Evidence That Levels of Presenilins (PS1 and PS2) Are Cooperatively Regulated by Competition for Limiting Cellular Factors*

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Mutations in two related genes, PS1 and PS2, account for the majority of early onset cases of familial Alzheimer's disease. PS1 and PS2 are homologous polytopic membrane proteins that are processed endoproteolytically into two fragments in vivo. In the present report we examine the fate of endogenous PS1 and PS2 after overexpression of human PS1 or PS2 in mouse N2a neuroblastoma cell lines and human PS1 in transgenic mice. Remarkably, in N2a cell lines and in brains of transgenic mice expressing human PS1, accumulation of human PS1 derivatives is accompanied by a compensatory, and highly selective, decrease in the steady-state levels of murine PS1 and PS2 derivatives. Similarly, the levels of murine PS1 derivatives are diminished in cultured cells overexpressing human PS2. To define the minimal sequence requirements for "replacement" we expressed familial Alzheimer's disease-linked and experimental deletion variants of PS1. These studies revealed that compromised accumulation of murine PS1 and PS2 derivatives resulting from overexpression of human PS1 occurs in a manner independent of endoproteolytic cleavage. Our results are consistent with a model in which the abundance of PS1 and PS2 fragments is regulated coordinately by competition for limiting cellular factor(s).

Alzheimer's disease (AD) is a neurodegenerative disorder characterized by the presence of numerous senile plaques and neurofibrillary tangles in the cerebral cortex and hippocampus of affected individuals (1). Familial early onset AD (FAD) is associated with mutations in the amyloid precursor protein (APP) gene on chromosome 21 (for review, see Ref. 2), the PS1 gene on chromosome 14 (3), and the PS2 gene on chromosome 1 (4, 5). Mutations in PS1 are causative in ∼25% of pedigrees with FAD. The mechanisms by which mutations in PS1/PS2 predispose individuals to FAD have not been defined. However, levels of highly fibrillogenic Aβ42 species are elevated in fibroblast-conditioned medium and plasma from carriers of PS1/PS2 mutations compared with unaffected family members (6). These studies have been confirmed by analysis of transfected cells and transgenic mice expressing FAD-linked PS1/PS2 variants (7–10). Collectively, these studies support the view that mutations in PS1 and PS2 cause AD by increasing the extracellular concentrations of highly fibrillogenic Aβ42 peptides.

PS1 and PS2 are polytopic membrane proteins (11) that share extensive amino acid sequence identity. In previous efforts, we documented that PS1 is subject to endoproteolytic cleavage and that the preponderant PS1-related species that accumulate in vivo are the 28-kDa NH2-terminal (NTF) and 17-kDa COOH-terminal (CTF) derivatives; the accumulation of NTF and CTF is highly regulated, saturable, and stoichiometric (12). These studies have led to the suggestion that the endoproteolytically generated PS derivatives are the functional units in vivo. Recent studies indicate that PS2 is also endoproteolytically processed (10, 13). Although the biological significance of PS1/PS2 endoproteolysis is unclear, an FAD-linked variant that lacks sequences encoded by exon 9 (14) fails to be cleaved in vivo (12).

In this report we examine the fate of mouse PS1 and PS2 in mouse neuroblastoma cell lines that overexpress human PS1 or PS2. Remarkably, murine PS1 and PS2 derivatives fail to accumulate in these cells. These observations in cultured cells have been confirmed by the demonstration that murine PS2 is "replaced" by human PS1 derivatives in brains of transgenic mice expressing human PS1. Arguing against the view that replacement of murine PS1 and PS2 derivatives in cultured cells may be the result of artifacts associated with protein overexpression, we document that neither the steady-state levels of murine PS1/PS2 mRNA, synthetic rates of total membrane-bound or soluble protein, nor the steady-state levels of endoplasmic reticulum (ER) resident proteins, i.e. calnexin and BiP, are altered in these cell lines. Interestingly, overexpression of the PS1E9 variant in neuroblastoma cells also resulted in failed accumulation of murine PS1 derivatives, findings that suggest that endoproteolytic processing is not obligatory for replacement. In summary, our results are consistent with a model in which overexpressed human PS1 or PS2 competes with newly synthesized endogenous PS1/PS2 for limiting cellular component(s) that regulate endoproteolysis of full-length PS1/PS2 polypeptide and/or accumulation of the resulting derivatives.

