Articles

Presenilin 1 Regulates the Processing of β-Amyloid Precursor Protein C-Terminal Fragments and the Generation of Amyloid β-Protein in Endoplasmic Reticulum and Golgi†

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ABSTRACT: Progressive cerebral deposition of the amyloid β-protein (Aβ) is believed to play a pivotal role in the pathogenesis of Alzheimer's disease (AD). The highly amyloidogenic 42-residue form of Aβ ($Aβ_{42}$) is the first species to be deposited in both sporadic and familial AD. Mutations in two familial AD-linked genes, presenilins 1 (PS1) and 2 (PS2), selectively increase the production of $Aβ_{42}$ in cultured cells and the brains of transgenic mice, and gene deletion of PS1 shows that it is required for normal γ-secretase cleavage of the β-amyloid precursor protein (APP) to generate Aβ. To establish the subcellular localization of the PS1 regulation of APP processing to Aβ, fibroblasts from PS1 wild-type (wt) or knockout (KO) embryos as well as Chinese hamster ovary (CHO) cells stably transfected with wt or mutant PS1 were subjected to subcellular fractionation on discontinuous Iodixanol gradients. APP C-terminal fragments (CTF) were markedly increased in both endoplasmic reticulum- (ER-) and Golgi-rich fractions of fibroblasts from KO mice; moreover, similar increases were documented directly in KO brain tissue. No change in the subcellular distribution of full-length APP was detectable in fibroblasts lacking PS1. In CHO cells, a small portion of APP, principally the *N*-glycosylated isoform, formed complexes with PS1 in both ER- and Golgi-rich fractions, as detected by coimmunoprecipitation. When the same fractions were analyzed by enzyme-linked immunosorbent assays for $Aβ_{total}$ and $Aβ_{42}$, $Aβ_{42}$ was the major Aβ species in the ER fraction ($Aβ_{42}$:$Aβ_{total}$ ratio 0.5−1.0), whereas absolute levels of both $Aβ_{42}$ and $Aβ_{40}$ were higher in the Golgi fraction and the $Aβ_{42}$:$Aβ_{total}$ ratio was 0.05−0.16 there. Mutant PS1 significantly increased $Aβ_{42}$ levels in the Golgi fraction. Our results indicate PS1 and APP can interact in the ER and Golgi, where PS1 is required for proper γ-secretase processing of APP CTFs, and that PS1 mutations augment $Aβ_{42}$ levels principally in Golgi-like vesicles.

Extracellular amyloid deposits composed of the 40- and 42-residue amyloid β-proteins (Aβ)[1] are an early and invariant neuropathological feature of Alzheimer's disease (AD). Missense mutations in the β-amyloid precursor protein (APP) cause increased production of both $Aβ_{40}$ and $Aβ_{42}$ or a selective increase in $Aβ_{42}$ alone in primary and transfected cells, transgenic mice, and humans bearing such mutations (*1*−*6*). Mutations in two other genes linked to early onset familial AD, presenilin (PS) 1 and 2, also selectively enhance the production of $Aβ_{42}$ in transfected cells and the brains of transgenic mice (*7*−*11*) and in the brain tissue (*12*) and plasma (*5*) of gene carriers. The elevation of $Aβ_{42}$ appears to be a primary result of the expression of mutant PS genes that does not require any other features of the AD state, as it occurs even in nonneural, nonhuman cells (*9*).

The mechanism by which mutant PS causes $Aβ_{42}$ elevation is unknown. De Strooper et al. (*13*) reported that PS1 is

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[1] Abbreviations: Aβ, amyloid β-protein; PS, presenilin; AD, Alzheimer's disease; APP, β-amyloid precursor protein, ER: endoplasmic reticulum; FBS, fetal bovine serum; CHO, Chinese hamster ovary; BFA, brefeldin A; IP, immunoprecipitation; KO, knockout; CTF, C-terminal fragment; DMEM, Dulbecco's modified Eagle's medium; EDTA, ethylenediaminetetraacetic acid; PBS, phosphate-buffered saline; HEPES, *N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid; ELISA, enzyme-linked immunosorbent assay; SDS, sodium dodecyl sulfate; PVDF, poly(vinylidene difluoride); ECL, enhanced chemiluminescence; Iodixanol, 5,5′-[(2-hydroxyl-1,3-propanediyl)bis(acetylimino)bis[*N*,*N*′-bis-(2,3-dihydroxypropyl)-2,4,6-triiodo-1,3-benzenedicarboxamide].

