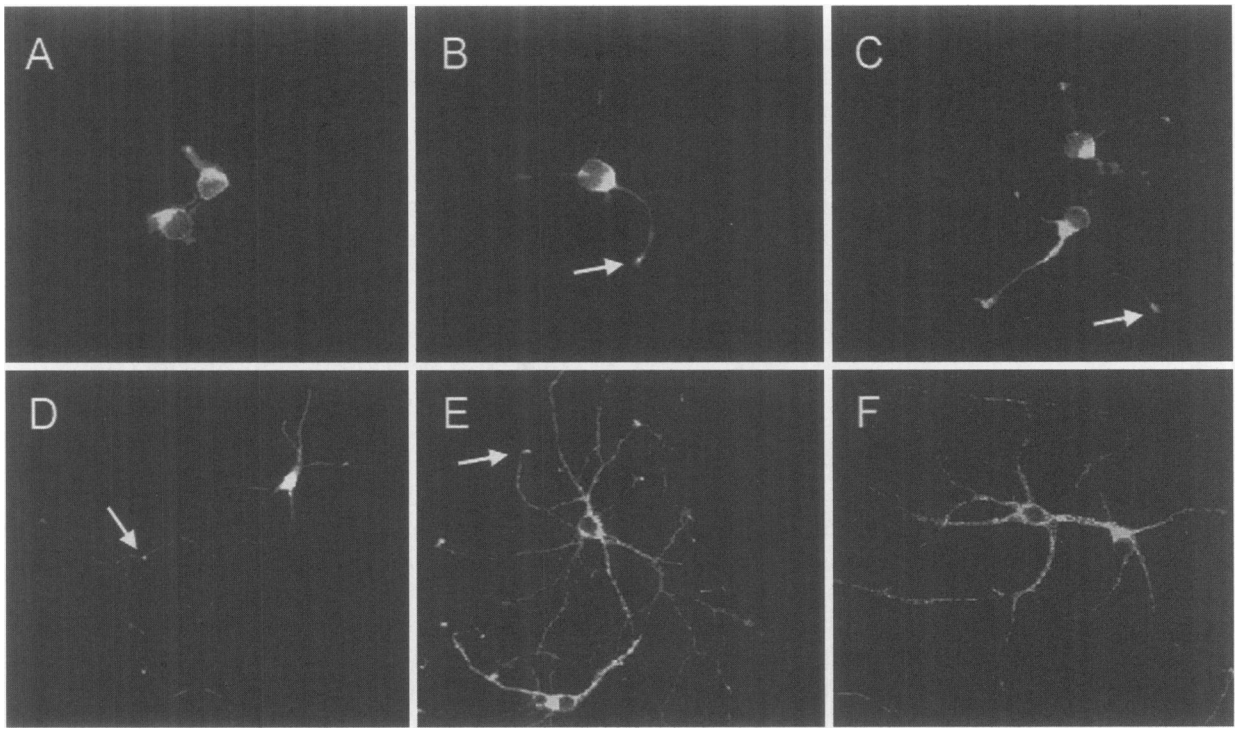

Fig. 3. Subcellular localization of PS1-CTF and -NTF in nontransgenic hippocampal neurons. Cultures examined at stage 1 (A), stage 2 (B), stage 3 (C), stage 4 (D), and stage 5 (E) with the PS1 C-terminal–specific antibody 17.2 showed the perinuclear labeling, as well as labeling within the processes, which extends to the growth cones. Comparable immunofluorescence was obtained with the N-terminal–specific Ab14 as shown in the fully differentiated cells (F). Arrows indicate the PS1 immunoreactivity that was observed at the termini of the expanding processes.

bly attributable to one or more post-translational modifications such as endoproteolysis and/or phosphorylation.

