Mutations in genes encoding presenilins (PS1 and PS2) cosegregate with the majority of early onset cases of familial Alzheimer's disease. PS1 and PS2 are polytopic membrane proteins that undergo endoproteolytic cleavage to generate stable NH2- and COOH-terminal derivatives (NTF and CTF, respectively). Several lines of evidence suggest that the endoproteolytic derivatives are likely the functional units of PS in vivo. In the present report, we examine the disposition of PS NTF and CTF assemblies in stable mouse N2a neuroblastoma cell lines expressing human PS polypeptides. We show that exogenous expression of PS1 NTFs neither assemble with endogenous CTF nor exhibit dominant negative inhibitory effects on the endogenous PS1 cleavage and the accumulation of derivatives. In cells co-expressing PS1 and PS2, PS1- and PS2-derived fragments do not form mixed assemblies. In contrast, cells expressing a chimeric PS1/PS2 polypeptide form stable PS1 NTF-PS2 CTF assemblies. Moreover, expression of chimeric PS1/PS2 polypeptides harboring a familial early onset AD-linked mutation (M146L) elevates the production of Aβ42 peptides. Our results provide evidence that assembly of structural domains contained within NH2- and COOH-terminal regions of PS occur prior to endoproteolytic cleavage.

Alzheimer’s disease (AD), a progressive neurodegenerative disorder, is the most common cause of dementia in the elderly. AD is characterized pathologically by the presence of senile plaques and neurofibrillary tangles in the brains of affected individuals (1). The predominant constituent of senile plaque is 40–43-amino acid β-amyloid peptides (Aβ), which are derived from proteolytic processing of a type I integral membrane glycoprotein, called the amyloid precursor protein (APP) (Ref. 2; reviewed in Ref. 3). Approximately 10% of all cases of AD, classified as familial early onset AD (FAD) (age of onset <60 years), is caused by autosomal dominant inheritance of mutations in genes encoding APP (for a review, see Ref. 4). presenilin 1 (PS1) (5) and presenilin 2 (PS2) (6–8). Expression of FAD-linked APP mutations leads to increased production of highly fibrillogenic Aβ species ending at residue 42 (or 43) (Aβ42) (reviewed in Ref. 3). In addition, expression of FAD-linked PS1 and PS2 variants alters APP processing in a manner that leads to elevated production of Aβ42 (9–14). Thus, genetic mutations in all the three genes that cosegregate with FAD increase the production of the pathogenic Aβ42 peptides. The role that PS plays in facilitating proteolytic cleavage of APP at the γ-secretase site is not known, but PS1-deficient neurons exhibit selective defects in the production of Aβ (15, 16).

PS1 and PS2 (PS) are homologous polytopic membrane proteins (17) that are subject to endoproteolytic cleavage (12, 18–20). In previous efforts, we documented that PS1-derived 28-kDa NH2-terminal (NTF) and 17-kDa COOH-terminal fragments (CTF) are the preponderant PS1-related species that accumulate in vivo (18). The PS NTF and CTF accumulate to 1:1 stoichiometry, and the absolute levels of PS-derived fragments are established by a highly regulated and saturable mechanism such that overproduction of human PS fragments led to diminution of mouse PS fragments (18). In transfected cells overexpressing PS, only a small fraction of full-length PS is converted to stable fragments (t1/2 > 12 h); excess full-length PS polypeptides are rapidly degraded (t1/2 ~ 1 h) (18, 21–25). Moreover, accumulation of PS1 and PS2 fragments are coordinately regulated, providing strong evidence that association with limiting cellular components regulates the abundance of PS fragments (21). More recently, using in situ chemical cross-linking and co-immunoprecipitation approaches, we demonstrated that the NTF and CTF of either PS1 or PS2 can be coisolated (26). The accumulated data support our view that the more stable endoproteolytic derivatives are the biologically functional forms of PS. Arguing against this notion, Citron et al. (27) reported that expression of a truncated PS1 species corresponding to the human PS1 NTF with a FAD mutation failed to increase Aβ42 production; the authors suggested that the pathogenic effect of mutant PS is elicited by the full-length molecule and not the endoproteolytic derivatives.

