Regulation of Amyloid Precursor Protein Processing by Presenilin 1 (PS1) and PS2 in PS1 Knockout Cells

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The presenilin 1 (PS1) and PS2 proteins are thought to play roles in processing of amyloid precursor protein (APP), but the nature of this role is not fully understood. Recent studies have shown that PS1 is necessary for cleavage of APP at the γ-secretase site. We now show that PS1 and PS2 participate in other aspects of APP processing. Fibroblasts generated from PS1 knockout mice have increased levels of the APP cleavage products, secreted APP (APPs), and APP C-terminal fragments, but lower secretion of APPs and Aβ. We have also observed that loss of PS1 prevents protein kinase C or extracellular regulated kinase from increasing production of the APP cleavage products, APPs, and APP C-terminal fragments. Transfection of PS1 −/− cells with PS1 restores the responsiveness of APP processing to protein kinase C and extracellular regulated kinase from increasing production of the APP cleavage products, APPs, and APP C-terminal fragments. Transfection of PS1 −/− cells with PS2 is also able to correct the defects in APP secretion, which suggests that the PS2 also has the ability to regulate APP processing. Finally, transfection of the truncated PS2 construct, Alg3, into cells lacking PS1 increases APP C-terminal fragments. This suggests that Alg3 can interfere with the processing of APP by PS2. These data point to roles for both PS1 and PS2 in regulating APP processing and suggest that the role of these proteins also includes coupling APP to signal transduction pathways.

The PS11 protein is thought to play an important role in Alzheimer’s disease (AD). Mutations in PS1 have been linked to familial Alzheimer’s disease (1–3). The mutations in PS1 and PS2 that are linked to familial AD most likely cause AD by increasing production of Aβ1–42 (4, 5). The Aβ1–42 peptide is a particularly hydrophobic form of Aβ that rapidly aggregates in patients with PS1 or PS2 mutations is thought to cause familial AD by accelerating the accumulation of Aβ aggregates and the formation of neuritic plaques, which are one of the principal pathologic components in AD.

Aβ is generated by cleavage of its parent protein, amyloid precursor protein (APP). During its processing, APP is alternatively cleaved to generate either Aβ or a secreted form of APP termed APPsa. The cleavages that generate Aβ occur at two positions. The putative protease that cleaves APP at the C terminus of the Aβ domain is termed γ-secretase, whereas cleavage at the N terminus of the Aβ domain is carried out by a putative protease termed β-secretase (7, 8). Cleavage by the putative protease, α-secretase, occurs at a site corresponding to Aβ1–16 and generates a form of APP that is normally secreted, termed APPsa (9, 10). In addition, both pathways generate C-terminal fragments of APP that are internalized and degraded. Cleavage by β-secretase also generates APPβ, which is similar to APPsa except that it lacks Aβ1–16. Secretion of APPsa and Aβ is tightly coupled to the activity of signal transduction systems (11). Activation of protein kinase C (PKC) or extracellularly regulated kinase (ERK) stimulates APPsa secretion and inhibits Aβ secretion (12–14).

The mechanism through which mutations in PS1 alter APP processing is poorly understood. Recent studies suggest that there is a direct connection between APP processing and PS1. PS1 may form a complex with APP in the endoplasmic reticulum (15). PS1 also appears to be required for at least one step in the processing of APP. DeStrooper et al. (16) recently showed that PS1 is required for γ-secretase activity, which is a proteolytic cleavage that generates the C terminus of the Aβ (16). Naruse et al. (17) have confirmed this observation, and Wolfe et al. (18) have shown that mutation of PS1 can interrupt this process.

Although deficits in Aβ production in cells lacking PS1 were observed by both groups (16, 17), several important questions were raised by these studies. Although APP processing is known to be regulated by protein kinase C, neither group studied the effects of loss of PS1 on the regulation of APP processing. In addition, neither group investigated whether PS2, a close homologue of PS1, could compensate for any of the defects associated with loss of PS1. Finally, DeStrooper et al. (16) observed normal basal APP secretion in the cells lacking PS1, whereas Naruse et al. (17) observed that cells lacking PS1 secrete more Aβ than wild-type cells.