MATERIALS AND METHODS

Generation of Expression Plasmids—CMV-based mammalian expression plasmids pCB6PS1 and pCB6PS2, encoding human PS1 and PS2, respectively, and mouse prion promoter-based PS1 expression plasmid MoPrP.PS1 were generated as described (12). Plasmids encoding COOH-terminally truncated PS1 polypeptides PS1D91Δ and PS1D140Δ were generated by polymerase chain reaction as follows. Antisense
oligonucleotide 5′-CCGGATCCGTTCTCAGAGTCTTTCCAGGAGCCGCAGACACAGCAGTCTGATCT-3′, complementary to sequences encoding amino acids 356–361 of human PS1, 7 amino acids (RFLEERP) of amyloid precursor-like protein 1, a stop codon, and containing a BamHI site at its 5′-end, were incubated in sense primer 5′-GGATCCATT-GTTGTCATGATCTAC-3′, which encodes PS1 amino acids 143–148 and plasmid pBEPS1. The polymerase chain reaction products were digested with PstI and BamHI, and the 460- or 590-base pair fragments were ligated to PstI-BamHI pCB6PS1 “vector” fragment. The polymerase chain reaction inserts were sequenced using Sequenase (U. S. Biochemical Corp.).

Antibodies—We utilized three antibodies specific for PS1 in this study. Ab14 is a polyclonal antiserum specific for amino acids 3–15 of human and mouse PS1 (12) (provided by Dr. Mary Seeger and Sam Gandy, Cornell University Medical College, New York); αPS1 is a rat monoclonal antibody specific for amino acids 21–70 of human PS1 (28) (provided by Dr. Allan I. L evey, Emory University School of Medicine, Atlanta); and αPS1Loop is a polyclonal antiserum that reacts with epitopes in the hydrophilic loop domain (amino acids 263–407) of human and mouse PS1 (12). To generate antibodies against PS2, New Zealand White rabbits were immunized with polyacrylamide gel-purified region of mouse PS1 (nucleotides 1759–1942; GenBank accession no. U57324), mouse APP cDNA, or pBEPS1 mouse PS1 (nucleotides 1759–1942; GenBank accession no. U57324), mouse APP cDNA, or pBEPS1 mouse N2a neuroblastoma cell lines that harbor butyrate-inducible CMV expression plasmids containing human PS1 cDNA (PS1 lines). After induction of transgene expression with butyric acid for 48 h, expression of PS1 was assessed by Western blotting using aPS1Loop, a polyclonal antiserum that recognizes epitopes in the hydrophilic loop domain of PS1 (12). Consistent with previous studies (8, 12), we detected ~16-kDa murine PS1 CTF in four independent G418-resistant N2a lines harboring “empty” plasmid vector (N2a lines) (Fig. 1A, lanes 1–4), whereas a ~43-kDa full-length human PS1 polypeptide and ~18-kDa CTF were detected in three independent PS1 lines (Fig. 1A, lanes 5–7). The striking difference in the intensity of CTF signal in human PS1-expressing lines compared with N2a lines is a reflection of the ~2–3-fold higher avidity of aPS1Loop for human PS1 CTF relative to murine PS1 CTF (12). The human PS1-derived CTF exhibits slightly retarded mobility compared with the murine PS1 CTF on SDS-PAGE (8, 12). At first glance it appears that overexpression of ~18-kDa human PS1 CTF led to diminished levels of endogenous ~16-kDa mouse PS1 CTF, and the vast majority of the CTF in PS1 lines is derived from human PS1. To confirm this observation, we fractionated protein extracts from two N2a lines and two PS1 lines on 16% SDS-PAGE to resolve the mouse and human CTF (Fig. 1B). As we expected, the mouse CTF was replaced by human CTF in PS1 lines.