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required for normal Aβ generation from APP CTFs, strongly supporting the involvement of PS1 in the γ-secretase cleavage of APP. PS2 and APP were found to interact in COS cells transiently transfected with these two genes (*14*), and we found that not only PS2 but also PS1 can participate in protein complexes with a small fraction of APP in stably transfected human cells (*15*). We were able to detect the interaction at endogenous levels of both proteins in untransfected cells, and experiments with brefeldin A or temperature block suggested that the association might occur in the ER and early Golgi (*15*). Previous reports suggest that Aβ_{42} peptide can be generated early in the secretory pathway, e.g., in ER, intermediate vesicles, and/or Golgi (*16*−*19*). To establish the locus of PS1 involvement in APP processing and the sites in which mutant PS acts, we subjected fibroblasts of mice lacking PS1, as well as CHO cells stably coexpressing APP and wt or mutant PS1, to subcellular fractionation. We found that in the absence of PS1, APP C-terminal fragments (CTF) accumulated in ER- and Golgi-type vesicles. In the CHO cells, complexes containing PS1 and APP could be recovered from these vesicles. Moreover, levels of Aβ_{42} were significantly elevated in Golgi-rich fractions of cells expressing mutant PS1. Taken together, these data are consistent with the direct involvement of wt and mutant PS1 in the critical γ-secretase cleavage of APP in ER, Golgi, and perhaps intermediate vesicles.

MATERIALS AND METHODS

Fibroblast Cultures. Mouse fibroblasts were cultured as described with modifications (*20*). Individual embryos were isolated from pregnant PS1 heterozygous (+/−) mice at E15. Head, liver, and internal organs were discarded, and carcasses were transferred to a 10 mL syringe (with luer lock) containing 3 mL of trypsin and incubated at 37 °C for 3 min. The contents of the syringe were discharged through a 20-gauge needle into a 10 cm culture dish and incubated at 37 °C for 3 min. Ten milliliters of DMEM was added, and the suspension was pipetted up and down several times to dissociate the tissue. After culturing for 48 h, the fibroblasts were ready to be split for further experiments. The amounts of PS1 protein in wt (+/+) vs KO (−/−) fibroblasts were established by immunoprecipitation−Western blotting of samples with PS1 antibodies.

Antibodies. PS1 polyclonal antibody X81 and monoclonal antibody N15 (gift of R. Tanzi) were raised against residues 1−81 and 12−69, respectively (*21*). Polyclonal antibody C7 is directed against $APP_{676-695}$ (APP_{695} numbering) (*22*). Monoclonal antibodies 8E5 and 13G8 are directed against $APP_{444-592}$ and $APP_{676-695}$, respectively. Monoclonal antibodies 5A3 and 1G7 were raised to APP_s purified from the medium of human APP-transfected CHO cells (*23*). Anti-calnexin antibody was purchased from Stressgen (Victoria, British Columbia, Canada).

Subcellular Fractionation. CHO cells stably cotransfected with wt APP_{751} and either wt PS1 ($PS1_{WT-1}$) or M146L mutant PS1 ($PS1_{ML-2}$) were generated and grown as described previously (*15*). Cell line C99 stably overexpresses the last 99 amino acids of APP (D. Watson and DJS, unpublished data). Five 15-cm dishes of cells ($\sim 1 \times 10^8$ cells) were detached with 20 mM EDTA in PBS. All of the following steps were carried out at 4 °C. Cells were pelleted and resuspended in 3 mL of homogenization buffer containing 10 mM HEPES, pH 7.4, 1 mM EDTA, and 0.25 M sucrose plus a protease inhibitor cocktail (5 μg/mL leupeptin, 5 μg/mL aprotinin, 2 μg/mL pepstatin A, and 0.25 mM phenylmethanesulfonyl fluoride) (Sigma). Cells were disrupted by 10 strokes in a Dounce homogenizer followed by 5 passages through a 27-gauge needle. Nuclei and unbroken cells were pelleted by centrifugation at 1500*g* for 10 min. The pellets were further extracted by resuspending in 1.5 mL of homogenization buffer and centrifuging at 1500*g* for 10 min. The postnuclear supernatants from both spins were combined and then centrifuged for 1 h at 65000*g*. The resultant vesicle pellets were resuspended in 0.8 mL of homogenization buffer. Discontinuous Iodixanol gradients were made according to Graham et al. (*24*) with some modifications. Iodixanol was diluted to a final concentration of 50% Iodixanol, 0.25 M sucrose, 1 mM EDTA, and 10 mM HEPES, pH 7.4, as the gradient stock solution. Different densities of Iodixanol were established by diluting this stock with 0.25 M sucrose homogenization buffer. Gradients were then set up in 13 mL Beckman SW41 centrifuge tubes by underlayering with a syringe and metal needle as follows: 2.5% Iodixanol, 1 mL; 5%, 2 mL; 7.5%, 2 mL; 10%, 2 mL; 12.5%, 0.5 mL; 15%, 2 mL; 17.5%, 0.5 mL; 20%, 0.5 mL; and 30%, 0.3 mL. The resuspended vesicle fractions were loaded on top of the gradients and centrifuged in a SW41 rotor at 40 000 rpm for 2.5 h (4 °C). The resulting gradient was collected in 1 mL fractions. Iodixanol gradient material (OptiPrep, 60% w/v) was purchased from Gibco.

