Presenilins (PS1/PS2) play a critical role in proteolysis of β-amyloid precursor protein (βAPP) to generate β-amyloid, a peptide important in the pathogenesis of Alzheimer’s disease. Nevertheless, several regulatory functions of PS1 have also been reported. Here we demonstrate, in neuroblastoma cells, that PS1 regulates the biogenesis of βAPP-containing vesicles from the trans-Golgi network and the endoplasmic reticulum. PS1 deficiency or the expression of loss-of-function variants leads to robust vesicle formation, concomitant with increased maturation and/or cell surface accumulation of βAPP. In contrast, release of vesicles containing βAPP is impaired in familial Alzheimer’s disease (FAD)-linked PS1 mutant cells, resulting in reduced βAPP delivery to the cell surface. Moreover, diminution of surface βAPP is profound at axonal terminals in neurons expressing a PS1 FAD variant. These results suggest that PS1 regulation of βAPP trafficking may represent an alternative mechanism by which FAD-linked PS1 variants modulate βAPP processing.

Alzheimer’s disease (AD) is characterized by the excessive generation and accumulation of β-amyloid peptides (Aβ). The amyloidogenic Aβ peptide is proteolytically derived from the β-amyloid precursor protein (βAPP) within the secretory pathway by distinct enzymatic activities known as β- and γ-secretase (1, 2). Full-length βAPP is synthesized in the endoplasmic reticulum (ER) and transported through the Golgi apparatus. The major population of secreted Aβ peptides is generated within the trans-Golgi network (TGN) (3–5), also the major site of βAPP residence in neurons at steady state. βAPP can be transported in TGN-derived secretory vesicles to the cell surface if not first proteolyzed to Aβ or an intermediate metabolite. At the plasma membrane βAPP is either cleaved to produce a soluble molecule, sβAPP (6) or, alternatively, reinternalized within clathrin-coated vesicles to an endosomal/lysosomal degradation pathway (7, 8). Thus, the distribution of βAPP between the TGN and cell surface has a direct influence upon the relative generation of sβAPP versus Aβ. This phenomenon makes delineation of the mechanisms responsible for regulating βAPP trafficking from the TGN relevant to understanding the pathogenesis of AD.

Expression of autosomal dominant variants of either βAPP, presenilin 1 (PS1), or presenilin 2 (PS2) results in increased Aβ42 production and predispose individuals to early onset familial Alzheimer’s disease (FAD) (9–11). Presenilins (PSs), multitransmembrane proteins, accumulate as endoproteolyzed heterodimers of N- and C-terminal fragments and associate with other membrane proteins (e.g. nicastrin, APH-1, and PEN-2) to form high molecular weight complexes (9, 12–16). Several lines of evidence suggest that presenilin complexes play a crucial role in intramembranous γ-secretase cleavage of type I membrane proteins including βAPP and the signaling receptor, Notch-1. For example, genetic ablation of PS1, PS2, or other components of the PS complex dramatically impairs Aβ secretion and production of the Notch derivative, S3/NICD, in cells (14, 15, 17, 18). Mutations of conserved transmembrane aspartate residues in PSs result in loss-of-function leading to reduced Aβ secretion (19, 20). Finally, biochemical fractionation studies closely link PSs with γ-secretase activity, and γ-secretase inhibitors can be photo-cross-linked to PS1 and PS2 (21, 22).

Although it has generally been accepted that PSs are essential for γ-cleavage, it has not been firmly established that PSs are the catalytic component of the enzyme complex. For example, recent studies (23) have shown that the production of Aβ42 in early compartments of the secretory apparatus is unpai red in the absence of PSs. Whereas the hypothesis remains attractive that PSs are the γ-secretase, several reports indicate that PSs mediate additional physiological functions, including roles in calcium homeostasis, neurite outgrowth, apoptosis, and synaptic plasticity (24), and some of these functions are influenced by FAD-linked PS mutations. PS1 has been implicated in regulating intracellular trafficking and maturation of selected transmembrane proteins. PS1 deficiency significantly affects trafficking of the tyrosine kinase receptor TrkB, as well as the PS1-interacting protein ICAM-5/teclephalin (25, 26). Indeed, evidence has emerged to support the notion that PS1 may facilitate γ-secretase cleavage of substrates via regulating the maturation and intracellular trafficking of substrates and/or...
components of the γ-secretase complex. For example, expression of the PS1 aspartate variants leads to accumulation of βAPP C-terminal fragments (CTFs) as well as full-length βAPP at the cell surface (20, 27). Several recent studies (28, 29) further demonstrate that PS1 regulates the maturation and cell surface accumulation/trafficking of nicastrin.