We proposed earlier that the PS1 NTF and CTF are likely the “functional” units since the accumulation of these derivatives is coregulated (12). Hence, and in view of the replacement of murine PS1 CTF by human PS1 CTF in PS1 lines (Fig. 1B), we anticipated that human PS1 NTF would also replace murine PS1 NTF. To examine this issue, we performed Western blots with Ab14, an antiserum raised against amino acids 1–25 of PS1. We and others have noted that the human PS1 NTF exhibits accelerated migration relative to the mouse PS1 NTF on SDS-PAGE (7, 8, 12); as we expected, Western blot analysis revealed a small but highly reproducible difference in the mobility of PS1 NTF in all PS1 lines compared with N2a lines (data not shown). From these analyses we conclude that the preponderant processed derivatives that accumulate in butyrate-induced PS1 lines are derived from the human PS1 polypeptide. Moreover, in all three PS1 lines examined, the human NH2- and COOH-terminal derivatives only accumulated to ~2-fold over N2a lines despite the notable accumulation of noncleaved, full-length human PS1 polypeptides. These results are consistent with our earlier studies in which we documented that in brains of transgenic mice expressing high levels of human PS1 mRNA and full-length protein, the human NTF and CTF accumulated to saturable levels with concomitant replacement of mouse NTF and CTF derivatives (12).

Overexpression of Human PS1 in Mouse Neuroblastoma Cells Compromises Accumulation of Endogenous Murine PS1—To examine the metabolism of PS1, we generated stable mouse N2a neuroblastoma cell lines that harbor butyrate-inducible CMV expression plasmids containing human PS1 cDNA (PS1 lines). After induction of transgene expression with butyric acid for 48 h, expression of PS1 was assessed by Western blotting using aPS1Loop, a polyclonal antiserum that recognizes epitopes in the hydrophilic loop domain of PS1 (12). In contrast to PS1 lines derived from the human PS1 TgM1 and TgM1-M1-HI plasmids, whose human PS1 mRNA and full-length protein expression levels were determined by Northern blotting using human PS1 cDNA probes (12). At first glance it appears that overexpression of ~18-kDa human PS1 CTF led to diminished levels of endogenous ~16-kDa mouse PS1 CTF, and the vast majority of the CTF in PS1 lines is derived from human PS1. To confirm this observation, we fractionated protein extracts from two N2a lines and two PS1 lines on 16% SDS-PAGE to resolve the mouse and human CTF (Fig. 1B). As we expected, the mouse CTF was replaced by human CTF in PS1 lines.

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Polypeptides bound nonspecifically by a represent lysates prepared from PS1 lines. Full-length human PS1 or PS2 polypeptide (∗ separation of human (∗) lines stably transfected with human PS1 cDNA (PS1 lines). Panel A, aliquots of N2a and PS1 lysates were fractionated on 16% SDS gels for better separation of human (Hu) and murine (Mo) CTFs. Panel C, aliquots of lysates shown in panel A were analyzed by immunoblotting with αPS2Loop. Lane 1 represents lysates prepared from stable cell line expressing human PS2; lanes 2–5 represent lysates prepared from N2a lines; lanes 6–8 represent lysates prepared from PS1 lines. Full-length human PS1 or PS2 polypeptide (FL) and human (Hu) and murine (Mo) CTFs are indicated. Polypeptides bound nonspecifically by αPS2Loop antiserum are marked by asterisks. Molecular mass standards are listed in kDa.

Coordinate Regulation of PS1 and PS2 Levels

Fig. 2. Overexpression of human PS1 compromises accumulation of endogenous PS2 derivatives in transgenic mice. Panel A, detergent extracts (50 µg of protein) prepared from transgenic mice (R8-1 and S8-4; lanes 3–6) or nontransgenic littermates (NTg; lanes 1 and 2) were analyzed by immunoblotting with αPS1Loop antiserum. Human (Hu) and murine (Mo) PS1-derived CTFs are indicated. Panel B, membrane proteins (50 µg) prepared from nontransgenic (lanes 2 and 3) and transgenic mice (lanes 4–7) were analyzed by immunoblotting with αPS2Loop antiserum. Lane 1 represents an aliquot of lysate prepared from stable cell line expressing human PS2. PS2-derived CTF is indicated.