In this report, we examine the assembly of PS1 and PS2 derivatives in stable mouse N2a neuroblastoma cell lines expressing full-length PS, recombinant PS1 NTF, or chimeric PS1/PS2 polypeptides. We demonstrate that transgene-derived COOH-terminally truncated human PS polypeptides that correspond to PS1 NTF (residues 1–298) fail to co-assemble with endogenous PS1 CTF and are rapidly degraded. In cells co-

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expressing PS1 and PS2, processed fragments derived from full-length PS polypeptides form assemblies consisting entirely of either PS1 NTF-CTF or PS2 NTF-CTF. Based on these results, we conclude that the association of NH2 and COOH domains of the precursor PS polypeptide precedes endoproteolytic cleavage and that PS1 and PS2 do not form heteromultimeric assemblies. Interestingly, fragments derived from proteolytic processing of a chimeric PS1/PS2 precursor polypeptide are capable of forming stable heteromeric PS1 NTF-PS2 CTF assemblies. Furthermore, expression of a FAD-linked M146L mutant PS1/PS2 chimera elevated the production of a PS2/PS1 chimera. Our results are consistent with a model where stabilization of intramolecular interaction(s) between domains within the NH2- and COOH-terminal regions of PS1 is a prerequisite for endoproteolytic processing.

MATERIALS AND METHODS

Generation of Expression Plasmids—Expression plasmids encoding human PS1 (pCB6PS1), PS2 (pCB6PS2), FAD-linked PS variants, and PS2Loop antisera (26). A rat anti-human PS2Loop antiserum (21). A rat monoclonal antibody (mAb) specific for human PS1 (30) (provided by Dr. Allan I. Levey, Emory University School of Medicine, Atlanta) was used to immunoprecipitate the mouse NTF was detected in several independent stable N2a cell lines (Fig. 1A, top, lanes 3–5). Consistent with previous reports (10, 11, 18, 21), the recombinant human PS1 NTF exhibited accelerated migration relative to the mouse PS1 NTF on SDS-polyacrylamide gel electrophoresis (lane 1) and co-migrated with the human NTF derived from full-length human PS1 (lane 2). Previously, we reported that the expression of human full-length PS1 in mouse N2a cells resulted in a decrease in the levels of murine PS1-derived NTF and CTF (21). To examine whether the expression of recombinant human NTF abrogated accumulation of endogenous murine PS1 derivatives, we performed Western blots using aPS1Loop, an antibody that reacts with epitopes in the hydrophilic loop domain of PS1 (18). The steady-state levels of mouse PS1 CTF in three independent N2a lines examined were essentially identical to N2a cells transfected with empty vector (21) (Fig. 1A, bottom), indicating that human NTF did not interfere with the accumulation of mouse CTF.

RESULTS

Transgene-derived Human PS1 NTF Fails to Assemble with Endogenous PS1 CTF—In previous studies, we and others reported that the NTF and CTF derived from endoproteolysis of PS1 (or PS2) are noncovalently associated to each other in vivo (26, 36, 37). To further characterize the NTF and CTF assemblies, we generated stable mouse N2a neuroblastoma cell lines transfected with a plasmid that encodes human PS1 residues 1–298 (analogous to NTF). Expression of transgene-derived human PS1 NTF was assessed by Western blotting using PS1NT, a polyclonal PS1 antisera that recognizes epitopes in the NH2 terminus of PS1 (26). Transgene-derived human PS1 NTF was readily detected in several independent stable N2a cell lines (Fig. 1A, top, lanes 3–5). Conditioned media from cells transfected with different cDNA, the ratio of Aβ42/total Aβ was calculated for each sample and statistically examined using analysis of variance followed by Fisher LSD test and expressed as mean ± S.E.