Subcellular Localization of Endogenous Murine PS1 in Hippocampal Neurons

The subcellular localization of PS1 was examined by immunofluorescence microscopy during neuronal development through stages 1–5 as described by Dotti et al. (53). At 4 hr after plating, cultures labeled with the C-terminal antibody 17.2 revealed that PS1 was largely confined to the perinuclear space within the cell body of these stage 1 neurons, but labeling could also be observed within newly extending processes (Fig. 3A). From stages 2 through 4 (Fig. 3B–D), intense immunoreactivity was observed within the cell body and extending into the hillocks of some neuritic processes. In addition, an intense punctate staining was observed within a subset of the developing growth cones of these early- to mid-stage neurons (Fig. 3B–D, arrows). During later stages, PS1 immunoreactivity began to appear along the shafts of some of neuritic processes but the intensity was substantially weaker than that present around the nucleus and at growth cones (see, for example, Fig. 3D).

At fully differentiated stage 5, the neuronal PS1 staining within the perinuclear space was similar to less developed neurons (Fig. 3E). In contrast to the early stages, labeling within the processes on day 12 had a more well-defined punctate appearance, which was significantly more intense than, for example, the stage 4 cultures (compare Fig. 3E to Fig. 3D). This intense, possibly vesicular, staining was also observed in many more processes in the fully developed neurons and displayed a diffuse pattern in the larger dendrites that contrasted with the widely scattered staining in the narrow axon-like processes. These findings are consistent with the data from studies of endogenous rat PS1 and PS2 in cultured neurons as described by Capell and colleagues (51). Visualization of endogenous PS1-NTF with Ab14 revealed an identical pattern of PS1 immunolocalization in stages 1–4 (not shown). In fully differentiated stage 5 cultures,

the PS1-NTF displayed a similar perinuclear staining and punctate vesicular structures within the processes as well as the intense growth cone localization (Fig. 3F). Some full-length PS1 was observed by Western blotting, which could account for this observation. However, the majority of the PS1 is in the cleaved form and therefore this overlap of CTF and NFT staining within the developing hippocampal neurons is consistent with the observation that the fragments remain associated following endoproteolytic cleavage (7–9).

Colocalization of PS1 with Cellular Organelle Markers

In order to define further the distribution of PS1 within hippocampal neurons, a series of subcellular markers were used in conjunction with the PS1 antibodies. To investigate the organelles contributing to the perinuclear reactivity of PS1, we performed double labeling using antibodies to endoplasmic reticulum and Golgi-localized proteins. The endoplasmic reticulum marker BiP was found primarily within the perinuclear region as well as in discrete vesicular profiles within the initial segments of both dendrites and axon-like processes (Fig. 4A). Staining for the PS1-CTF (Fig. 4B) in the same cell indicated a partial overlap with BiP (Fig. 4C), which is consistent with the subcellular localization of PS1 in transfected and wild-type cells (19–21). However, the murine neurons did not display a complete overlap between BiP and endogenous PS1. This suggests that PS1 may be contained within an ER subcompartment. The *cis*-Golgi network (CGN) marker p58 also displayed strong perinuclear staining (Fig. 4D) as expected (47). Double labeling for p58 and the PS1-CTF using antibody 17.2 (Fig. 4E) revealed a virtually complete overlap of the two staining patterns (Fig. 4F). This PS1-Golgi immunoreactivity was apparently restricted to the *cis*-component since double labeling with antibodies to TGN38 and PS1 indicated little obvious codistribution (data not shown).

The localization of human and murine PS1 within processes was examined using antibodies to the cytoskeletal protein, MAP2, and the growth cone protein, GAP43. In fully differentiated hippocampal neurons, MAP2 staining was widely distributed within dendrites (Fig. 5A) as reported (53–55). Double labeling with antibodies to MAP2 and PS1 indicated an extensive colocalization of the two proteins within the MAP2-reactive processes (Fig. 5A). However, the PS1 immunoreactivity was also apparent in the MAP2-negative axons (Fig. 5A, arrows). This indicates that PS1 maintains a widespread level of expression within various types of processes.