Saturable Accumulation of PS2 Derivatives and Effects on Accumulation of PS1 Derivatives—Our demonstration that overexpression of PS1 led to compromised accumulation of PS2 suggests that the homologous molecules competed, at some level, with limiting cellular components responsible for endoproteolysis and/or stabilization of the resulting derivatives. To provide additional support for this hypothesis, we generated stable N2a cell lines transfected with butyrate-inducible CMV promoter-driven human PS2 expression plasmids (PS2 lines) and examined the levels of endogenous PS1 derivatives in these lines. Four independent PS2 lines were examined which accumulated human CTF and low levels of full-length human PS2 (Fig. 3A, lanes 5–8). Although murine PS2 CTF appears to be weakly detectable in N2a lines (Fig. 3A, lanes 1–4 and 9–12), the CTF was readily detectable after extended exposures of the blots (see Fig. 1C). Upon treatment of cells with butyric acid, high levels of human PS2 full-length polypeptide accumulated in PS2 lines, but the levels of CTF remained unchanged relative to uninduced cells (compare Fig. 3A, lanes 5–8 and 13–16). These results confirm that, like PS1 CTF, the PS2 CTF also
Coordinate Regulation of PS1 and PS2 Levels

Overexpression of human PS2 compromises accumulation of endogenous PS1 derivatives. Panel A, detergent lysates were prepared from N2a lines (lanes 1–4 and 9–12) or independent stable cell lines transfected with human PS2 cDNA (PS2 lines; lanes 5–8 and 13–16). Aliquots of lysates were analyzed by immunoblotting with aPS2Loop antiserum. Lanes 1–8 represent lysates prepared from uninduced cells; lanes 9–16 represent lysates prepared from cells after induction of transgene expression with butyric acid for 48 h. Full-length PS2 polypeptide (FL) and PS2 CTF are indicated. A ~150-kDa polypeptide bound nonspecifically by aPS2Loop antiserum is marked by an asterisk. Panel B, aliquots of lysates prepared from butyrate-induced N2a lines (lanes 1–4) and PS2 lines (lanes 5–8) were analyzed by immunoblotting with Ab14 or aPS1Loop antiserum. Endogenous PS1-derived NTF and CTF are indicated.

As described above, murine PS1 and PS2 mRNA were unaffected by overexpression of human PS1 or PS2. Nevertheless, it was uncertain whether the biosynthetic rate of murine PS1 and PS2 might be compromised. Unfortunately, our currently available antibodies cannot distinguish between human and murine PS1/PS2. Moreover, we have attempted to examine the biosynthesis of murine PS1 and PS2 in N2a lines following short 20-min pulse labeling with [35S]methionine and immunoprecipitation using PS1-specific antibodies Ab14 and aPS1Loop or PS2-specific antibody, aPS2Loop. Using this pulse labeling/immunoprecipitation strategy, we have failed to recover newly synthesized [35S]methionine-labeled murine PS1 or PS2 (data not shown), findings entirely consistent with several earlier reports that indicated that endogenous PS1 or PS2 polypeptides are not detectable in untransfected NT2 neurons, HEK293, Chinese hamster ovary, and COS cells even after continuous labeling for 2–4 h (9, 17–21). Thus, the synthetic rates of endogenous murine, primate, and human PS1 and PS2 polypeptides are well below the limits of detection using conventional pulse labeling/immunoprecipitation assays. Having failed to detect newly synthesized murine PS1/PS2, we sought to examine whether induced expression of human PS1 or PS2 in N2a lines or PS2 lines, respectively, affected the biosynthesis of total membrane-bound or soluble proteins. For these studies, N2a lines, PS1 lines, or PS2 lines were induced with butyric acid for 48 h and then pulse labeled for 20 min with [35S]methionine in the presence of butyric acid. Cells were lysed in hypotonic buffer and fractionated into soluble and membrane fractions. 50-μg aliquots of each sample were fractionated by electrophoresis on one-dimensional SDS-PAGE, and labeled polypeptides were revealed by autoradiography. The profiles of newly synthesized soluble or membrane-bound proteins in N2a lines, PS1 lines, and PS2 lines were essentially indistinguishable (Fig. 4B, lanes 1–8). To examine the biosynthesis of a