Immunoprecipitation and Western Blotting. Mouse brains were homogenized in 1% NP-40 lysis buffer containing 50 mM Tris, pH 7.6, 150 mM NaCl, 2 mM EDTA, and the protease inhibitor cocktail (described above) in a final concentration of 100 mg/mL. Fibroblast cells were lysed in the same lysis buffer and subjected for immunoprecipitation and Western blotting. Subcellular fractions were lysed by the addition of NP-40 to a final concentration of 1% and aliquoted for either coimmunoprecipitation or ELISA. Immunoprecipitations were performed as described (*15*, *21*), and the precipitates were washed in a buffer containing 50 mM Tris, pH 7.6, 500 mM NaCl, 2 mM EDTA, and the protease inhibitor cocktail (described above) for 15 min at 4 °C, followed by additional washes with the same buffer but containing 150 mM instead of 500 mM NaCl. The immunoprecipitated proteins were eluted with 2× sample buffer containing 40% glycerol, 6% SDS, and 6% β-mercaptoethanol, electrophoresed on 8−16% Tris−glycine gels (Novex), and transferred to PVDF membrane (*21*). Western blotting using ECL detection was performed as suggested by the manufacturer (Amersham).

ELISA. Aβ sandwich ELISAs were performed as described (*6*, *25*). The capture antibodies were 266 (to Aβ residues 13−28) for $A\beta_{total}$ and 21F12 (to Aβ residues 33−42) for the Aβ_{42} species. The reporter antibody was biotinylated 3D6 (to Aβ residues 1−5) in both assays. None of these antibodies shows significant cross-reaction with full-length APP or $APP_{s-\alpha}$ in the ELISA.

RESULTS

Presenilin Is Required for APP CTF Processing in Brain. Individual PS1 knockout (KO) and wild-type (wt) embryo

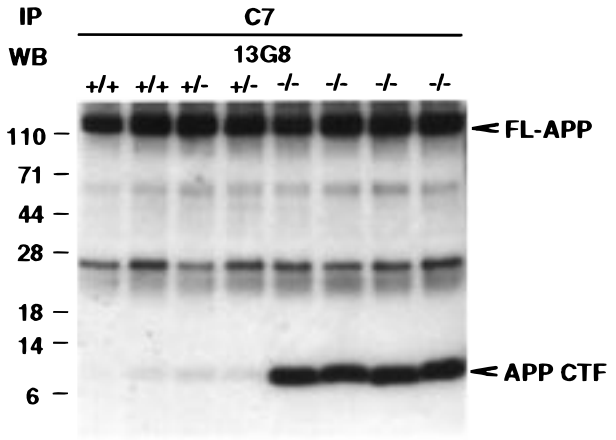

FIGURE 1: Accumulation of APP CTF in PS1 KO mouse brains. Individual mouse embryo brains from wt (+/+), heterozygous (+/−) and knockout (−/−) mice were harvested, homogenized in 1% NP-40 lysis buffer, and immunoprecipitated (IP)/Western blotted (WB) with APP antibodies C7 and 13G8, respectively. Ten milligrams of brain homogenate was used for each immunoprecipitation, and the resultant pellet was resuspended in SDS sample buffer and loaded in one lane. MW standards ($\times 10^3$) are indicated on the left.

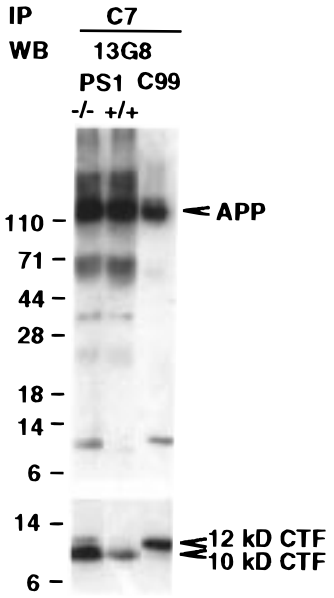

FIGURE 2: Accumulation of APP CTF in PS1 KO mouse fibroblasts. Fibroblasts from individual embryos were cultured and subjected to IP−Western blotting with C7 and 13G8. Upper panel: The APP bands in all three lanes are the endogenous holoproteins in each cell line. Lower panel: Longer exposure of the bottom half of the gel in the upper panel. The 12 kDa APP CTF in −/− cells comigrates with authentic 12 kDa APP CTF from a CHO cell line overexpressing a construct encoding the last 99 amino acids of APP (C99). The 10 kDa (α-secretase-generated) and 12 kDa (β-secretase generated) CTFs are indicated.