PS1 distribution within neurite terminals was investigated using antibodies to GAP43 and PS1. GAP43 immunoreactivity was observed in axons and extending growth cones (Fig. 5B), consistent with previous investigations (48,55,56). Immunolabeling for PS1 demonstrated a partial coincidence of staining for the two proteins within the axonal processes. It is also possible that the discrete axonal staining could represent synapse-like structures containing PS1, since GAP43-positive domains can represent axon-dendrite contacts. This possibility would be consistent with the observation that intense PS1 immunoreactivity within the growth cones often overlapped with the GAP43 immunoreactivity (Fig. 5B, arrow). While some PS1-positive but GAP43-negative terminals were observed, these represented only a small percentage of the terminals. Double labeling for PS1 and synaptophysin, a synaptic vesicle protein of nerve terminals, did not significantly overlap (not shown). This observation is consistent with the failure of PS1 to cofractionate with small synaptic vesicles (S. Petanceska and S. Gandy, unpublished results).

Comparing the Subcellular Localization of Human Wild-Type and Mutant PS1

The distribution of PS1 in the transgenic neurons expressing human wtPS1 and the M146L mutant was also investigated to determine whether the human proteins localized in a markedly different fashion than did the endogenous murine proteins. These studies were undertaken using the human-specific MAb NT1. In this case, fully differentiated neurons displayed a perinuclear staining similar to that seen with the control, nontransgenic cultures (Fig. 6). In contrast to the staining observed with the polyclonal antisera, NT1 revealed immunoreactivity within the processes which appeared to be more intense and punctate as compared to the pattern present in neurons expressing endogenous mouse PS1. Normal nontransgenic mouse hippocampal neurons did not show any immunoreactivity with NT1, indicating that the labeling observed in the