Based on these observations, we investigated the potential role of PS1 in regulating intracellular trafficking of full-length βAPP through the secretory pathway. We previously established a cell-free system in which we can reconstitute both the formation of Aβ and the trafficking of βAPP/Aβ from the ER or the TGN (3, 5, 30–32). By utilizing this system, we directly examined individual cellular processes involved in PS1 regulation of βAPP trafficking. We report here that PS1 deficiency or expression of loss-of-function variants led to robust formation of βAPP-containing vesicles, concomitant with increased maturation and/or cell surface accumulation of βAPP. In contrast, vesicle formation from the TGN and the ER was impaired in cells expressing FAD-linked PS1 mutants, resulting in a reduction of βAPP delivery to the cell surface. We also observed a profound reduction of surface βAPP at axonal terminals in neurons harboring an FAD PS1 mutation (A246E), compared with neurons expressing wild type (wt) PS1. Taken together, these results suggest that PS1 can modulate metabolism of βAPP via regulating βAPP trafficking within the secretory pathway and thus affect Aβ generation by controlling the availability of substrate βAPP to appropriate secretases.

EXPERIMENTAL PROCEDURES

Cell Lines—Mouse N2a neuroblastoma cells doubly transfected with cDNAs encoding human βAPP harboring the “Swedish” double mutant (βAPPswede) and human wt PS1 or various PS1 mutants (11, 30) were maintained in medium containing 50% Dulbecco’s modified Eagle’s medium, 50% Opti-MEM, supplemented with 5% fetal bovine serum, antibiotics, and 200 μg/ml G418 (Invitrogen). Immortalized PS1−/− fibroblasts (33) were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum and antibiotics. To circumvent potential variability between fibroblasts established from PS1−/− and PS1+/− embryos, immortalized PS1−/− fibroblasts were transfected with wt PS1 expression plasmid, and stable transfecteds were isolated in 400 μg/ml Zeocin (Invitrogen). To express βAPPsw, PS1−/− fibroblasts and wt PS1 transfecteds were infected with βAPPsw retrovirus produced from the mouse NIH3T3-based PT67 packaging cell line, and stable expressers (designated PS1−/− and wt) were isolated in medium containing 200 μg/ml puromycin (Calbiochem), and ~150–200 colonies were pooled for further studies. Blastocyst-derived wt and PS1−/−/IP2−− cells have been described previously (28).

Neuronal Culture—Human wt PS1 (line 17-3) or FAD-linked PS1 A246E mutant transgenic mice (line 16-4) (34) were mated with heterozygous PS1 null mice (PS1+/−) (35). Double heterozygous F1s were then intercrossed to generate offspring that express human wt or A246E PS1 in the PS1−/− background. Genotypes were determined by PCR amplification as described previously (34). Primary neuronal cultures were derived from the cerebral cortices of embryonic day 17 (E17) mouse embryos from the above matings. Dissociated neurons were plated (~5 × 10^4 cells per chamber) on poly-d-lysine (Sigma)-precoated 8-chamber slides (Lab-Tek) in serum-free neurobasal medium with N2 supplement as well as B27 supplement (Invitrogen) and cultured for 7 days. Media were replaced every 2 days with the addition of 16.5 mg/ml uridine and 6.7 mg/ml 5-fluoro-2′-deoxyuridine to prevent proliferation of glial cells.