Assembly of Presenilins

We addressed this issue in our stable N2a cell lines expressing human PS1 NTF. A PS1NT-reactive species co-migrating with the mouse NTF was visible in lysates of stable N2a cells expressing low levels of recombinant human NTF (Fig. 1A, top, compare lanes 1 and 3). However, the mouse NTF and the recombinant human NTF were not sufficiently resolved on...
PS1 NTF studies demonstrated that after repeated immunodepletion of accumulation of mouse PS1 derivatives. From these results, we conclude that the NTF. Furthermore, the short half-life of transgene-derived hu-

nence for the presence of stable pools of endogenous NTF in were obtained in cell lines expressing low or high levels of 6-h cycloheximide treatment (Fig. 1). The slower migrating PS1 NT-reactive species, which corresponds to recombinant human NTF, degraded rapidly (1/2 h) after the addition of cycloheximide. The slower migrating aPS1Loop-reactive band probably represents the phosphorylated form of PS1 CTF (36, 43). The high molecular weight PS1NT-reactive species (lane 5) represent nonspecific aggregation of overexpressed human NTF. The small decrease in the CTF signal in lane 5 is not reproducible and is seen only in samples with aggregated human NTF.

SDS-polyacrylamide gel electrophoresis to convincingly demonstra-tate whether or not the expression of human NTF influenced the accumulation of mouse NTF. Therefore, we used an alternate strategy that takes advantage of the findings that while the recombinant human NTF is short lived (25), endogenous PS-derived NTF has a half-life of several hours (22, 23). We incubated stable N2a cell line PS1298.6, which expresses high levels of human NTF (Fig. 1B, lane 1), with cycloheximide to arrest protein synthesis and examined the decay of human NTF over a 6-h interval. Immunoblot of cell lysates collected at various time points revealed that the faster migrating PS1NT-reactive species, which corresponds to recombinant human NTF, degraded rapidly (1/2, 1.5 h) after the addition of cycloheximide. The slower migrating PS1NT-reactive species, which corresponds to mouse PS1 NTF was readily visible after 1 h of cycloheximide treatment and was the only remaining PS1 NTF after 4 h of cycloheximide treatment (Fig. 1B, top). Reprobing of the blots using aPS1Loop antiserum revealed that the levels of mouse CTF remained unchanged during the 6-h cycloheximide treatment (Fig. 1B, bottom). Similar results were obtained in cell lines expressing low or high levels of human PS1 NTF (data not shown). These results provide evidence for the presence of stable pools of endogenous NTF in N2a cells expressing high levels of recombinant human NTF. Furthermore, the short half-life of transgene-derived human NTF in our stable N2a cell lines is entirely consistent with previous studies (25). From these results, we conclude that the expression of transgene-derived human NTF failed to interfere with the endoproteolysis of endogenous murine PS1, or the accumulation of mouse PS1 derivatives.

We then examined whether the transgene-derived human NTF associated with endogenous mouse PS1 CTF. Previous studies demonstrated that after repeated immunodepletion of PS1 NTF-CTF complexes, only recombinant human NTF re-

mained in the lysates of human 293 cells expressing human PS1 NTF (25). We were interested in directly testing whether we could isolate complexes containing human PS1 NTF and mouse PS1 CTF using a human PS1-specific antibody. To dis-


tinguish between the transgene-derived human NTF and en-


dogenous mouse NTF, we used a rat mAb that reacts specifi-


cally with the human PS1 NH2 terminus (21, 30). Stable N2a cells expressing human PS1 NTF were lysed under nonadenaturing conditions, and lysates were used for co-immunoprecipitation analyses using PS1NT and aPS1Loop (PS1L), PS2NT, or aPS2Loop (PS2L) antibodies as indicated. SOD1 and normal rabbit serum (NRS) were used as negative controls. After fractionation by SDS-polyacrylamide gel electrophoresis and transfer to membranes, blots containing NTF immunoprecipitates were sequentially probed with aPS1Loop (top panels, lanes 1–4) and aPS2Loop antibodies (bottom panels, lanes 1–4). Blots containing CTF immunoprecipitates were sequentially probed with PS1NT (top panels, lanes 5–8) and PS1NT antibodies (bottom panels, lanes 5–8).