brains (*26*) were collected, and fibroblast cultures from PS1 KO and wt whole embryos were established. The absence of PS1 protein in the brains and fibroblasts of the KO mice was confirmed by immunoprecipitation (IP)−Western blotting with PS1 antibodies (data not shown). Mouse brains were lysed and precipitated with APP polyclonal antibody C7 followed by Western blotting with APP monoclonal antibody, 13G8. The levels of full-length APP molecules were unchanged in both brain tissue and fibroblasts of PS1-deleted mice (Figure 1). However, a marked accumulation of APP CTF was observed in the brains of the homozygous KO mice (Figure 1). While we assume that this strong CTF band reflects increases in both 10 and 12 kDa CTFs as reported by De Strooper et al. (*13*) in primary neurons from PS1 KO mice, we have only observed with certainty an increase in the 10 kDa CTF in brain; both are observed in fibroblasts (see below). A modest increase in CTF occurred in heterozygous (+/−) mouse brains, indicating that partial reduction of PS1 expression is sufficient to alter APP CTF catabolism. When the fibroblasts were utilized for the same immunoprecipitation−Western blotting experiments, similar results were obtained (Figure 2). Increases of both 10 and 12 kDa APP CTF were observed compared to fibroblasts from the wild-type littermates, again without change in holoAPP. The 12 kDa CTF from PS1 KO fibroblasts comigrated with the corresponding protein of CHO cell line C99 (D. Watson and D.J.S., unpublished data), which overexpresses a cDNA encoding the last 99 amino acids of APP (beginning at the β-secretase site). These results clearly indicate that, in the absence of PS1, both the 10 and 12 kDa APP CTF accumulate in brain due to decreased processing by γ-secretase, which is in full agreement with the results of De Strooper et al. (*13*) in cultured neurons of a different PS1 KO mouse line.

Lack of PS1 Results in Accumulation of APP CTF in ER and Golgi. To examine the subcellular locations in which PS1 affects γ-secretase cleavage of APP, both PS1 KO fibroblasts and CHO cells stably expressing wt or mutant human PS1 [$PS1_{WT-1}$ and $PS1_{ML-2}$ (*15*)] were subjected to fractionation. Membrane vesicles were separated by fractionation on discontinuous Iodixanol sucrose gradients (see Materials and Methods). A total of 12 fractions were collected from each gradient for each cell line, and each fraction was analyzed by Western blotting. ER-rich fractions from CHO cells were identified with an antibody against the ER marker protein, calnexin (Figure 3A). Fractions 1−4, the densest fractions, had the strongest immunoreactivity for calnexin, indicating that these fractions contained ER vesicles. This finding was supported by Western blotting with APP monoclonal antibodies 5A3/1G7, which can detect both *N*- and (*N* + *O*)-glycosylated APP proteins (Figure 3B). Fractions 1−3 uniformly showed only *N*-glycosylated APP proteins. The lack of further glycosylation of these immature APP isoforms indicates that they primarily reside in the ER, as reported previously (*27*−*29*). From fraction 4 to fraction 8, both *N*- and (*N* + *O*)-glycosylated APP proteins were observed. The appearance of (*N* + *O*)-glycosylated APP demonstrated the passage of APP into the Golgi compartment, where this posttranslational modification is completed (*27*). Furthermore, these same fractions were rich in galactosyltransferase activity (not shown), an enzyme selectively localized to Golgi vesicles (*30*). Although a discontinuous gradient was used for separation, some overlap of ER and Golgi markers was observed in fraction 4 (Figure 3A,B). On the basis of multiple gradient runs that gave highly similar results, we concluded that fractions 1−3 were markedly enriched in ER vesicles and fractions 5−8 were enriched in Golgi-type vesicles. Fraction 4 contained both ER and Golgi vesicles.

When the fractions of PS1 wt (+/+) or KO (−/−) fibroblasts were immunoprecipitated with C7 and blotted with 13G8, we found that the distribution of full-length APP

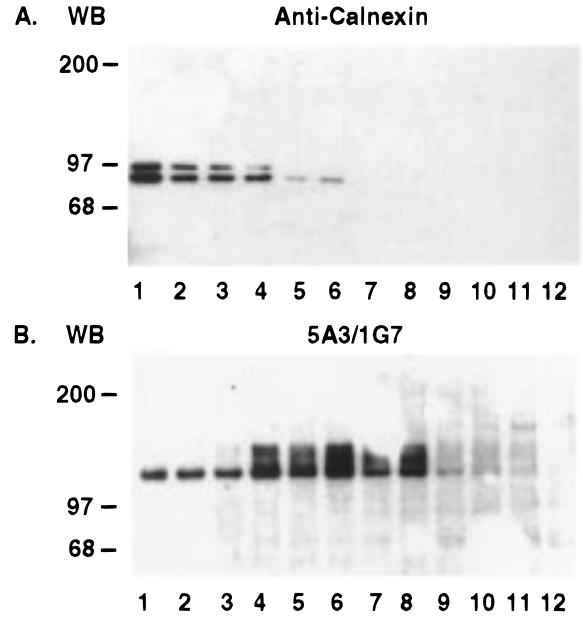

FIGURE 3: Characterization of subcellular fractions prepared by discontinuous iodixanol sucrose gradient fractionation. CHO cells stably coexpressing human APP and wt presenilin were fractionated as described in Materials and Methods, and fractions were analyzed by either Western blotting (this figure) or combined IP−Western blotting (Figure 5). (A) Fractions were blotted with an antibody against the ER marker protein calnexin. (B) The same fractions as in panel A were immunoblotted with APP monoclonal antibodies 5A3/1G7. Fractions 1−3 are rich in ER vesicles, which contain calnexin and solely *N*-glycosylated APP; fractions 4−8 are rich in Golgi vesicles which contain both *N*- and (*N* + *O*)-glycosylated APP. Fraction 4 represents a transition fraction in the discontinuous gradient and contains both ER and Golgi proteins.