We have now investigated these questions. Consistent with the published literature, we find that cells lacking PS1 secrete less Aβ. In addition, we find that cells lacking PS1 are unable to increase secretion of APP following treatment with agents that stimulate PKC or ERK. This occurs despite their ability to
activate components of the ERK cascade. We also observe that overexpression of PS2 can compensate for the lack of PS1.

**MATERIALS AND METHODS**

**Cell Lines, Antibodies, and Plasmids—**293 HEK cells were grown in high glucose Dulbecco's modified Eagle's medium plus 10% fetal bovine serum supplemented with 500 μg/ml G418, as needed. Fibroblasts were grown in Dulbecco's modified Eagle's medium plus 10% calf serum. The antibodies used for the analyses include 22C11 (0.1 μg/ml, Roche Molecular Biochemicals, against the N terminus of APP), E610 (1:1000, Senetek, against Aβ1–40), CT15 (1:5000, against the C terminus of APP, provided by Eddie Koo, University of California, San Diego), and PS1loop (1:1000, provided by Sam Sisodia, University of Chicago). The PS1 and PS2 plasmids were provided by John Hardy (Mayo Clinic, Jacksonville, FL). Alg3 was provided by Luciano D’Adamio (NIAID, National Institutes of Health). The plasmids were cloned into pcDNA3 vectors.

**Immunoblotting—**Cells were harvested with 1% SDS, 20 mM Tris, pH 7.4, 2 mM β-glycerophosphate, 1 μM 4-(2-aminoethyl)benzenesulfonyl fluoride. Protein was determined using the BCA assay (Pierce), and 30 μg per lane was run on 14% polyacrylamide gels and transferred to nitrocellulose (200 mAmp, 6 h). The nitrocellulose was incubated 1 h in 5% milk phosphate-buffered saline, washed, incubated overnight in 1° antibody, washed, and then incubated for 3 h in peroxidase-coupled 2° antibody and developed with chemiluminescent reagent (DuPont).

**Secreted APP—**Cells were plated at 10° cells/well in 35-mm dishes. The following day, the cells were washed with serum-free medium, transferred to 1 ml of Opti-MEM (Life Technologies, Inc.) without serum (as described below), and then treated with 1 μM phorbol 12-myristate 13-acetate (PMA) or 10 ng/ml epidermal growth factor (EGF) for 1 h. The medium was then collected and centrifuged and 50 μl of heparin-agarose (Bio-Rad) was added for a 1 h, 4°C incubation in order to bind the APP. The heparin-agarose was pelleted, washed four times with phosphate-buffered saline, 1% Triton X-100, mixed with 25 μl of Laemmli sample buffer, boiled for 5 min, and immunoblotted. The resulting films were quantitated by video densitometry using the NIH Image program.

**Aβ Enzyme-linked Immunosorbent Assay—**The assay was performed as described previously (19). For total Aβ measurement, we used the anti-Aβ1–40 antibody 3160 as the capture antibody and peroxidase-coupled 4G8 as the detection antibody (19). Assays specific to Aβ40 were done by Christopher Eckman as described by Suzuki et al. (19).

**Transfections—**DNA was transfected into freshly plated cells using LipofectAMINE Plus (Life Technologies, Inc.). Transfections were performed in Opti-MEM (Life Technologies, Inc.) using a ratio of 2 μg of DNA:6 μl of LipofectAMINE:9 μl of Plus reagent per 1 ml of Opti-MEM. For detection of Elk, the Elk Pathdetect system (Stratagene) was used. Luciferase activity was assayed and quantitated using a Turner luminometer.

**Metabolic Labeling—**35-mm dishes containing 1 million cells each were preincubated in methionine-free, serum-free medium for 30 min. The cells were then incubated with 250 μCi of [35S]methionine in fresh methionine-free, serum-free medium for 30 min and then washed and incubated in normal growth medium. At the indicated times, the cells were lysed in lysis buffer (phosphate-buffered saline, pH 7.4, 1% Nonidet P-40, protease inhibitors), and the lysates were cleared by centrifugation. The lysates were then incubated with CT15 (1:500) for 1 h, washed three times in lysis buffer, and incubated with protein A (25 μl, 1 h). The conjugates were then centrifuged, washed three times in lysis buffer, electrophoresed on 12% polyacrylamide gels, dried down, and then exposed to film.