Preparation of Permeabilized N2a Cells—It has been well established that incubation of cells at 15 °C (36) or 20 °C (30) leads to an accumulation of membrane and secretory proteins in the ER and TGN, respectively. To assay βAPP trafficking from TGN, cells were pulse-labeled with [35S]methionine (500 μCi/ml) for 15 min at 37 °C, washed with phosphate-buffered saline (prewarmed to 20 °C), and chased for 2 h at 20 °C in prewarmed complete media. To assay βAPP trafficking from the ER, cells were pulse-labeled for 4 h at 30 °C. For both types of preparations, cells were permeabilized at the termination of incubation as follows. Cells were first incubated at 4 °C in “swelling buffer” (10 mM KCl, 10 mM HEPES, pH 7.2) for 10 min. The buffer was discarded and replaced with 1 ml of “breaking buffer” (90 mM KCl, 10 mM HEPES, pH 7.2) after which the cells were broken by scraping with a rubber policeman. Cells were centrifuged at 800 × g for 5 min, washed in 5 ml of breaking buffer, and resuspended in 5 volumes of breaking buffer. This results in >95% cell breakage as evaluated by trypan blue staining. Broken cells (cell-free system) were incubated in a final volume of 300 μl containing 2.5 mM MgCl2, 0.5 mM CaCl2, 110 mM KCl, cytosol (30 μg protein) prepared from N2a cells (32, 37), and an energy-regenerating system consisting of 1 μM ATP, 0.02 μM GTP, 10 μM creatine phosphate, 80 μg/ml creatine phosphokinase, and a protease inhibitor mixture. Incubations were carried out at 37 °C for various times (15–120 min) to observe the kinetics of protein trafficking.

Formation of Nascent Secretory Vesicles in Permeabilized Cells and Immunoprecipitation—Following incubation of cell-free systems, vesicles were separated from membrane fractions by centrifugation at 11,000 rpm for 30 s at 4 °C in a Brinkmann centrifuge (Brinkmann Instruments). Vesicle (supernatant) and membrane (pellet) fractions were then intercrossed to generate offspring that express human wt or A246E PS1 APP, nicastrin, and Notch-1, suggest that PS1 regulates the formation of APP-containing vesicles from the TGN, cells were transferred to 37 °C for various time intervals. Cells were then incubated at 4 °C with 0.5 mg/ml anti-N-1-hydroxy-succinimidyl biotin (Pierce) to biotinylate cell surface proteins. Biotinylated and non-biotinylated proteins were first separated into two fractions by binding to streptavidin-agarose beads (Pierce), and (APP from each fraction was immunoprecipitated with antibody 369 (7, 38) and analyzed by SDS-PAGE. Each experiment was performed at least three times. Band intensities were analyzed and quantified using NIH ImageQuant software, version 1.52.

Biotinylation and Detection of Cell Surface βAPP—Stably transfected N2a cells were labeled with [35S]methionine (500 μCi/ml) for 10 min at 37 °C and chased for 2 h at 20 °C in complete medium to accumulate labeled proteins in the TGN. To study vesicle trafficking from TGN, cells were transferred to 37 °C for various time intervals. Cells were then incubated at 4 °C with 0.5 mg/ml sulfo-N-hydroxysuccinimidyl biotin (Pierce) to biotinylate cell surface proteins. Biotinylated and non-biotinylated proteins were first separated into two fractions by binding to streptavidin-agarose beads (Pierce), and (APP from each fraction was immunoprecipitated with antibody 369 (7, 38) and analyzed by SDS-PAGE (39).

Immunofluorescence Confocal Microscopy—For staining of full-length βAPP, N2a cells grown in chamber slides were incubated at 4 °C with primary antibody 6E10 (diluted 1:1000 in growth medium, Senetek) for 1 h at 4 °C. Cells were then permeabilized with secondary antibodies and FITC-conjugated Vicia villosa agglutinin (1:100, Vector Laboratories Inc.), cells were fixed with 4% formaldehyde at room temperature for 15 min. Cultured neurons were fixed twice with 4% formaldehyde for 15 min twice. For surface staining of full-length βAPP, cells were directly incubated with primary antibody mAb348 (1:100 dilution, Roche Molecular Biochemicals) at 4 °C overnight. In some experiments, neurons were permeabilized by ice-cold methanol for 2 min prior to overnight incubation with mAb348 or anti-GAP43 antibody (1:4000). Immunofluorescence staining was examined by confocal microscopy (LSM510, Zeiss).