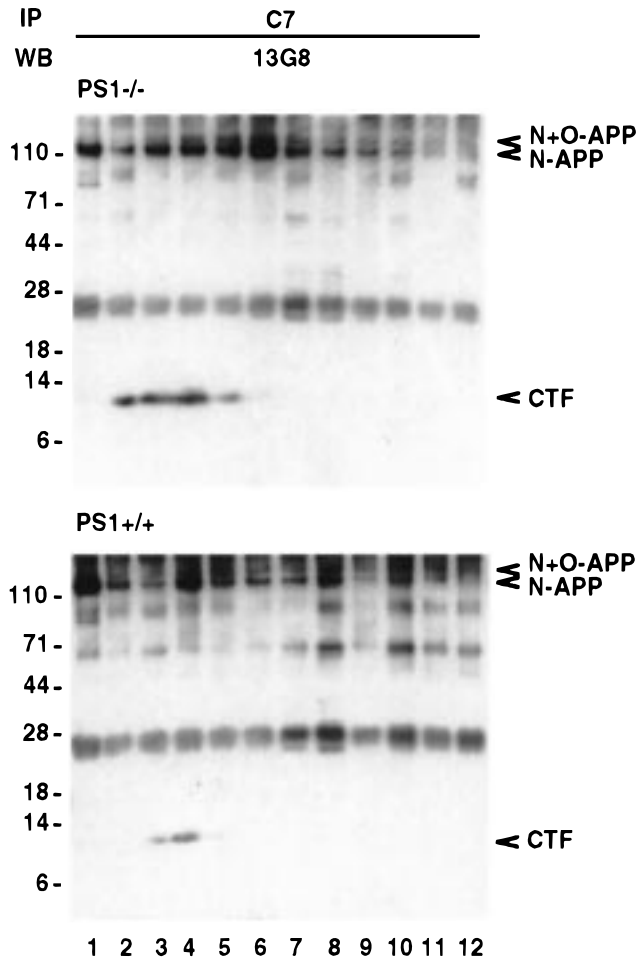

FIGURE 4: Accumulation of APP CTF in ER- and Golgi-rich fractions of PS1 KO fibroblasts. Fibroblasts from PS1 KO vs wt littermate embryos were subjected to discontinuous Iodixanol gradient fractionation. Each fraction was immunoprecipitated with C7 followed by Western blotting with 13G8. Immature *N*-glycosylated APP was detected in the ER-rich fractions 1−3. Mature (*N* + *O*)-glycosylated APP was distributed principally in fractions 4−8, which are enriched in Golgi vesicles. The distribution of full-length APP in KO fibroblasts was unchanged from that in PS1 KO fibroblasts. The APP CTF in PS1 KO fibroblasts were mainly located in the ER and denser Golgi fractions, with much less CTF found in these fractions from wt fibroblasts.

was not changed by the complete absence of PS1 (Figure 4). The *N*-glycosylated APP was mainly located in ER (fraction 1−3), and (*N* + *O*)-glycosylated APP was located in Golgi fractions (4−8) and to some extent in the lightest vesicle fractions (9−12). Compared to fractions from PS1wt cells, a marked accumulation of APP 10 kDa CTF was observed in both ER and Golgi of the KO fibroblasts. The 12 kDa CTF was not unequivocally detected, probably due to the low amount of this species compared to the 10 kDa CTF in the individual fractions. These data suggest that PS1 is required for proper γ-secretase cleavage of APP in ER and Golgi vesicles.

Presenilin Proteins Form Complexes with APP in the ER and Golgi. CHO cells overexpressing APP and PS1 were fractionated as above, and aliquots of the gradient fractions were immunoprecipitated with presenilin antibody X81, followed by Western blotting with PS antibody N15 (for detection of PS distribution) or APP antibody 8E5 [for detection of APP that coimmunoprecipitates with presenilins (*15*)]. When the fractions from $PS1_{WT-1}$ cells were immunoprecipitated with X81 and blotted with the PS1 monoclonal antibody N15, the characteristic 43−45 kDa PS1 holoproteins (*9*) were principally localized in the ER, while PS1 endoproteolytic fragments were mainly localized in the Golgi, with a smaller amount of the fragments present in the ER (Figure 5A). Analysis of the mutant $PS1_{ML-2}$ cell line showed a highly similar distribution of PS1 molecules in these compartments, and no consistent difference between wt and mutant PS1 was observed (not shown).