**RESULTS**

**APP Secretion Is Reduced in Fibroblasts Lacking PS1—**SV40-transformed fibroblasts were generated from E14 PS1 knockout mice (20, 21). Two types of cell lines were used, a line hemizygous (+/-) for PS1 and a line nullizygous (-/-) for PS1. The absence of PS1 in the nullizygous mice was assessed by polymerase chain reaction (not shown) and confirmed by immunoblotting with an antibody against PS1 using anti-PS1loop (Fig. 1A).

Next, we took the PS1 +/- and PS1 -/- lines and examined the secretion of APP under basal conditions and after treatment with two agents known to stimulate APP secretion by selectively activating α secretase, PMA and EGF (22, 23). The fibroblast cell lines were preincubated in serum-free medium, transferred to fresh serum-free medium, and treated with 1 μM PMA or 10 ng/ml EGF for 1 h. The secreted APP was precipitated and immunoblotted with 22C11, which is an antibody capable of detecting murine APP, as well as the human APPα, APPβ, and amyloid precursor-like proteins. The PS1 +/- cells showed a 50 ± 4% (p < 0.05) increase in APP secretion following stimulation with PMA, whereas the PS1 -/- cells showed no increase (6 ± 14%) in secreted APP (Fig. 1, B and C). Following treatment with EGF, the PS1 +/- cells also showed a robust increase in APP secretion, whereas the PS1 -/- cells showed no increase and instead showed a reduction in APP secretion (Fig. 1D), suggesting an impairment in the stimulation of APP secretion by EGF and PMA. To determine whether APP secretion was also impaired in nontransformed cells lacking PS1, we examined the APP secretion in primary embryonic fibroblasts generated from E14 mice that were homozygous (+/+), heterozygous (+/-), or nullizygous (-/-) for PS1. The primary embryonic fibroblasts were then plated at a density of 250,000 cells per 35-mm dish, and the secretion of APP was examined under basal conditions or following treatment with 1 h with 1 μM PMA or 10 ng/ml EGF. The results paralleled those seen with the transformed cell lines. PMA and EGF were able to increase APP secretion in primary embryonic fibroblasts expressing PS1 but were unable to increase APP secretion in primary embryonic fibroblasts lacking PS1 (Fig. 2, A and B). The basal level of APP secretion was similar for all cells. Thus, both primary cells and transformed lines lacking PS1 are unable to increase APP secretion.
following stimulation with agents that activate PKC or ERK.

To determine whether loss of PS1 specifically inhibited APPs secretion, we transiently transfected the PS1+/– and PS1−/− lines with varying amounts of a human APP expression construct driven by a cytomegalovirus promoter. This allowed use of the antibody 6E10, which recognizes the Αβ1–16 sequence that is specific to human APP and is present in APPs but absent from APPβs and amyloid precursor-like proteins. Two days later, we incubated the cells with fresh medium for 1 h and precipitated and immunoblotted the secreted human APP. Parallel transfections with a constitutively active pGL3 luciferase reporter showed equal transfection efficiencies between the two cell lines. The PS1+/– fibroblasts transfected with human APP showed dose-dependent increases in secreted human APP. The PS1−/− line showed a lower level of APP secretion at each dose and reached a plateau at a total level of secreted human APP that was half that of the PS1+/– line, as determined by densitometry (Fig. 2C). These results suggest that fibroblasts lacking PS1 have impaired secretion of APPs.

APP secretion has been shown to be regulated by the ERK cascade (22). Absence of ERK signaling could also explain the inability of PS1−/− cells to increase APP secretion. Hence, we examined EGF signaling in the PS1−/− and PS1+/– cell lines in order to determine whether the ERK signaling cascade was functioning. To examine this question, we tested the ability of EGF to activate Elk, a member of the MAP kinase cascade. The PS1+/– and PS1−/− cell lines were transfected with plasmids containing luciferase driven by a Gal4 upstream activating site and a chimeric Elk1/Gal4 construct. The following day, the cells were treated with 10 ng/ml EGF for 24 h, and then luciferase activity was examined. EGF increased luciferase activity 4–6-fold above basal levels in both PS1+/– and PS1−/– lines (Fig. 2D). These results indicate that EGF is capable of activating Elk in the PS1−/– cells. In contrast, EGF does not stimulate APP secretion in PS1−/– cells (Fig. 1D). These results indicate that loss of ERK/Elk signaling does not account for the inability of EGF to stimulate APP secretion in PS1−/– cells.