RESULTS

PS1 Deficiency Leads to Increased βAPP Transport from TGN to Plasma Membrane and from ER to Golgi—It has been well established that PS1-deficient neurons fail to secrete Aβ but accumulate intracellular βAPP C-terminal fragments (25, 40). In addition to affecting γ-secretase activity, recent observations (25, 28, 29) that PS1 deficiency or loss-of-function alters the maturation and cell surface accumulation of certain membrane proteins, such as βAPP, nicastrin, and Notch-1, suggest a potential role for PS1 in intracellular protein trafficking. To support further the notion that wt PS1 may have a direct regulatory effect on βAPP trafficking through secretory compartments, we assessed the formation of βAPP-containing vesicles from the TGN and from the ER in PS1−/− fibroblasts using a cell-free reconstitution system.

The formation of βAPP-containing vesicles from the TGN was examined using a cell-free system that has been used extensively to study βAPP trafficking and Aβ generation (see “Experimental Procedures” and Refs. 3, 5, and 32). This cell-free system has been used to investigate nascent secretory vesicle budding from a variety of cells (30, 31), and the integrity of TGN stacks and derived vesicles has been demonstrated by electron microscopy (37). To study trafficking of βAPP-containing vesicles from the TGN, we labeled wt and PS1−/−/IP2−− fibroblasts with [35S]methionine and then incubated the cells
at 20 °C, a temperature at which transport of proteins, including βAPP, from the ER to the TGN is unimpaired. However, under these conditions, the egress of secretory vesicles from the TGN is blocked, thus allowing labeled βAPP (and other membrane proteins) to accumulate in the TGN (3, 5, 32). This experimental design ensures that TGN-specific vesicle biogenesis is measured. In our cell-free trafficking assays, following permeabilization and incubation at 37 °C, budding of βAPP-containing vesicles from the TGN was greatly increased at all time points examined in preparations that lacked PS1 when compared with preparations from cells that express wt PS1 (Fig. 1, a and b). After 2 h of incubation, the amount of βAPP transported out of the TGN was 54.2% higher in PS1−/− cell preparations compared with preparations from PS1 wt cells (i.e. 21.9 versus 14.2% of the total labeled βAPP in the TGN).

We next tested whether PS1 deficiency might affect βAPP vesicle transport from the ER to Golgi utilizing a modified cell-free system in which βAPP-containing post-ER vesicles were reconstituted. In this case, cells were labeled with [35S]methionine at 15 °C to accumulate labeled βAPP within the ER (see “Experimental Procedures” and Ref. 5), followed by permeabilization and incubation at 37 °C to initiate vesicle release. The budding of βAPP-containing ER vesicles was accelerated (~2-fold) in PST−/− cells compared with wt cells (Fig. 1, c and d).

**Fig. 1. PS1 deficiency accelerates βAPP transport from the TGN and from the ER.** a–d, PS1−/− fibroblasts expressing human βAPPsw e alone (PS1−/−) or coexpressing βAPPsw e and wt human PS1 (PS1WT) were labeled for 15 min with [35S]methionine at 37 °C and chased for 2 h at 20 °C to accumulate labeled βAPP in the TGN. Alternatively, cells were labeled for 4 h at 15 °C to accumulate labeled βAPP within the ER. Permeabilized cells were prepared and incubated at 37 °C for various times to allow the formation of post-TGN (a and b) or post-ER vesicles (c and d). Labeled βAPP was immunoprecipitated from nascent vesicles or the donor compartments and analyzed by SDS-PAGE and autoradiography (a and d). The kinetics of βAPP-containing vesicle formation is presented as percent of vesicle budding (b and d); data represent mean ± S.E. from three independent experiments. e, the kinetics of ER budding of FGFR-containing vesicles was analyzed as above. Data represent mean ± S.E. of three experiments. f, samples of cell lysates with equal amount of protein (50 μg protein/lane) prepared from wt and PS1−/−/PS2−/− cells were analyzed by Western blotting using C-terminal βAPP antibody CT15.
PS1 Regulates βAPP Trafficking