**Assembly of Presenilins**

**Fig. 1.** Characterization of transgene-derived human NTF expressed in stably transfected mouse N2a cells. A, detergent lysates prepared from stable N2a cell lines transfected with empty vector (lane 1), full-length PS1 cDNA (lane 2), or human PS1 NTF cDNA (lanes 3–5) were fractionated by SDS-polyacrylamide gel electrophoresis and ana-

lyzed by immunoblotting with PS1NT (top) or aPS1Loop (bottom). Full-

length human PS1 (FL), human (Hu), and murine (Mo) PS1 NTF and CTF are indicated. B, stable N2a cells expressing human PS1 NTF were incubated in culture medium containing cycloheximide (30 μg/ml) for the intervals indicated. Detergent lysates prepared after the incubation period were analyzed by immunoblotting with PS1NT (top) or aPS1Loop (bottom). C, stable N2a cells expressing human PS1 NTF were lysed under nonadenaturing conditions, and lysates were used to co-immunoprecipitate PS1 NTF using polyconal PS1NT antiserum, a PS1 mAb (which reacts specifically with human PS1 NH2-terminal epitopes), or control Myc and SOD1 antibodies. The resulting immunoprecipitates and total lysates (corresponding to 1/20 of the volume used for immuno-

precipitations) were probed with PS1NT (top panels) or aPS1Loop (bottom panels). PS1 NTF and CTF are indicated by arrowheads. The slower migrating aPS1Loop-reactive band probably represents the phosphorylated form of PS1 CTF (36, 43). The high molecular weight PS1NT-reactive species (lane 5) represent nonspecific aggregation of overexpressed human NTF. The small decrease in the CTF signal in lane 5 is not reproducible and is seen only in samples with aggregated human NTF.

**Fig. 2.** PS1 and PS2 derivatives do not form mixed complexes. A, detergent lysates prepared from cells transfected with empty vector or PS2 cDNA were analyzed by immunoblotting with aPS2Loop (lanes 1–3) or a mixture of PS1NT and aPS1Loop (lanes 4–6). Full-length human PS2 (FL) and endoproteolytic PS1 and PS2 derivatives (arrow-

heads) are indicated. B, stable N2a cells expressing low levels of human PS2 were lysed under nonadenaturing conditions, and lysates were used for co-immunoprecipitation analyses using PS1NT, aPS1Loop (PS1L), PS2NT, or aPS2Loop (PS2L) antibodies as indicated. SOD1 and normal rabbit serum (NRS) were used as negative controls. After fractionation by SDS-polyacrylamide gel electrophoresis and transfer to membranes, blots containing NTF immunoprecipitates were sequentially probed with aPS1Loop (top panels, lanes 1–4) and aPS2Loop antibodies (bottom panels, lanes 1–4). Blots containing CTF immunoprecipitates were sequentially probed with PS1NT (top panels, lanes 5–8) and PS1NT antibodies (bottom panels, lanes 5–8).

**Cells Co-expressing PS1 and PS2 Do Not Form heteromeric PS1-PS2 Assemblies—**Both PS1 and PS2 have been reported to form heteromeric assemblies and migrate as high molecular weight complexes on size exclusion columns and velocity den-

sity gradients (29, 36, 37). However, it is not clear whether PS1 derivatives form heteromeric complexes with PS2 derivatives. To address this issue, we used a stable N2a cell line transfected with human full-length PS2 cDNA. As we reported previously (21), high level expression of human PS2 in mouse N2a cells results in the diminution of mouse PS1 derivatives (Fig. 2A, lane 6). Therefore, we chose a cell line that expresses low levels of human PS2 (lane 2) for the present studies; PS1 derivatives are only partially replaced in this cell line (compare lanes 4 and 5). We performed co-immunoprecipitation analyses using PS1 and PS2 antibodies: PS1 derivatives were immunoprecipitated
using antibodies PS1nt and αPS1Loop; PS2 NTF and CTF were immunoprecipitated using PS2nt and αPS2Loop, respectively. In parallel, polyclonal SOD1 antisera and normal rabbit serum were used in control immunoprecipitations. The immunoprecipitates were analyzed by sequential immunoblotting using PS1 and PS2 antisera. As expected, PS1nt and αPS1Loop antisera co-immunoprecipitated PS1 CTF and NTF, respectively (Fig. 2A, top panels, lanes 1 and 5), but failed to co-purify PS2 derivatives (Fig. 2B, bottom panels, lanes 1 and 4). Furthermore, PS2nt and αPS2Loop antisera co-immunoprecipitated PS2 CTF and NTF, respectively (Fig. 2B, bottom panels, lanes 2 and 6), but failed to co-purify PS1 derivatives (Fig. 2B, top panels, lanes 2 and 6). These results demonstrate that the derivatives resulting from endoproteolysis of PS1 and PS2 form either PS1 NTF-PS1 CTF or PS2 NTF-PS2 CTF assemblies but do not form mixed complexes containing the derivatives from both of the precursor polypeptides.