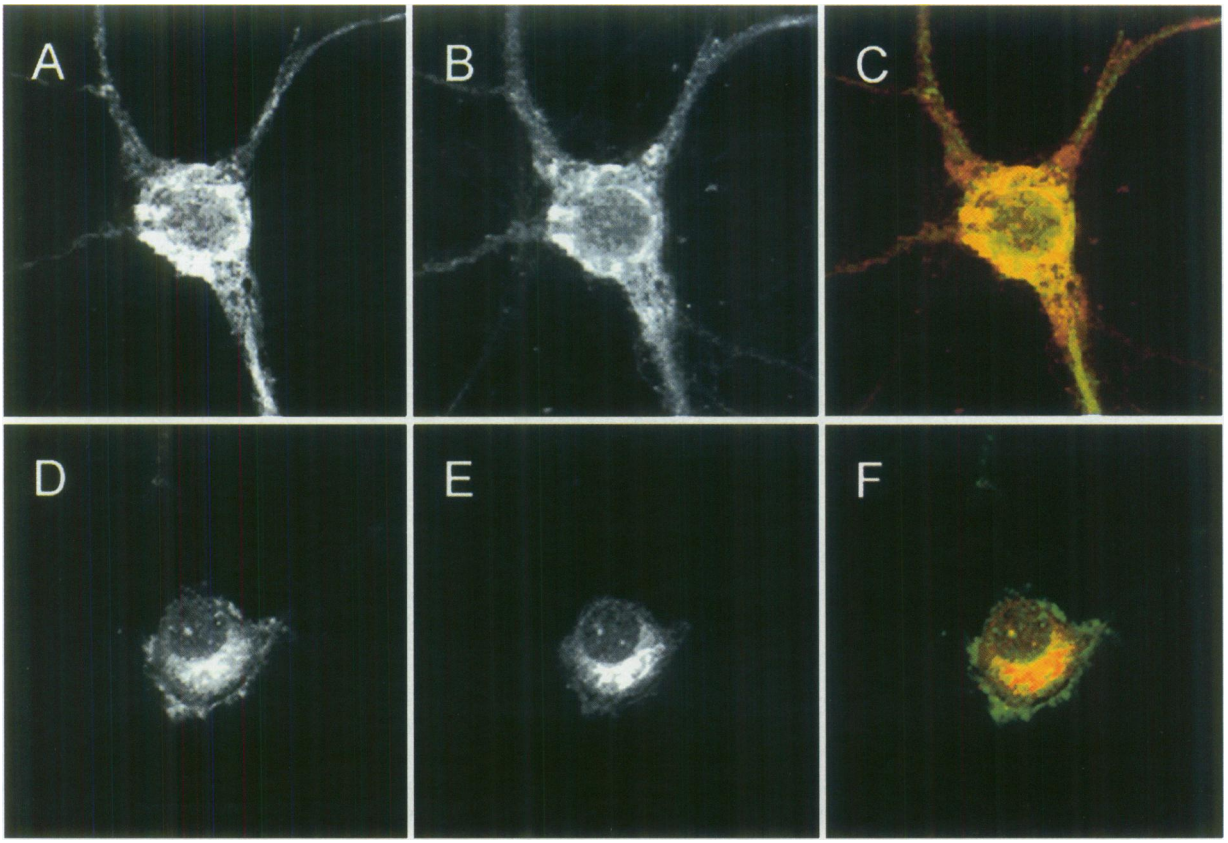

Fig. 4. Colocalization of PS1 with markers to the endoplasmic reticulum (ER) and CGN compartments. Differentiated neurons (stage 5) were double labeled with a monoclonal antibody to the ER protein, BiP (A), and the PS1 C-terminal fragment using the 17.2 antiserum (B) with a color overlay (C), with BiP in green and PS1 in red. Similar cultures were examined with the CGN marker, p58 (D) and the 17.2 antiserum (E) to demonstrate the degree of overlap between the two proteins. A color overlay with p58 in green and PS1 in red is shown in F.

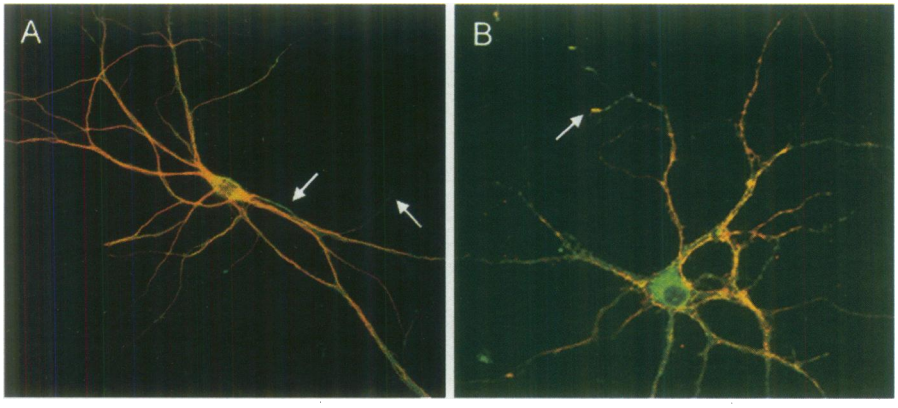

Fig. 5. Colocalization of PS1 with neuronal markers. Differentiated neurons were double labeled with a monoclonal antibody to microtubule-associated protein 2 (MAP2) (red) and with the PS1 C-terminal specific antibody 17.2 (green). The unique axonal distribution of the PS1 protein is indicated by the arrows (A). Similar cultures were double labeled with a monoclonal antibody to the growth cone marker GAP43 (red) and PS1 (green; antibody 17.2) indicating the overlap of staining within the extending processes and terminals (arrow) (B).

transgenic cultures was limited to the human protein (not shown). This is consistent with the Western blots probed with the NT1 antibody where no immunoreactive bands were observed for endogenous mouse PS1. These observations indicate that the introduction of transgenic PS1,