accumulated to saturable levels regardless of the levels of full-length PS2 polypeptides. As we had predicted, the levels of endogenous murine PS1 NTF and CTF were also diminished markedly in cells overexpressing human PS2 (Fig. 3B). Thus PS1/PS2 fragment accumulation appears to be cross-regulated. The biochemical mechanism(s) that underlie this aspect of PS biology remain elusive. Nevertheless, these data offer several models including one in which full-length PS1 and PS2 compete for a limiting cellular pool of factors necessary for trafficking to intracellular compartments wherein endoproteolytic processing occurs, and another in which stabilization of PS1 and PS2 derivatives is regulated by the association of shared, but limiting, cellular factors.

Overexpression of Human PS1 or PS2 Leads to a Highly Selective Diminution of Murine PS1/PS2 Fragment Levels—It is well established that overloading of ER and/or other membrane compartments of the secretory pathway by overexpressed membrane-bound proteins can profoundly influence the stability/trafficking of endogenous membrane-bound molecules. Because PS1 and PS2 largely reside in the ER (16–19), it is very conceivable that overexpression of either polypeptide could severely compromise the synthesis or steady-state levels of endogenous PS1, PS2 and/or other ER resident polypeptides. To document the specificity of the effects of human PS1/PS2 overexpression on murine PS1/PS2 accumulation, we performed several control experiments.

The simplest interpretation of our demonstration that overexpressed human PS1/PS2 replaced endogenous murine PS1/PS2 is that transcripts encoding murine PS1/PS2 are diminished. To address this issue, we performed Northern blot analysis of total cellular RNA isolated from N2a lines, PS1 lines, and PS2 lines after 48 h of incubation in butyric acid. We document that although there appears to exist some variability in the steady-state levels of murine PS1 or PS2 mRNA among the cell lines examined, these differences were independent of transgene expression and no more pronounced than the variable expression of control endogenous mRNAs encoding β-tubulin or APP (Fig. 4A). These results provide strong evidence that diminished levels of murine PS1/PS2 polypeptides in cells overexpressing human PS1/PS2 cannot simply be accounted for by diminished steady-state levels of murine PS1/PS2 transcripts.

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specific membrane-bound protein, we performed immunoprecipitation analysis of membrane fractions with an antibody, D2I, which uniquely recognizes APLP2, a type I transmembrane protein largely localized in the ER and Golgi apparatus (8). Fifty-μg aliquots of soluble (lanes 1–4) and membrane proteins (lanes 5–8) were fractionated by SDS-PAGE and visualized by autoradiography. Lanes 9–12 represent APLP2 immunoprecipitated from membrane fractions using polyclonal antiserum, D2I (23). Arrowheads indicate polypeptides corresponding to APLP2.

To confirm that overexpression of human PS1 or PS2 selectively compromises the steady-state accumulation of murine PS1/PS2, we examined the expression of other resident proteins in the ER, a compartment in which PS1/PS2 accumulates (16–19). We demonstrate that the steady-state levels of calnexin, a membrane-bound protein that transiently interacts with a variety of newly synthesized membrane-bound and secretory proteins (24), was unaffected in cells overexpressing human PS1 or human PS2 (Fig. 5, top panels). Moreover, the levels of BiP/GRP78, a lumenal ER resident protein that is induced by accumulation of misfolded ER proteins (25–27), remained unchanged after induced expression of PS1 or PS2 (Fig. 5, bottom panels).