The Endoproteolytic Derivatives from a PS1/PS2 Chimeric Polypeptide Can Form Functional PS1/PS2 Assemblies—PS1 and PS2 have similar protein structures, share a high degree of amino acid sequence identity, and undergo endoproteolytic cleavage, and the resulting fragments derived from each precursor associate with each other. Despite these similarities, our results indicate that fragments derived from endoproteolytic cleavage of PS1 and PS2 fail to form mixed complexes. The absence of complexes containing PS1 NTF and PS2 CTF or PS2 NTF and PS1 NTF strongly suggests that the noncovalent association between the NH2- and COOH-terminal structural domains of the precursor polypeptide may precede endoproteolytic cleavage. If this prediction were correct, one would expect that endoproteolysis of a chimeric polypeptide containing PS1 NTF and PS2 CTF should result in the generation of processed PS1 NTF and PS2 CTF derivatives that can be co-immunoprecipitated. To test this hypothesis, we generated stable N2a cell lines transfected with an expression plasmid encoding a chimeric polypeptide corresponding to amino acids 1–280 of human PS1 fused to amino acids 287–448 of human PS2. Expression of the PS1/PS2 chimera was assessed in several cell lines by Western blotting using PS1nt and αPS2Loop antisera (data not shown). The chimeric polypeptide was endoproteolytically processed to generate fragments corresponding to PS1 NTF and PS2 CTF, and in cell lines expressing high levels of the PS1/PS2 chimera, endogenous PS1 derivatives were replaced (Fig. 3A). Thus, the metabolism of the PS1/PS2 chimera was similar to that of PS1 or PS2, and the accumulation of the resulting derivatives (PS1 NTF and PS2 CTF) was similar to that of the fragments derived from full-length PS1 or PS2. Next, we performed co-immunoprecipitation studies to assess whether the fragments derived from endoproteolytic processing of the PS1/PS2 chimera could be co-immunoprecipitated. For these studies, we chose a stable cell line in which the endogenous mouse PS1 derivatives are incompletely replaced. Nondenaturing lysates were subject to immunoprecipitation with PS1 and PS2 antibodies. As expected, PS1nt antisera co-immunoprecipitated endogenous murine PS1-derived CTF (Fig. 3B, top panels, lane 1). Interestingly, reprobing of the blot using αPS2Loop antisera revealed that PS1nt antisera also co-purified PS2 CTF (bottom panels, lane 1). Furthermore, αPS1Loop antisera co-precipitated the slower migrating mouse PS1 NTF, whereas αPS2Loop antisera co-precipitated the faster migrating human PS1 NTF (top panels, lanes 7 and 8, respectively). Because endogenous murine PS2 NTF is not detectable in total lysates prepared from the PS1/PS2 cell line used for this experiment (bottom panel, lane 12), we failed to detect any signal for PS2 CTF or NTF in co-precipitations (bottom panels, lanes 2 and 8, respectively). These results demonstrate that the PS1 NTF and PS2 CTF are capable of forming mixed complexes when derived from cleavage of a chimeric polypeptide and strongly support our view that interaction between NH2- and COOH-terminal regions of a precursor PS polypeptide precedes endoproteolysis.

Next we examined whether the chimeric PS1 NTF-PS2 CTF assembly is stable. To address this issue, we incubated cells expressing PS1/PS2 chimera with cycloheximide to block protein synthesis. In parallel, we incubated cells expressing full-length PS1 or PS2 with cycloheximide. Detergent lysates prepared at the end of the incubation period were analyzed by immunoblotting using PS1 and PS2 antisera. As expected from previous studies (21–24), in stable N2a cells expressing PS1 the full-length PS1 molecules were rapidly degraded, while the NTF and CTF were stable (Fig. 4A). Similar results were obtained in stable cell lines expressing PS2 (data not shown). Analysis of lysates from stable N2a cells expressing the PS1/PS2 chimera revealed that full-length PS1/PS2 polypeptides were also rapidly degraded, while the endoproteolytic fragments derived from the chimera remained stable over a 6-h incubation period in the presence of cycloheximide (Fig. 4A). From these results, we conclude that endoproteolytic fragments derived from the processing of PS1/PS2 chimera are long lived.