In PS1/H9252 studies, the amount of mature (glycosylated/sialylated) trafficking, budding of fibroblast growth factor receptor (FGFR)−containing vesicles from the ER was determined in parallel and remained unchanged by PS1 deficiency (Fig. 1c). Collectively these results suggest that PS1 selectively regulates βAPP trafficking from ER and TGN compartments.

To support further the notion that βAPP is transported more efficiently out of the ER and Golgi compartments in the absence of PS, we examined the patterns of βAPP maturation in detergent lysates prepared from PS1−/−/PS2−/− cells and wt fibroblasts. As expected from the PS1+/−-permeabilized cell studies, the amount of mature (glycosylated/sialylated) βAPP in PS1−/−/PS2−/− cells was significantly higher than that in PS1 wt cells (Fig. 1f), indicative of increased residence and transit of βAPP through the late secretory compartments. However, alternation in βAPP glycosylation/maturation was much less dramatic in PS1−/− cells than in PS1+/−/PS2−/− cells (data not shown). Although the underlying mechanism is not clear, this may be attributed to differential roles of PS1 and PS2 in APP trafficking versus maturation.

Loss-of-Function PS1 Mutants Accelerate βAPP Trafficking from the ER without Altering TGN Vesicle Budding—To confirm further that loss of PS1 activities results in accelerated βAPP trafficking through the secretory pathway, N2a cells expressing loss-of-function PS1 variants were examined to assess their effects on intracellular transport of βAPP. Previous studies demonstrated that a single amino acid substitution in PS1 transmembrane domain 7 (D385A) (19, 20) or deletion of the first two PS1 transmembranes (Δ1,2) (41) lowers Aβ secretion and leads to accumulation of βAPP βCTFs. Unexpectedly, βAPP-containing vesicle budding from the TGN was not changed by the expression of PS1 loss of function mutations (D385A or Δ1,2) (~35% of maximal βAPP vesicle budding in both D385A and Δ1,2 versus ~38% in PS1 wt cells) (Fig. 2, a and b). However, the trafficking of βAPP from the ER to Golgi was significantly increased in these loss-of-function PS1 mutants (Fig. 2, c and d). The maximal level of vesicle budding was increased by ~1.5- (for D385A) to 2-fold (for Δ1,2) when compared with PS1 wt; this increase in budding is similar to that observed in PS1-deficient cells. As a result of accelerated ER trafficking, the total amount of βAPP residing in TGN membrane in loss-of-function PS1 mutants was increased compared with that in wt TGN membrane (Fig. 2a), although the rate of budding of βAPP-containing vesicles from the TGN was unchanged (Fig. 2b).

Together, these results suggest that the loss of function for PS1 in γ-cleavage of βAPP and the complete absence of PS1 protein differentially regulate vesicle biogenesis from the TGN, although the efflux of βAPP molecules from the ER is enhanced under both conditions.

Loss-of-Function PS1 Mutations Increase the Amount of Full-length βAPP Delivered to the Plasma Membrane—Despite the lack of marked differences in TGN vesicle biogenesis, increased ER to Golgi trafficking of βAPP observed in the loss-of-function mutant cells may be sufficient to elevate the steady-state levels of βAPP at the cell surface in intact cells. In addition, as recently reported (42), a delay in the internalization of surface-bound βAPP may further facilitate increased surface accumulation of βAPP. Indeed, live staining of Δ1,2 cells using monoclonal antibody 6E10 revealed an obvious increase in the amount of surface-bound βAPP compared with PS1 wt cells. The amount of total surface glycoproteins is identical in the two