To document more fully the specificity of the murine PS1/PS2 replacement phenomenon, we examined two stable N2a cell lines transfected with the identical butyrate-inducible CMV expression vector harboring human APP cDNA (APP lines). We documented that the expression of APP in APP lines (Fig. 6A, lanes 3 and 4) had no effect on the accumulation of endogenous PS1 derivatives (Fig. 6A, compare lanes 5 and 6, N2a, with lanes 7 and 8, APP). Finally, to confirm that the loss of murine PS1 and PS2 polypeptides after induced expression of human PS1 or PS2 expression is neither an artifact of transient protein overproduction nor indirect consequence of butyrate-mediated changes in gene expression, we generated several stable N2a cell lines harboring PS1 cDNA under the transcriptional control of the mouse prion protein promoter, which is constitutively active in N2a cells (8). Constitutive expression of human PS1 in N2a cells also resulted in the loss of murine PS1 derivatives (Fig. 6B, compare N2a.1 with PS1.11 lines). Taken together, these results support our view that loss of murine PS1 or PS2 polypeptides in cells overexpressing human PS1 or PS2 (Figs. 1–3) is not the result of artifact(s) associated with overloading the secretory pathway with the polytopic PS1 or PS2 polypeptide.

Relationship between Endoproteolysis and Replacement of Endogenous PS1—One potential mechanism by which overexpression of human PS1 or PS2 might lead to the loss of endogenous PS1 or PS2 derivatives and concurrent replacement with human PS1 derivatives is by competing with limiting levels of the protease responsible for endoproteolytic cleavage. To test this hypothesis, we generated stable N2a lines harboring a modified PS1 cDNA encoding a COOH-terminally truncated PS1 polypeptide (PS1Δ), in which the last 107 amino acids of human PS1 (including part of the large hydrophilic loop domain, the last two transmembrane domains, and the COOH terminus) were deleted. Although the PS1Δ polypeptide contains the site of cleavage (21) and extends 60 amino acids COOH-terminal to the cleavage site, immunoblot analysis of PS1Δ cell lines (Fig. 7A, left panel, lanes 2–4) using a monoclonal antibody specific for human PS1 NH2-terminal region (8, 28) failed to reveal a ~29 kDa human PS1 NTF that is nor-

FIG. 4. Murine PS1/PS2 mRNA and overall protein synthesis are unaffected by overexpression of human PS1/PS2. Panel A, Northern blot analysis of mouse PS1 and PS2 mRNA. Total RNA was isolated from stable N2a lines, PS1 lines, and PS2 lines after induction of transgene expression with butyric acid for 48 h. Aliquots of 10 μg of RNA were fractionated on agarose gels and stained with ethidium bromide (EtBr). Nitrocellulose membranes containing transferred RNA were sequentially hybridized with mouse PS1 (MoPS1), APP, β-tubulin, and mouse PS2 (MoPS2) cDNA. The blots were exposed to film for 5 h for tubulin, 16 h for mouse PS1 and APP, or processed by PhosphorImaging for 2 days for mouse PS2. Thick and thin dashes represent the positions of 28 S and 18 S rRNA, respectively. Panel B, protein biosynthesis in stable cell lines. Two N2a lines (N2a.1 and N2a.2) and cells overexpressing human PS1 (PS1.9) or human PS2 (PS2.12) were pulse labeled with [35S]methionine for 20 min and lysed in hypotonic buffer. Fifty-μg aliquots of soluble (lanes 1–4) and membrane proteins (lanes 5–8) were fractionated by SDS-PAGE and visualized by autoradiography. Lanes 9–12 represent APLP2 immunoprecipitated from membrane fractions using polyclonal antiserum, D2I (23). Arrowheads indicate polypeptides corresponding to APLP2.

FIG. 5. Replacement of murine PS1/PS2 is a selective effect. Aliquots of lysates prepared from butyrate-induced cell lines described in Figs. 1A and 3A were analyzed by immunoblotting using antibodies specific for calnexin (top panels) and BiP/GRP78 (bottom panels).
mally generated in stable cell lines expressing full-length PS1 (Fig. 7A left panel, lane 6). Thus, PS1 361Δ polypeptides are not subject to endoproteolytic cleavage. Interestingly, and despite the accumulation of PS1 361Δ molecules to levels comparable to full-length PS1 in PS1.9 cells (Fig. 7A, lanes 6), the steady-state levels of murine PS1 derivatives remained unchanged in three independent stable cell lines after a 48-h induction of PS1 361Δ expression (Fig. 7A, right panel, lanes 2–4). Essentially identical results were obtained by analysis of stable lines expressing PS1 361Δ, a truncated PS1 polypeptide lacking the COOH-terminal 64 amino acids of human PS1 (data not shown). These studies suggested that endoproteolysis might be a prerequisite for replacement of murine PS1 derivatives.