Transfection with PS1 Rescues the PS1−/− Phenotype—To verify that the deficits in APP secretion from the PS1−/– cells resulted specifically from lack of PS1, we examined whether transfecting in PS1 restores normal secretion of APP. PS1−/− cells were transfected with the human APP expression vector (1 μg) and either wild-type PS1 (1 μg) or a control plasmid (β-galactosidase, 1 μg). Two days after transfection, the cells were preincubated in serum-free medium for 2 h and then placed in fresh serum-free medium +/- 1 μM PMA for 1 h. The APP was precipitated, and human APP was immunoblotted with the 6E10, which recognizes human APP and therefore identifies only APP secreted from the transfected cells. PS1−/– cells transfected with PS1 increased APP secretion during stimulation with PMA (Fig. 3A, lane 2); in contrast, the PS1−/– control cells showed no change in APP secretion in response to PMA treatment (Fig. 3A, lane 4). Because each point was analyzed in triplicate, we also quantitated the results by densitometry in Fig. 3B. The ability of PS1 to restore normal patterns of APP secretion indicates that the defect in APP secretion seen in the PS1−/– cells results specifically from the lack of PS1 expression.

To determine whether the effect of the rescue was directly related to the presence or absence of PS1, rather than a result of overexpression of both PS1 and APP, we determined whether transfecting with PS1 rescues endogenous APP secretion. PS1−/– cells were transfected with either wild-type PS1 (2 μg) or a control plasmid (β-galactosidase, 2 μg). Two days after transfection, the cells were preincubated in serum-free medium for 2 h and then placed in fresh serum-free medium with or without 1 μM PMA for 1 h. The APP was immunoprecipitated and immunoblotted using 22C11, which detects the native mouse APP secretion. Transfecting PS1−/– cells with human PS1 restored their ability to increase APP secretion after stimulation by PMA (Fig. 3C, lanes 3 and 4). The ability of transfected PS1 to restore normal secretion of endogenous APP confirms that this is a specific function of PS1.

Cells Lacking PS1 Have Higher Levels of Cellular APP Cleavage Products—To determine whether APP secretion was reduced because of less cleavage of cellular APP or less secretion of APP, we immunoblotted cell lysates with antibodies to the N and C termini of APP separately. The antibody 22C11 binds to the N terminus of APP and therefore recognizes both holo-APP and APPs (the APP C-terminal fragment migrates at 16 kDa during electrophoresis). In contrast, the antibody CT15 binds to the C terminus of APP and recognizes holo-APP and the APP C-terminal fragment, but not APPs (the APP C-terminal fragment migrates at about 16 kDa during electrophoresis). To control for loading, we reprobed the immunoblots with anti-actin antibody. Immunoblots with 22C11 showed 26% more APP/APPs reactivity in the lysates from PS1−/– cells than in the lysates from PS1+/– cells after normalizing for loading by quantitating staining with the anti-actin antibody (Fig. 4, A, upper panel, and C). To investigate whether the increased reactivity in the PS1−/– cells resulted from increased levels of holo-APP or increased levels of APPs, we probed immunoblots of cellular lysates with antibody CT15, which does not detect APPs. Unlike the results

Fig. 2. A, immunoblot using antibody 22C11 of secreted APP from primary cultures of PS1+/+, +/−, and −/− murine fibroblasts following stimulation with 10 ng/ml EGF for 1 h. EGF did not increase secretion of APP in the −/−/B, quantitation of the absorbance of the APPs reactivity from the PS1+/+, +/−, and −/− cells shows that EGF stimulates APP secretion only in cells from PS1 expressing animals. *, p < 0.05; n = 3 for each point. C, immunoblot using the antibody 6E10 to detect secreted APPs from PS1+/+, +/−, and −/− cell lines that had been transfected with varying amounts of a human APP expression vector. Secretion of APP was reduced in the −/− cell line. D, measurement of ERK/Elk activity using luciferase constructs in PS1+/+, +/−, and −/− cell lines following stimulation with 10 ng/ml EGF for 24 h. *, p < 0.0001.
Regulation of APP Processing by PS1 and PS2