**Fig. 2.** βAPP trafficking from the ER and the TGN in cells expressing loss-of-function mutations of PS1. Stable N2a cells coexpressing βAPPsw and wt PS1 (WT) or PS1 harboring either D385A or Δ1,2 mutations were used. Budding assays were performed as described in Fig. 1. Quantitative data represent mean ± S.E. from three independent experiments.

d). To assess whether PS1 deficiency selectively affects βAPP trafficking, budding of fibroblast growth factor receptor (FGFR)-containing vesicles from the ER was determined in parallel and remained unchanged by PS1 deficiency (Fig. 1c). Collectively these results suggest that PS1 selectively regulates βAPP trafficking from ER and TGN compartments.

To support further the notion that βAPP is transported more efficiently out of the ER and Golgi compartments in the absence of PS, we examined the patterns of βAPP maturation in detergent lysates prepared from PS1−/−/PS2−/− cells and wt fibroblasts. As expected from the PS1+/−-permeabilized cell studies, the amount of mature (glycosylated/sialylated) βAPP in PS1−/−/PS2−/− cells was significantly higher than that in PS1 wt cells (Fig. 1f), indicative of increased residence and transit of βAPP through the late secretory compartments. However, alternation in βAPP glycosylation/maturation was much less dramatic in PS1−/− cells than in PS1+/−/PS2−/− cells (data not shown). Although the underlying mechanism is not clear, this may be attributed to differential roles of PS1 and PS2 in APP trafficking versus maturation.

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Loss-of-Function PS1 Mutations Increase the Amount of Full-length βAPP Delivered to the Plasma Membrane—Despite the lack of marked differences in TGN vesicle biogenesis, increased ER to Golgi trafficking of βAPP observed in the loss-of-function mutant cells may be sufficient to elevate the steady-state levels of βAPP at the cell surface in intact cells. In addition, as recently reported (42), a delay in the internalization of surface-bound βAPP may further facilitate increased surface accumulation of βAPP. Indeed, live staining of Δ1,2 cells using monoclonal antibody 6E10 revealed an obvious increase in the amount of surface-bound βAPP compared with PS1 wt cells. The amount of total surface glycoproteins is identical in the two
accumulate labeled ° allows trafficking of /H9252 incubated at 37 ° ° plasma membrane. Cell surface proteins were then biotinylated at 4 ° for 15 min. Biotinylated and non-biotinylated proteins were separated in intact cells. Cells were pulse-labeled for 10 min with [35S]methionine at 37 ° and chased for 20 ° to allow transport of APP to the plasma membrane. To test this hypothesis we stained live N2a cells with monoclonal antibody 6E10 to visualize surface-bound βAPP and βAPP C-terminal fragments and FITC-conjugated V. villosa agglutinin to label all glycoproteins. As shown in Fig. 5a, the intensity of βAPP immunofluorescence on the cell surface of mutant cells was markedly reduced as compared with PS1 wt cells. V. villosa agglutinin staining of surface glycoproteins was comparable between PS1 wt and mutant cells (Fig. 5a). These findings are consistent with the results from permeabilized cell experiments described above and suggest that FAD-linked PS1 mutants impair the trafficking of βAPP to the cell surface.

To examine directly the delivery of newly synthesized full-length βAPP trafficking from the TGN to the plasma membrane, we carried out pulse-chase labeling in combination with cell-surface biotinylation. As shown in Fig. 5, b and c, in PS1 wt cells, 9.6% of nascent βAPP that accumulated in the TGN during incubation at 20 ° left the TGN and traveled to the cell surface after a 2-h chase at 37 °. However, the amount of newly synthesized βAPP that accumulated on the cell surface at various chase time periods was much lower in PS1 (ΔE9) mutant cells, with only 3–5% of nascent βAPP molecules delivered to the plasma membrane even after 2 h of chase. Therefore, both immunofluorescence and biochemical studies indi-
cate that the levels of full-length βAPP delivered from TGN to plasma membrane are diminished in cells expressing FAD-linked PS1 variants, most likely by delaying the budding of βAPP-containing vesicles from the TGN and the ER.