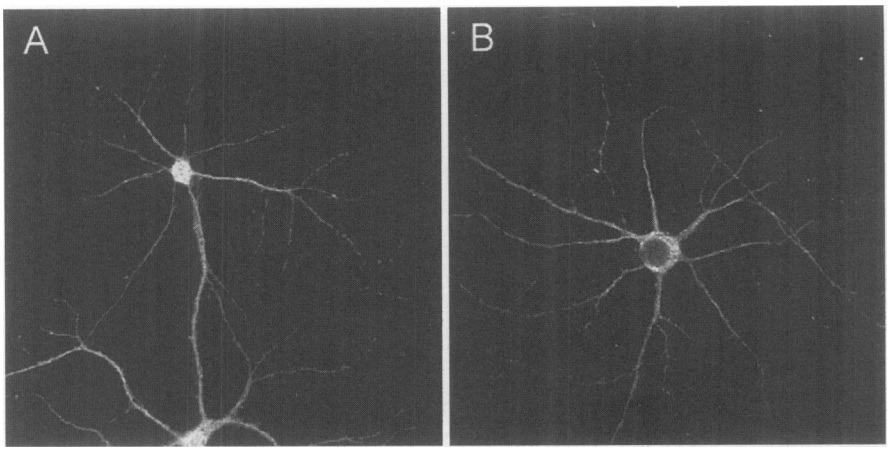

Fig. 6. Immunolabeling of human PS1 in transgenic neuronal cultures. The expression and subcellular distribution of the transgenic PS1 were examined independently of the patterns of endogenous murine protein in cells expressing the wtPS1 (A) and M146L (B) mutant proteins using the human-specific antibody NT1. Labeling comparable to that of the endogenous mouse PS1 was observed, although with a more punctate distribution.

either wild-type or mutant, does not cause any obvious abnormalities in the patterns of PS1 distribution.

Discussion

In the present study, we have examined the cell biology of PS1 in hippocampal neurons derived from wild-type mice as well as neurons from transgenic mice expressing wild-type or mutant human PS1. The objective of this investigation was to determine the processing and subcellular distribution of mutant and wild-type PS1 at various developmental stages. A comparative study of the endogenous mouse and human PS1 transgenic neurons allowed us to determine whether there might be any obvious differences in the behavior of the normal human PS1 protein or in a mutated counterpart. In addition, this investigation allowed us to establish the feasibility of using these neuronal cultures to investigate the relationship of presenilins to AD-related pathobiology.

Previous investigations have revealed that PS1 undergoes endoproteolytic cleavage at or near Met-292 which is located within the cytoplasmic loop (52). This results in the formation of an ~30 kD PS1-NTF and an ~20 kD PS1-CTF fragment (5,52). The majority of PS1 exists in a cleaved state which appears to be highly stable as shown by the extended half-lives of the endoproteolytic fragments (5,7,49,52). In contrast, PS1-FL is rapidly degraded, possibly by a proteasome-mediated pathway (57). Examination of the transgenic and nontransgenic cultured neurons indicated that endogenous murine and transgenic human wild-type and mutant PS1 were all processed in a comparable fashion, based upon immunoblotting of major fragments using a variety of antibodies. In all cases, PS1-NTFs and CTFs behaved similarly in expression and cleavage state at all stages of development. Detectable levels of the full-length PS1 were only observed at the final stages of differentiation. We found an indistinguishable pattern for the M146L mutant (i.e., early appearance of the endoproteolytic fragments with same accumulation of the full-length protein at later stages). These results suggest that, at the level of PS1 processing, the human wild-type and mutant PS1 proteins behave similarly to the endogenous mouse protein.

The availability of a human PS1-specific MAb permitted us to follow the processing of the transgenic proteins without interference from the endogenous murine PS1. Consistent with previous findings, the human wtPS1 appeared as proteolytic fragments. However, compared to murine PS1, the level of the full-length human PS1 was considerably lower, even in fully differentiated neurons. The reduction in human PS1-FL may be due either to the expression level or to preferential cleavage of the human protein. Another unique feature of the human PS1 protein was the presence of electrophoretic heterogeneity of its N-terminal fragments, which appeared as a major species at ~33 kD and a less prominent and slightly more rapidly migrating species. This was also observed using the cross-reactive polyclonal antibodies. The origin and potential biological function of this minor species is unknown but our investigations with the human-specific antibody have revealed that both fragments are derived from the human PS1 protein, and may therefore originate from an alter-

native cleavage pathway. Multiple N-terminal fragments have been observed in differentiating rat cultures (51) and in the developing human brain (45,52). Alternatively, they may arise from distinct post-translational modifications such as phosphorylation (7,58). These observations demonstrate not only that the post-translational modification of transgenic PS1 is developmentally regulated but also that human PS1 metabolism in neuronal cultures from transgenic animals parallels that of the human brain.