Finally, we examined whether the PS1/PS2 chimera could influence APP processing and elevate the production of Aβ42 peptides. For these studies, we constructed expression plasmids encoding PS1/PS2 chimeric polypeptide that harbors the FAD-linked M146L substitution. COS cells were co-transfected with plasmids encoding wild-type or mutant PS1/PS2, and APP (or C100; see “Materials and Methods”). In parallel, we also transfected cells with plasmids encoding wild-type or mutant full-length PS1 or PS2. The levels of secreted Aβ x-40 and x-42 peptides in conditioned media of transfected cells were quantified using a two-site ELISA assay as described (33–35). To account for variations in the levels of Aβ40 and Aβ42 due to differences in transfection efficiencies of different expression plasmids, we calculated the ratio Aβ x-42/total Aβ for each expression construct and compared the relative ratios (32, 27). Results of these analyses are presented in Fig. 4B. As reported previously (10, 12, 13), expression of mutant PS1 (Aβ x-42/total
Aβ ratio PS1 wild-type was $10.30 \pm 0.7$ versus PS1 M146L, 18.16 $\pm 1.2$ or PS2 (wild-type was $14.51 \pm 0.8$ versus N141I, 26.72 $\pm 1.8$) elevated the secretion of Aβ 42 peptides. The levels of Aβ 42 in cells transfected with PS1/PS2 chimera lacking FAD-linked mutation were comparable with that of wild-type PS2. In contrast, expression of the PS1/PS2 chimera harboring the FAD-linked M146L mutation markedly elevated the levels of Aβ 42 in the conditioned medium (wild-type PS1/PS2 was 14.37 $\pm 1.8$ versus M146L PS1/PS2, 23.16 $\pm 1.9$). Thus, we argue that chimeric PS1/PS2 polypeptides harboring FAD-linked PS1 M146L are effective in influencing Aβ42 production. Collectively, these results document that the endoproteolytic fragments derived from PS1/PS2 chimera can co-assemble into stable heteromeric assemblies, and PS1/PS2 polypeptides harboring FAD-linked mutation were capable of elevating the production of Aβ42 peptides.

**DISCUSSION**

Our understanding of the nature of the functional PS “unit” is still very incomplete. Knowledge about the subunit stoichiometry is essential for further studies on the structure and function of the PS complex. In the present study, we exclusively focused on the composition and stoichiometry of PS NTF and CTF complexes immunoprecipitated from stable mouse neuroblastoma cells that express recombinant human PS1 NTF, full-length PS2, or a chimeric PS1/PS2 polypeptide. These studies demonstrate that transgene-derived recombinant PS1 NTF neither influenced the metabolism of endogenous PS1 polypeptides nor associated with endogenous PS1-derived CTF. PS1 and PS2 fragments derived from the cleavage of co-expressed full-length PS1 and PS2 do not form mixed heteromeric assemblies. In contrast, endoproteolysis of a PS1/PS2 chimera generates stable PS1 NTF-PS2 CTF assemblies. The results of our studies provide an important insight: an intramolecular association(s) between domains of the PS NTF and CTF is established prior to endoproteolytic cleavage. Our conclusion is consistent with previous gel filtration chromatography and velocity gradient analyses, which revealed that full-length PS and the PS-derived NTF and CTF are components of high molecular weight complexes (29, 36, 37). Independently, two studies demonstrated that complexes containing PS1 NTF and CTF were larger than the complexes containing full-length PS1 (29, 37). Interestingly, a FAD-linked PS1 deletion mutant that fails to undergo endoproteolytic cleavage (18, 38) was found to be in a high molecular weight complex, similar in size to that of the cleaved fragments, suggesting that cleavage was not necessary for the assembly of stable PS complexes (37). Nevertheless, attempts to bypass the requirement for endoproteolysis by co-expressing truncated PS2 polypeptides, which correspond to FAD-linked mutant NTF and CTF, did not lead to elevated Aβ42 production (32).