An FAD-linked PS1 Variant Causes a Profound Reduction of Cell Surface-bound βAPP at Axons and Axonal Terminals in Primary Neurons—Previous studies (44–48) have demonstrated that βAPP is axonally transported by the kinesin-mediated, fast anterograde component. It has also been reported (49) that both full-length and processed derivatives of βAPP accumulate at presynaptic terminals of cortical neurons. We further assessed whether FAD-linked mutations affect the distribution of full-length βAPP on the surface of primary neurons.

Embryonic cortical neurons from PS1 knockout mouse embryos rescued with comparable levels of expression of either wt PS1 (WT) or PS1 harboring one of the indicated FAD-linked mutations were used in these experiments. Vesicle budding experiments were performed as described in the legend to Fig. 1. Quantitative data represent mean ± S.E. from three independent experiments. e, budding of NCAM-containing vesicles was examined in parallel from WT and mutant cells and quantified.

Fig. 4. FAD-linked PS1 mutants delay βAPP transport from the TGN and the ER. a–d, stable N2a cells coexpressing βAPPsw and either wt PS1 (WT) or PS1 harboring one of the indicated FAD-linked mutations were used in these experiments. Vesicle budding experiments were performed as described in the legend to Fig. 1. Quantitative data represent mean ± S.E. from three independent experiments. e, budding of NCAM-containing vesicles was examined in parallel from WT and mutant cells and quantified.
length βAPP was identical in PS1 wt and A246E neurons (Fig. 6, top panels). In addition, intracellular localization of growth-associated protein 43 (GAP43), a protein associated with growth cone membranes, was similar in wt and mutant PS1 neurons (Fig. 6, bottom panels). These data suggest that the FAD-linked mutants selectively impair the targeting or fusion of βAPP-containing vesicles to the plasma membrane, especially at axons and axonal terminals of neurons.

**DISCUSSION**

To date, the mechanisms by which PSs exert their effects on βAPP metabolism are not fully understood, although multiple lines of evidence support a direct role of PS1 in facilitating γ-secretase cleavage of βAPP, Notch, and other substrates (1, 50). On the other hand, it has been reported (24) that PS1s may play multiple physiological roles such as those in calcium homeostasis, neuronal development, neurite outgrowth, apoptosis, synaptic plasticity, and tumorigenesis. Recent evidence indicates a novel function of PS1 in regulating intracellular trafficking of a selected set of proteins including those associated with PS1 and βAPP metabolites (25, 26, 42). In the present report, we demonstrate the following: 1) PS1 deficiency leads to the accelerated trafficking of βAPP from both the TGN and the ER; 2) loss-of-function PS1 mutants with impaired γ-secretase function increase ER biogenesis of βAPP-containing vesicles without affecting TGN budding and eventually elevate the amount of βAPP transported to the plasma membrane; 3) FAD-linked PS1 mutants impair βAPP trafficking from the TGN to the plasma membrane, as well as from the ER to Golgi, resulting in delayed delivery of βAPP to the cell surface; 4) a profound reduction of surface βAPP at axonal terminals of neurons that express FAD-linked PS1 mutants. Taken together, these results indicate that the role of PS1 in facilitating γ-secretase processing of APP extends beyond its putative function in the catalytic process.

The above findings are consistent with a model in which PS1 might regulate βAPP metabolism by altering βAPP trafficking (Fig. 7). Our model proposes that PS1 provides a retention
signal, which guides βAPP delivery to appropriate compartments where γ-secretase processing occurs (Fig. 7a). Gain-of-function FAD mutants (Fig. 7c) may direct sustained retention of βAPP-containing vesicles and consequently increase the availability of βAPP to cleavage enzymes resident in the TGN and/or ER, the major intracellular sites for Aβ generation. However, the possibilities that PS1 may recruit certain cytosolic trafficking factors (such as phospholipase D1 and/or Rab11) to TGN and/or ER membrane and thereby regulate βAPP transport cannot be excluded.