Further investigations of the biology of the murine and transgenic PS1 were performed to determine their distribution and subcellular localization during development. As with endoproteolysis, comparisons of PS1 localization in the different cultures indicated that the endogenous and wild-type human transgenic PS1 were distributed in a similar manner. Both human and murine PS1 were initially concentrated within the perinuclear space, and overlapped to a large extent with ER and CGN markers such as BiP and p58. This is consistent with previous observations in transfected cells and in rat neuronal cultures (20,21,51,59,60). In contrast to that observed in non-neuronal cells, PS1 distribution in hippocampal cultures was not restricted to perinuclear compartments but appeared to be much more widespread. At later stages of development, PS1 immunoreactivity was observed within the processes and appeared to be distributed in both axons and dendrites. It is possible that this virtually contiguous staining from the cell body into the processes is the result of ER-resident PS1 as it has been shown that the smooth ER can extend for long distances within neurites (54). However, the punctate labeling pattern is more suggestive of a discrete vesicular localization. These structures could, for example, reflect the association of PS1 with the machinery of anterograde and/or retrograde vesicular transport. Immunofluorescence studies with the human PS1-specific mAb NT1 revealed the most striking punctate staining. Because this antibody exclusively recognizes the human fragments and low levels of the full-length human PS1 protein was present in the cultures, these results may indicate that fragments of the human PS1 preferentially localize to vesicles and/or that the proteins are sequestered to particular compartments following cleavage.

Interestingly, some of the most intense PS1 immunostaining was observed within the extending growth cones as shown by overlap with GAP43 immunoreactivity. This concentration of PS1 at the process terminals raises the possibility that the presenilins are involved in synaptic contacts. The recent report that the PS1 cytoplasmic loop forms a stable complex with β-catenin and a novel *armadillo* protein is consistent with a proposed functional interaction in synaptic development (9,61). It has been demonstrated that cell–cell adhesion mediated by the cadherin–catenin complex is localized to synapses and may be responsible for maintaining the synaptic ultrastructure possibly via direct interactions with the actin network (62). This hypothesis is supported by recent fractionation studies demonstrating the association of endogenous rat PS1 with synaptic organelles and growth cone membranes (63). Although this requires further investigation, the growth cone localization of PS1 may be indicative of a larger functional complex with the catenins and other underlying cytoskeletal elements.

The object of our investigation was to determine the behavior of the presenilins in neuronal cultures and to ascertain whether any differences were readily apparent between the endogenous murine PS1 and the transgenic human wild-type or mutant proteins. Based upon immunoblotting and immunofluorescence studies at different stages of development, it appears that the wild-type human transgenic PS1 is generally handled in a fashion comparable to the endogenous mouse protein, although some minor differences in N-terminal processing were observed. This was also true for the human PS1 M146L mutant which was processed and localized in a similar fashion. This suggests that there are no overt differences in the metabolism or subcellular distribution of the mutant PS1 as compared to human wild type. Therefore, the pathological changes associated with PS1 mutations, such as the abnormalities in APP processing, must arise from more subtle mutation-related changes in presenilin function. The observed similarities in the biology of the transgenic PS1 proteins are also important in that they indicate that these cultures may be useful models to investigate further presenilin function and its relationship to AD-related pathology. Finally, the widespread distribution of the presenilins in various subcellular localization such as the ER, Golgi, and growth cones suggests that PS1 may be involved in several pathways. Use of hippocampal cultures should help to clarify both the normal and pathological functions of the presenilins.

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