When the fractions from $PS1_{WT-1}$ cells were immunoprecipitated with antibody X81 and blotted with APP monoclonal antibody 8E5, a small fraction of APP molecules was found to coimmunoprecipitate with PS1 (Figure 5B). The major form of coprecipitated APP was *N*-glycosylated; only a minor amount of (*N* + *O*)-glycosylated APP coprecipitated with PS1, and this was found in the Golgi fractions of the discontinuous gradients, as expected (Figure 5B). This result is consistent with our previous finding that brefeldin A treatment does not block APP−PS complex formation in cells (*15*). Analysis of fractions prepared identically from the mutant $PS1_{ML-2}$ cell line showed that some APP molecules consistently coprecipitated with mutant PS1 in both ER and Golgi fractions (Figure 5C). Therefore, in ER and Golgi, both wt and mutant PS1 can participate in protein complexes with APP, supporting a direct involvement of PS1 in APP processing.

Increased Intracellular $A\beta_{42}$ Generation in Cells Expressing Mutant Presenilins. Aliquots of the same vesicle fractions that were used for the above IP−Western analyses

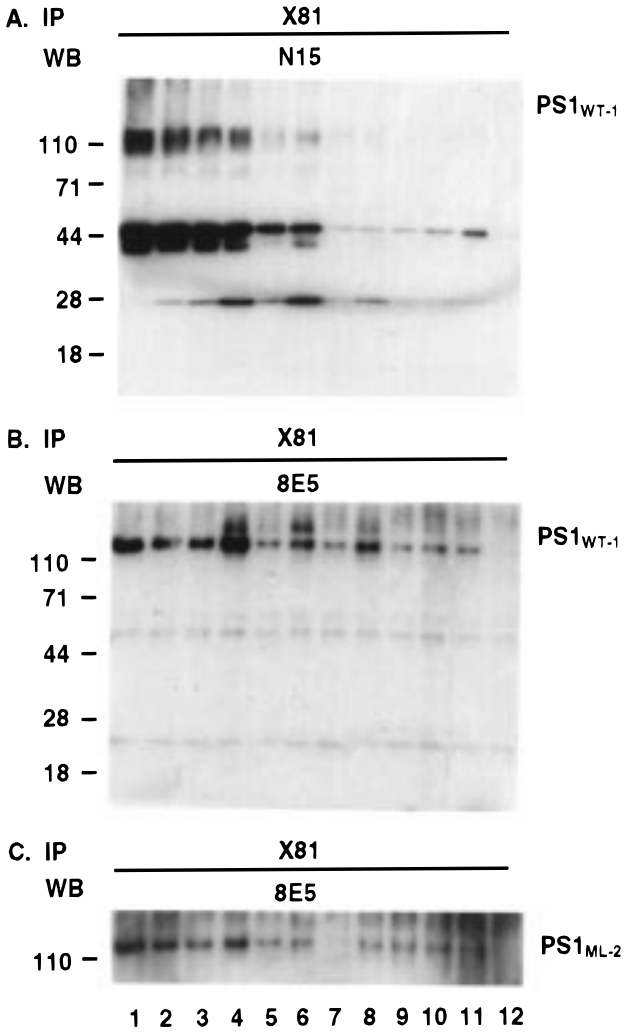

FIGURE 5: Complex formation between PS1 and APP in ER and Golgi vesicles. CHO cells stably coexpressing APP and PS1 were lysed and immunoprecipitated with the PS1 antibody (X81) followed by Western blotting with PS1 antibody N15 (A) or APP antibody 8E5 (B, C). (A) Most PS1 holoproteins (~43 kDa) reside in ER, while N-terminal PS1 fragments (~28 kDa) were localized to ER and Golgi vesicles, with greater relative abundance in the latter compartment. The band at ~110 kDa represents the characteristic oligomer of PS1 holoprotein described previously (e.g., refs *21* and *34*). (B) The *N*-glycosylated form of APP coprecipitates with wt PS1 in ER and Golgi fractions, with a small amount of (*N* + *O*)-glycosylated APP detected in the Golgi. (C) *N*-APP can also be coprecipitated with M146L mutant PS1 in both ER and Golgi vesicles.