Previously it was shown that PS1 deficiency causes missorting of select type I membrane proteins. For example, PS1 is required for the maturation and intracellular trafficking of nicastrin, an integral component of the γ-secretase complex (14, 28, 29, 52). Moreover, in PS1-deficient neurons, telencephalin/ICAM is translocated from the plasma membrane to large intracellular clusters (26). It is interesting to note that PS1 deficiency causes enhanced localization of βAPP at the cell surface but has the opposite effect on surface accumulation of nicastrin and telencephalin/ICAM.

To fully understand the regulation of βAPP metabolism by PS1, it would be important to determine whether PS1 exerts an effect on trafficking of βAPP CTFs similar to that on full-length βAPP. It has been shown that loss of PS1 results in the accumulation of the α-/β-CTF (25, 40). However, further studies on the subcellular distribution of CTFs in PS1-deficient cells indicate the complexity of intracellular trafficking of CTFs. For example, the generation of CTFs mostly occurs in the late secretory compartments, whereas βAPP CTFs accumulate in the ER, Golgi, and lysosomes (53). It has also been reported (53) that CTFs may accumulate in restricted and unpredicted intracellular compartments in PS1-deficient cells. Although our preliminary data indicate that PS1 may regulate intracellular trafficking of CTFs in a similar manner as its regulation of full-length βAPP (data not shown), vesicle budding assays (pulse-chase and low temperature incubations) have not yet distinguished between trafficking and production of CTFs in the ER and Golgi compartments. More rigorous studies are underway to establish appropriate cell lines (e.g., those overexpressing 6CFTFs) and optimal experimental conditions.

Much remains to be learned about the mechanisms by which PS1 regulates intracellular trafficking of βAPP. Based on our permeabilized cell data, budding of vesicles containing FGFR1 and NCAM is not influenced by PS1 function, indicating that PS1 exerts its regulation on select membrane proteins. One attractive model is that PS1 regulates the recruitment or the association of trafficking factors with cytoplasmic sorting signals within βAPP, thereby selectively regulating the sorting of βAPP to the surface. In the absence of PS function, βAPP-containing vesicles may be transported to the cell surface via the default constitutive pathway. On the other hand, FAD-linked mutations modify the interaction between βAPP and trafficking factors in a manner that interferes with efficient βAPP trafficking. In this regard, it has been reported that PS1 and PS2 associate with Rab11, a member of the GTP-binding protein family of membrane trafficking regulators implicated in protein transport along the biosynthetic and endocytic pathways (54). In addition, PS1 binds to Rab GDP dissociation inhibitor (RabGDI), a protein that functions in vesicular membrane transport to recycle Rab GTPases, and PS1 deficiency leads to impaired Rab-GDI membrane association (55). Furthermore, our preliminary data suggest that addition of phospholipase D1 to the cell-free budding assays prevents impaired βAPP trafficking from the TGN in cells expressing FAD PS1 mutations, whereas inhibition of phospholipase D1 activity by primary butanol diminishes the accelerated βAPP trafficking from the TGN in cells expressing loss-of-function PS1 mutations.

Finally, our studies demonstrate that FAD-linked PS1 mutants result in a decreased distribution of surface βAPP in axons and axonal terminals of neurons. This finding suggests that PS1 may specifically affect targeting to and/or fusion of βAPP-containing vesicles at the nerve terminals. As reported previously, full-length βAPP has been implicated in a number of physiological functions such as synapse formation, growth cone outgrowth, and axon guidance (55, 56). Furthermore, based on the demonstration that βAPP plays an essential role in axonal trafficking (47, 48), our findings that PS1 may regulate βAPP sorting along the axon have much broader implications as well. For example, impaired delivery of full-length βAPP to the cell surface at axonal terminals by FAD-linked PS1 variants may interfere with neurite initiation, elongation and branching, and synaptic plasticity. It is important to note that in AD presynaptic pathology is more severe than neuronal loss (57). By affecting vesicle transport and surface delivery of full-length βAPP, pathogenic PS1 mutants might directly modulate βAPP metabolism and, in addition, indirectly contribute to the pathogenesis and progression of AD.

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