Are Also Sensitive to the Amount PS1 Expression—The increased levels of APP C-terminal fragments seen in PS1 −/− cells indicates that loss of PS1 increases the levels of APP C-terminal fragments. To determine whether increasing expression of PS1 decreases levels of APP C-terminal fragments we immunoblotted cell lysates from 293 HEK cells stably transfected with wild-type PS1, FAD1 PS1, or control. Immunoblots with an antibody against PS1, PS1

(Fig. 6A). Immunoblots with the antibody CT15 showed that the levels of APP C-terminal fragments correlates with the levels of PS1. Cells stably transfected with wild-type PS1 showed lower levels of APP C-terminal fragments than control cells (Fig. 6, B and C). Cells stably transfected with FAD1 PS1 also showed lower levels of APP C-terminal fragments than control cells (data not shown). The correlation between APP C-terminal fragment levels and PS1 levels in 293 HEK cells shows that APP processing is affected by either increases or decreases in PS1 expression.

Aβ Secretion Is Reduced in Cells Lacking PS1—The reduction in γ-secretase cleavage of APP seen in cells lacking PS1 raises the possibility that Aβ secretion is reduced in cells that lack PS1. To examine Aβ secretion, we transfected PS1 −/− and PS1 +/− cells with an expression vector coding for human APP, immunoblotted the cell lysates with the human specific anti-APP antibody 6E10 to determine the level of expression of human APP in the cells, and then measured Aβ secreted into the medium from the cells by enzyme-linked immunosorbent assay. We were able to express human APP in both the PS1 +/− and −/− cells (Fig. 7A). Enzyme-linked immunosorbent assay measurements of Aβ secretion from the cells over a 24-h period showed that the cells expressing PS1 produced much more Aβ than the cells lacking PS1, even after normalizing for human APP expression (Fig. 7B). Thus, loss of PS1 reduces Aβ secretion.

Transfection with PS2 Rescues the PS1 −/− Phenotype—PS2 is a close homologue of PS1 and has been shown to bind APP, like PS1 (24). Whether PS2 also regulates APP processing is unknown. Cells lacking PS1 might be a useful system for investigating the role of PS2 because the absence of PS1 eliminates the possibility of PS1 activity masking potential actions of PS2. To investigate this issue, we examined the effects of increasing PS2 expression in the PS1 −/− cells on APP C-terminal fragments and secreted APP. PS1 −/− cells were transfected with human APP (1 μg/ml) and either 1 μg/ml of wild-type PS1 (lanes 1 and 2) or 2 μg/ml of wild-type PS1 (lanes 3 and 4). Two days after transfection, the cells were preincubated in serum-free medium for 2 h and then transferred to fresh serum-free medium and incubated with or without 1 μM PMA for 1 h. The secreted APP was isolated and immunoblotted with antibody 22C11. All transfections were done in triplicate, and representative lanes are shown.

seen with antibody 22C11, immunoblots with antibody CT15 did not show increased holo-APP activity in the lysates from PS1 −/− cells compared with the lysates from PS1 +/− cells (Fig. 4, B, upper panel, and C). The unchanged (or slightly reduced) holo-APP reactivity seen using the CT15 antibody contrasts with the increased reactivity seen using the 22C11 antibody and suggests that in PS1 −/− cells some of the holo-APP is cleaved, to yield APPs, but not secreted. The cleaved cellular APPs would not be detected by CT15, but the cleaved APP would be detected by 22C11, which also accounts for the increased reactivity seen in PS1 −/− cells with 22C11. Consistent with increased cleavage of APP in the PS1 −/− cells, we also observed that the amount of APP C-terminal fragments were higher in the PS1 −/− cell lysates (Fig. 4B, lower panel). The increased levels of cellular APPs and APP C suggest that cleavage of APP is increased in PS1 −/− cells, but that the APPs is not secreted.