Previously, we reported that an FAD-linked PS1 variant PS1 403Δ, which lacks exon 9-encoded residues (amino acids 290–319) (14), fails to undergo endoproteolysis (12). We reasoned that if endoproteolysis is obligatory for replacement, overexpression of human PS1ΔE9 molecules in N2a cells will not affect the levels of accumulated endogenous murine PS1 derivatives. To address this issue, we examined three stable N2a cell lines expressing the human PS1ΔE9 variant. In contrast to PS1 361Δ and PS1 403Δ molecules, we document that expression of PS1ΔE9 polypeptides compromised accumulation of endogenous PS1 derivatives in all three stable cell lines (Fig. 7B, lanes 3–5). Similar results were obtained in transgenic mice expressing PS1ΔE9 polypeptides (29). Collectively, these studies demonstrate that endoproteolysis is not entirely a prerequisite for replacement, a conclusion that may seem to contradict our earlier interpretation (derived from analysis of the PS1 361Δ and PS1 403Δ polypeptides) that endoproteolysis might be a prerequisite for replacement of murine PS1 derivatives. To resolve this conundrum, we propose that unlike the PS1 361Δ and PS1 403Δ polypeptides, the PS1ΔE9 variant (which in large part, resembles full-length PS1) is appropriately folded and subsequently interacts with the same limiting components involved in targeting of full-length PS1 to the cleavage pathway.

**DISCUSSION**

Mutations in two related genes, PS1 and PS2, account for the majority of early onset cases of FAD (30). PS1 and PS2 are multitransmembrane proteins that are expressed in brain and various peripheral tissues (3, 5, 12, 28, 31). In preceding efforts, we and others documented that PS1 is subject to endoproteolytic processing in vivo (12) and that the preponderant PS1-related polypeptides that accumulate in the brains of rodents, primates, and humans are NH2-terminal –28-kDa and COOH-terminal –17-kDa derivatives (7–9, 12, 28, 31). We also documented that in transgenic mice overexpressing human PS1, the two derivatives accumulate to saturable levels and at a 1:1 stoichiometry independent of the transgene-encoded mRNA (12). The molecular mechanism(s) underlying this fascinating regulatory aspect of PS biology are yet to be established. Neverthe-
less, endoproteolysis appears to be a common catabolic step for all presenilins, including human and murine PS2 (10, 13) and a homologous protein in C. elegans, termed SEL-12 (32). To these latter observations, the present report provides an important insight. We document that in cultured cells overexpressing human PS1, saturable accumulation of human PS1 derivatives is accompanied by a compensatory decrease in accumulated murine PS1 and PS2 derivatives, this despite the persistence of murine PS1/PS2 mRNAs. Similarly, overexpression of human PS2 in cultured cells compromised accumulation of murine PS1-derived fragments. These effects are not unique to cultured cells since overexpression of human PS1 polypeptides in brains of transgenic mice leads to the reduced accumulation of murine PS1 (12) and PS2 derivatives (this work). These observations are consistent with a model in which PS1 and PS2 molecules are stabilized and endoproteolytically processed by common, but limiting cellular factors, whereas excessively overexpressed PS polypeptides are rapidly degraded (Fig. 8). In support of this view, recent studies have shown that in cultured cells, the vast majority of transiently overexpressed full-length PS polypeptides are rapidly turned over (1/2 t1/2 ≈ 1 h), whereas the processed PS derivatives are long lived (1/2 t1/2 ≈ 24 h) (13, 21, 33).

Although the mechanisms that underlie this interesting aspect of PS stabilization are presently uncertain, this phenomenon is not without biological precedent. For example, overexpression of chicken class IV β-tubulin isotype in Chinese hamster ovary cells resulted in limited accumulation of chicken class IV β-tubulin polypeptides and was accompanied by a selective loss of endogenous CHO class IV (m-IV) β-tubulin isotype (34). In this latter case, the authors argued that a limiting cellular pool of factors that bind selectively to either class IV β-tubulin or class IV β-tubulin-enriched microtubules is responsible for stabilization of c-IV β-tubulin and replacement of m-IV β-tubulin isotype.