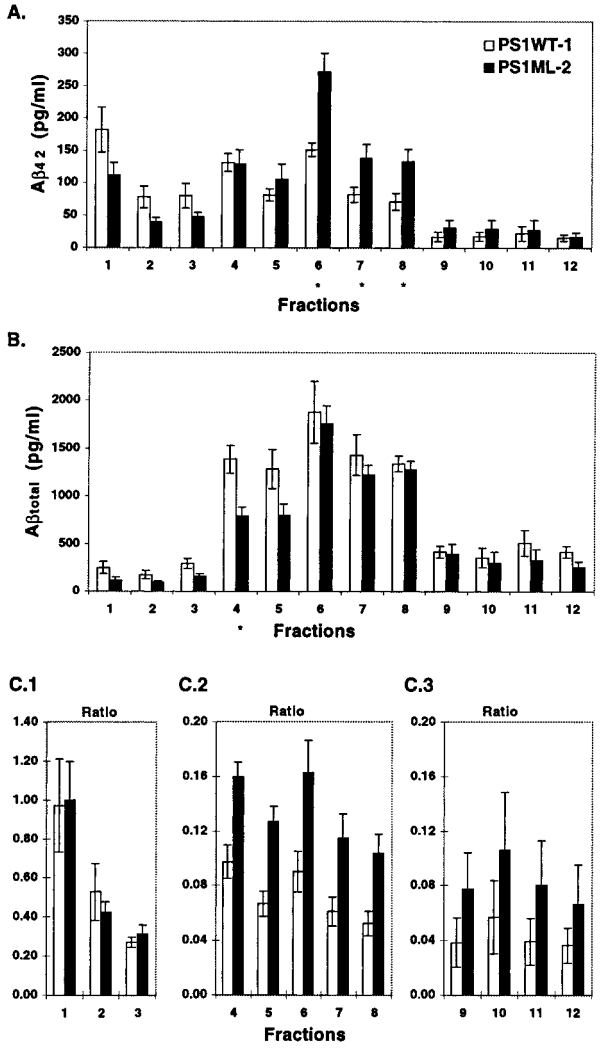

FIGURE 6: Determination of intravesicular Aβ levels by ELISA. Subcellular fractions as shown in Figures 3 and 5 were lysed in 1% NP-40 buffer and subjected to sandwich ELISA using capture antibody 21F12 for Aβ_{42} and capture antibody 266 for total Aβ. Biotinylated antibody 3D6 was the reporter in both assays. Values from five independent ELISA assays performed on five different fractionations were averaged, and the absolute values of Aβ_{42} and Aβ_{total} as well as the ratios of Aβ_{42}/Aβ_{total} were plotted. Student's *t*-test (two-tailed) was performed on all values. (A) Fraction 6−8 (*) showed increased Aβ_{42} in mutant PS1 cells ($p < 0.005$, 0.03, and 0.001, respectively). (B) Only fraction 4 (*) showed significantly increased Aβ_{total} in wt PS1 cells ($p < 0.03$). (C) Fractions 4−8 showed significant increases ($p < 0.001$, 0.001, and 0.002, 0.01, 0.001, respectively) of the ratio in mutant vs wt PS1 cells.

were examined for their concentrations of Aβ_{total} and Aβ_{42} by sensitive sandwich ELISAs. Five independent subcellular fractionations on APP plus wt or mutant PS1 double transfectants were performed, and the mean amounts of Aβ_{total} and Aβ_{42} were obtained for each fraction and plotted (Figure 6). Fractions from mutant PS1 cells showed a significant elevation of Aβ_{42} selectively in fractions 6−8, which are enriched in Golgi vesicles (two-tailed Student's *t* test, $p < 0.005$, 0.03, and 0.001, respectively); (Figure 6A). Both cell lines contained statistically similar levels of total Aβ, except for fractions 4, which showed significantly lower levels in mutant cells ($p < 0.03$, Figure 6B). The ratios of Aβ_{42}/Aβ_{total} for each fraction were calculated (Figure 6C). In ER-rich fractions (Figure 6C.1), the majority of Aβ was Aβ_{42}, with fraction 1 containing almost 100% Aβ_{42}. This ratio declined in successive ER-rich fractions, and in the Golgi-rich fractions, the ratio was less than 20% (Figure 6C.2) (note different scales on ordinates of panel C.1 vs panels C.2 and C.3). Increased Aβ_{42}/Aβ_{total} ratios were observed in the mutant PS1 cells beginning with fraction 4, so that the principal Golgi-rich fractions (5−8) had about 2-fold higher ratios in mutant than in wt cells (Figure 6C.2). These differences between wt and mutant PS1 subcellular fractions were statistically significant (two-tailed Student's *t* test; *p* values of fractions 4−8 were < 0.001, 0.001, 0.002, 0.01, and 0.001, respectively). The differences in the light fractions 9−12 were not statistically significant. Therefore, in CHO cells, mutant presenilins affected APP processing by selectively increasing the steady-state levels of Aβ_{42}, principally in the Golgi compartment.

We next used human kidney 293 cells stably double-transfected with the Swedish mutant form of APP and either wt or mutant PS1 to perform subcellular fractionation followed by ELISA determination of Aβ levels. A similar result was obtained: mutant Δexon 9 PS1 cells generated substantially more Aβ_{42} in the Golgi (fractions 5−8) than did wt cells. Fraction 5 showed a 3.3-($\pm$1.0) fold increase (mean $\pm$ SD) of the ratio Aβ_{42}/Aβ_{total}, and fractions 6−8 showed 1.6- ($\pm$0.3), 2.5- ($\pm$0.2), and 2.3- ($\pm$0.9) fold increases, respectively, in this ratio. Therefore, in both human kidney cells and CHO cells, mutant presenilins had a similar effect on APP processing by selectively increasing Aβ_{42} levels principally in Golgi-type vesicles.