Next, we metabolically labeled the PS1 −/− and PS1 +/− cells to compare the turnover times for the APP and C-terminal fragments. PS1 −/− and +/− cells (10⁶ cells/dish) were labeled with [35S]methionine for 30 min and then incubated for 0, 30, or 60 min in normal serum-free medium not containing radiolabeling activity, and CT15 was used for the immunoprecipitation. No difference was seen between the disappearance of holo-APP in the PS1 −/− cells versus the PS1 +/− cells (Fig. 5A); however, APP C-terminal fragments were more abundant at all time points in the PS1 −/− lysates compared with PS1 +/− lysates (Fig. 5B). The persistence of APP C-terminal fragments in the PS1 −/− cells suggests that loss of PS1 impairs the catabolism of these fragments.

The Levels of APP C-terminal Fragments in 293 HEK Cells

FIG. 3. Transfection of PS1 −/− cells with PS1 rescues APP secretion. A, PS1 −/− cells were transfected with a human APP expression vector (1 μg/ml) and either 1 μg/ml of wild-type PS1 (lanes 1 and 2) or 1 μg/ml of control plasmid (β-galactosidase) (lanes 3 and 4). Two days after transfection, the cells were preincubated in serum-free medium for 2 h, transferred to fresh serum-free medium, and incubated +/− 1 μM PMA for 1 h. The secreted APP was isolated and immunoblotted with antibody 6E10. All transfections were done in triplicate, and representative lanes are shown.
Terminal fragments were more abundant at all time points in the PS1 lysates from PS1 antibody to control for loading. The PS1 nonsblots were reprobed with anti-actin antibody 22C11, and then the immunoblot was then quantitated by video densitometry after subtracting the background for each lane. No difference was seen between the PS1 lane was then quantitated by video densitometry after subtracting the corresponding anti-actin band. **, $p < 0.003$; *, $p < 0.05$; $n = 3$ per group.

FIG. 4. Analysis of cellular APP expression in PS1 −/− and +/+ cells. A, lysates from PS1 −/− and +/+ were immunoblotted with the anti-APP N-terminal antibody 22C11, and then the immunoblots were reprobed with anti-actin antibody to control for loading. The PS1 −/− cells showed more 22C11 reactivity than the PS1 +/+ cells. B, the same lysates were also with an APP C-terminal antibody CT15, and then the immuno- blots were reprobed with anti-actin antibody. The APP C-terminal fragments (arrow, CTF) showed more reactivity in PS1 −/− cells than in PS1 +/+ cells, but the holo-APP bands (arrow, holo-APP) showed no increase in reactivity in the PS1 −/− cells. C, quantitation of the density of the bands. The density of each anti-APP band was determined and then normalized to the relative density of the corresponding anti-actin band. **, $p < 0.003$; *, $p < 0.05$; $n = 3$ per group.

FIG. 5. Metabolic labeling of PS1 −/− and +/+ cells with [35S]methionine. PS1 −/− or +/+ cells were pulsed with [35S]methionine for 30 min, and APP was immunoprecipitated with CT15. Each lane was then quantitated by video densitometry after subtracting the background for each lane. No difference was seen between the PS1 +/+ and −/− cell lines for the metabolism of holo-APP (A), whereas C-terminal fragments were more abundant at all time points in the PS1 −/− cells (B).

good system for investigating the action of Alg3 because the cells are unable to use PS1 to compensate for any potential inhibition of PS2 activity. To investigate this question we transfected PS1 −/− cells with Alg3 (2 μg) or control (β-galactosidase, 2 μg) and examined the expression of APP C-terminal fragments. Cells transfected with Alg3 showed increased levels of APP C-terminal fragments compared with control cells (Fig. 8C). This increase in APP C-terminal fragments contrasts with the decrease in APP C-terminal fragments seen after transfection with PS2. Thus, Alg3 and PS2 have opposing actions on APP C-terminal fragments. We also examined the effects of Alg-3 on secreted APP and found that Alg3 did not restore PMA responsiveness, unlike PS2 (data not shown). These data suggest that Alg3 interferes with the processing of APP by endogenous PS2. The ability of Alg3 to interfere with APP processing and with apoptosis suggests that it is a general inhibitor of PS2 function.