With the concern that overexpression of membrane-bound or secreteory proteins has a confounding affects on protein biogenesis in the ER and intracellular trafficking, we felt compelled to examine several aspects of ER metabolism in cells overexpressing PS1 and PS2 to confirm that the replacement of murine PS1/PS2 derivatives by human PS1 or PS2 is a highly specific and selective phenomenon. First, we documented that the biosynthesis of newly synthesized membrane-bound and soluble polypeptides in control cell lines and lines expressing human PS1 or PS2 was identical. Second, the steady-state levels of calnexin, an ER resident, integral membrane protein that facilitate folding and assembly of glycoproteins in the ER is identical in PS1, PS2, and control lines. Third, overexpression of PS1 or PS2 had no effect on the steady-state levels of BiP/GRP78, an ER luminal chaperone that is normally induced by the accumulation of misfolded proteins in the ER. Fourth, induced overexpression of APP, a type I integral membrane glycoprotein, had no effect on the accumulation of murine PS1 derivatives. Finally, expression of COOH-terminally truncated human PS1 polypeptides had no effect on the levels of endogenous murine PS1 derivatives, indicating that murine PS1 gene expression is unaffected by the presence of high levels of truncated human PS1 derivatives, indicating that murine PS1 gene expression is unaffected by the presence of high levels of truncated human PS1 derivatives. Thus, we conclude that compromised accumulation of murine PS1 and PS2 derivatives resulting from overexpression of human PS1 or PS2 is a highly selective effect.

In a variety of experimental settings it has become increasingly evident that the processed PS1/PS2 derivatives accumulate to saturable levels independent of the levels of full-length PS1/PS2 polypeptides (7–10, 12, 13). Moreover, in several lines of transgenic mice, processed human PS1 derivatives accumulate to saturable levels independent of the levels of PS1 mRNA (12). These observations have suggested that the cellular factor(s) involved in endoproteolysis or stabilization of PS1 derivatives may be limiting. Although little information is available regarding the molecular identity of the enzyme(s) responsible for PS1/PS2 endoproteolysis and factors responsible for fragment stability, we consider it unlikely that the regulated accumulation of PS fragments is dependent on competition for protease(s) responsible for cleavage. In support of this view, we demonstrate that expression of COOH-terminally truncated human PS1 polypeptides (PS1D291Δ and PS1ΔCTD), which contain the proteolytic cleavage site but fail to be cleaved, do not influence accumulation of endogenous murine PS1 fragments. On the other hand, expression of noncleavable FAD-linked PS1ΔE9 variant results in the replacement of murine PS1 derivatives in cultured cells (this work) and in transgenic mice (29). Interestingly, we and others have demonstrated that although the PS1ΔE9 variant efficiently rescues an egg-laying defect in C. elegans lacking SEL-12 (35, 36) the human PS1 lacking the loop and COOH-terminal domains fails to do so (36). Thus, it is highly likely that the functional rescue by the PS1ΔE9 variant is a reflection of its stabilization by successful interaction with limiting cellular factor(s) or targeting to cellular compartments that are normally occupied by endoproteolytic derivatives of PS (33).

The nature of subcellular compartments in which PS fragments accumulate has not been established. However, a major endoproteolytic site between amino acids 298 and 299 has been reported (21). With this information, it will be important to generate end-specific antibodies to the NH2 terminus of the CTF or the extreme COOH terminus of the NTF to discriminate full-length PS1 from its cleaved derivatives. These reagents will be enormously useful for biochemical, cell biological, and morphological studies of PS1 metabolism and trafficking. Moreover, characterization of the protein(s) involved in targeting PS to the cleavage pathway and identification of the enzyme(s) responsible for the endoproteolysis of PS will facilitate future efforts aimed at clarifying the limiting step(s) that regulate the accumulated levels of processed PS1/PS2 derivatives.

![Diagram of PS1/PS2 processing](image)
Coordinate Regulation of PS1 and PS2 Levels

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