DISCUSSION

More than 45 different missense mutations and one in-frame deletion have been identified to date in the PS1 gene in familial AD, and two missense mutations have been found in the PS2 gene (reviewed in ref *31*). Among more than 60 unrelated presenilin-linked pedigrees reported to date, 10 kindreds carry the M146L mutation in PS1, a mutation that has been shown by several laboratories to selectively increase Aβ_{42} production in both transfected cell lines and transgenic mice (*7*−*9*). Other mutations that occur at different sites in PS1, such as M146V, H163R, A246E, E280A, L286V, Δexon 9, and C410Y, and the two PS2 mutations have all been shown to increase Aβ_{42} production in various cell lines, including nonneural cells (*7*, *9*−*11*). Therefore, a selective alteration of γ-secretase processing of APP to yield more Aβ_{42} is believed to be a direct consequence of the expression of mutant presenilins.

In this study, we investigated the subcellular localization and mechanism of presenilins in regulating the processing of APP to Aβ. Our findings of increased APP CTF in PS1 KO mouse brain tissue and fibroblasts extend the report by De Strooper et al. (*13*) of a requirement for PS1 in the normal γ-secretase cleavage of APP. Using subcellular fractionation, we directly demonstrate the accumulation of APP CTF in ER- and Golgi-rich fractions, the same organelles in which the occurrence of APP−PS1 complexes could be observed in CHO cells overexpressing both proteins. Although both *N*- and (*N* + *O*)-glycosylated APP occur abundantly intracellularly (*27*−*29*), it was mainly *N*-glycosylated APP that underwent complex formation with PS1. These results in subcellular fractions are entirely consistent with our earlier results in whole lysates of the same stable cell lines (*15*) and with evidence of PS2−APP complexes in transiently transfected COS cells (*14*). The relative dearth of (*N* + *O*)-glycosylated APP interacting with PS1 or PS2 can be attributed to two possibilities: there is substantially less of this mature form of APP in the ER and early Golgi, compared to that in secretory vesicles or at the cell surface; or presenilins interact weakly or at low abundance with the mature APP molecules. Whether this latter explanation is true requires further investigation into the specific domains of APP that interact with the presenilins.

The pathobiological importance of the PS−APP interactions we detected in the ER and Golgi fractions is supported by the consistent difference in Aβ_{42} generation between wt and mutant presenilin-expressing cells that was observed in the same fractions. Compared to wt PS1-expressing cells, we observed a 2-fold increase of Aβ_{42}/Aβ_{total} in the Golgi fractions of mutant PS1 cells. In the ER, most Aβ molecules generated were Aβ_{42}, and there was almost no difference in Aβ_{42}/Aβ_{total} ratios between wt and mutant PS1 cells. The presence of mutant PS1 apparently alters the γ-secretase cleavage of APP in Golgi- and/or intermediate-type vesicles, which are known to principally contain the endoproteolytic fragments of PS1/2 that are thought to be the biologically active form, whereas the PS holoproteins are primarily localized to the ER (*32*). It should be noted that this and other studies of the subcellular localization of APP and PS have generally relied on stably transfected cells, because it is difficult to accurately localize the low-abundance presenilin proteins without transfection. However, we did previously detect small amounts of endogenous APP that coimmunoprecipitated with endogenous PS1 in whole lysates of nontransfected CHO cells (*15*). Moreover, the degree of elevation of Aβ_{42}/Aβ_{40} ratios observed in the media of cells transfected with mutant PS1/2 is closely similar to that measured directly in the primary fibroblast media and sera of humans bearing these mutations (*5*, *7*−*11*). Therefore, it is likely that the results of this and other studies using transfected molecules reflect in considerable part the situation under endogenous conditions.

Reports from several laboratories, including ours, have previously provided evidence that Aβ peptides (not specified as Aβ_{40} or Aβ_{42} at the time) can be generated in part in an early endosomal compartment following the reinternalization of some APP molecules from the cell surface (reviewed in ref *33*). Data presented in this and other recent studies (*16*−*19*) indicate that Aβ peptides, particularly Aβ_{42}, can also be generated early in the secretory processing of APP. The trafficking and processing of APP in neural and nonneural cells is complex, and the relative amounts of Aβ_{40} and Aβ_{42} produced in various subcellular loci are only beginning to be understood.

The critical involvement of PS1 in APP CTF processing in ER and Golgi shown here is consistent with the hypothesis that PS1 participates in protein complexes with APP and the currently unknown γ-secretase in these compartments (*15*). The various AD-causing mutations in PS1 may induce conformational changes of presenilin that favor cleavage of the APP CTF at residue 42. Lack of PS1 leads to a marked accumulation of APP CTF, as confirmed directly in brain tissue here. Further studies are necessary to understand the molecular mechanism of this crucial regulation of APP CTF processing by PS and whether other PS-binding proteins play a role in this process. The results should be important for designing therapeutic compounds to selectively inhibit the production of the highly amyloidogenic Aβ_{42} peptide.

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