Together with our present results, these studies indicate that the formation of a functional PS complex may be an ordered and stepwise process in which newly synthesized PS polypeptides establish intramolecular association(s) during the process of folding and subsequently bind to other proteins expressed at limiting levels that “stabilize” the PS complex; properly folded and “mature” PS molecules that have associated with limiting accessory molecules are then cleaved (or not in the case of PS1 lacking exon 9-encoded residues). The mechanisms involved in facilitating PS endoproteolysis are not known.

It is likely that both protein stabilization and endoproteolytic cleavage may rely on proper folding of the synthetic PS polypeptides. The lack of an intact COOH terminus, for example, might prevent proper folding and/or association with PS binding proteins and thereby lead to rapid degradation. In this regard, previously we showed that COOH-terminally truncated PS polypeptides, which lack the last two transmembrane domains and the cytoplasmic tail, failed to undergo endoproteolysis and were rapidly degraded (21). Our present results confirm studies by Steiner et al. (25) that recombinant transgene-derived PS1 NTF is not incorporated into complexes containing endogenous PS1 CTF and is rapidly degraded. In recent studies, it was reported that recombinant PS1 NTF with a FAD mutation failed to increase Aβ42 production, even after stabilization of recombinant NTF by incubation with proteasome inhibitors (25, 27). Based on the recombinant NTF studies, it was suggested that full-length PS molecules, and not the endoproteolytic derivatives, were the functional PS units in vivo (27). It is known that proper assembly contributes to the structural maturation as well as the function of oligomeric membrane proteins. Thus, our demonstration that exogenously expressed human NTF failed to assemble with endogenous CTF provides direct evidence that the recombinant NTFs were not incorporated into PS complexes. We argue that the transgene-derived NTF molecules are structurally quite different from NTF-CTF assemblies derived from full-length PS polypeptides; hence, their inability to increase Aβ42 production cannot be interpreted in a way that provides any meaningful information regarding the functional form of PS.

The outcome of co-expression of full-length and truncated membrane receptors has been investigated for several membrane proteins. In several instances, expression of truncated polypeptides disrupted the functional assembly and activity of endogenous proteins by a dominant negative mechanism (39). For example, expression of fragments derived from the extracellular NH2-terminal domains of α-, β-, and γ-subunits of the acetylcholine receptor blocked the assembly and cell surface expression of functional acetylcholine receptors (40). In analogous studies, co-expression of truncated and full-length gonadotropin-releasing hormone receptors resulted in the inhibition of gonadotropin-releasing hormone signaling via the full-length
receptor, probably because of specific interactions between the two receptor proteins resulting in impaired maturation of the stable receptor (41). In contrast to these studies, our analysis of the stable cell lines expressing transgene-derived human PS1 NTF shows that the expression of PS1 NTF did not have any dominant negative inhibitory effect on the cleavage of endogenous full-length PS1 or on the stable accumulation of endogenous PS1-derived fragments. Moreover, we and others showed that transgene-derived PS1 NTF cannot be co-isolated with endogenous PS1-derived CTF, excluding any direct physical interaction between truncated PS molecules and endogenous PS1. Together, these results strongly suggest that the COOH terminus of PS might provide information essential for protein folding and/or interaction with proteins that interact with PS. In this regard, a calcium-binding protein, termed calcinulin, has been shown to bind to the COOH-terminal 40 residues of PS (42).

As described above, PS1 and PS2 fragments derived from cleavage of co-expressed full-length PS1 and PS2 neither form mixed heteromeric assemblies nor exchange subunits to result in complexes containing PS1-NTF-PS2 CTF or PS2 NTF-PS1 CTF. Yet, overexpression of human PS1 resulted in diminution of endogenous PS2 derivatives (21). In studies presented here, we demonstrate that a chimeric polypeptide consisting of PS1 NTF and PS2 CTF undergoes endoproteolysis, and the resulting derivatives accumulate to saturable levels. Furthermore, expression of chimeric PS1/PS2 polypeptides harboring the FAD-linked M146L mutation resulted in the elevated production of Aβ42. Together, these findings indicate that PS1 and PS2 share common pathways of processing and likely interact with similar factors that modulate γ-secretase activity. Future efforts to identify individual polypeptide components of the oligomeric PS complex will be required to define the multiple steps involved in the regulation of assembly of the functional PS "unit".

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