DISCUSSION

PS1 was recently shown to be necessary for γ-secretase activity; however, its role in other aspects of APP processing is unclear (16). We show that PS1 is also required for the coupling of APP processing to signal transduction cascades and for an inhibitory regulation of cleavage of APP at the α-cleavage site. These roles of PS1 were demonstrated by evaluating cells deficient in PS1, cells with normal quantities of PS1, and cells overexpressing PS1. Our data show that the effects due to loss of PS1 extend beyond reduction of the γ-secretase cleavage. Cells lacking PS1 exhibit more cleavage of APP at α or β secretes sites under basal conditions, increased amounts of APP C-terminal fragments, increased amounts of intracellular APPs, and impaired coupling of APP processing to signal transduction cascades. These changes in APP processing occur in both clonal, SV40 transformed PS1 −/− cell lines and in primary cultures of PS1 −/− fibroblasts, which shows that the changes in APP processing are not due to the transformation process. Increasing PS1 expression produces the opposite effect of loss of PS1 and reduces APP C-terminal fragments both in PS1 −/− cells and in 293 HEK cells. Results from other studies have shown that increasing PS1 expression increases APP secretion (28). These reciprocal changes in APP processing that
are associated with increasing or decreasing PS1 levels points to a tight linkage between PS1 expression and APP processing. DeStrooper et al. (16) have shown that loss of PS1 leads to reduced cleavage of APP at the γ-secretase site. Our results and those of others confirm this observation (17, 29). Aβ secretion is reduced in PS1 −/− cells, which supports the observation that lack of PS1 reduces cleavage of APP by γ-secretase. Despite reduced Aβ secretion, the total amount of APP cleavage products is also increased in PS1 −/− cells, including increases in the levels of intracellular APPs and APP C-terminal fragments. The increase in APP C-terminal fragment and decrease in Aβ secretion that we have observed also been observed in neurons lacking PS1 and might be a general feature of cells lacking PS1 (16, 17, 29). We observed that fibroblasts lacking PS1 produce more APPs, which is in agreement with the observations of Naruse et al. (17). Interestingly, in the fibroblasts in our study, the APPs are not secreted, whereas neurons lacking PS1 do secrete the extra APPs that they produce (17). The increased levels of APP cleavage products in PS1 −/− cells suggests that PS1 regulates aspects of APP processing beyond the γ-secretase cleavage. An important line of future studies will be to examine the regulation of APPs in primary neurons.

A requirement for PS1 in the coupling of APP processing to signal transduction pathways has not previously been shown. Cells lacking PS1 show deficits in the coupling of APP processing to signal transduction. The PS1 −/− cells neither increase secretion of APP nor generate more APP C-terminal fragments after stimulating PKC and extracellular regulated kinase by treatment with PMA or EGF, respectively. The lack of responsiveness of APP processing to PMA or EGF could result from a reduced ability of the cell to cleave APP. However, transfecting APP into PS1 −/− cells does increase APP secretion, which indicates that PS1 −/− cells do have the capacity to increase APP cleavage and secretion, despite not responding to PMA or EGF. The lack of responsiveness of APP processing to PMA or EGF also cannot be explained by poor receptor or signal transduction signaling, because signaling from the EGF receptor through the extracellular regulated kinase cascade to Elk gene transcription remains intact in the cells lacking PS1. Given that the PS1 −/− cells are able to increase APP secretion after transfection of PS1 and that EGF signaling remains strong in the PS1 knockout cells, the unresponsiveness of APP processing in PS1 −/− appears to result from deficits in the coupling of APP processing to signal transduction. Thus, PS1 appears to be required for normal cleavage and secretion of APP, including coupling signal transduction cascades.

Cells expressing mutant PS1 associated with familial AD also are unable to increase APP secretion after stimulation with PMA. However, this phenotype appears to differ from the phenotype associated with loss of PS1. Unlike PS1 −/− cells, cells expressing mutant PS1 have no changes in levels of APP C-terminal fragments or basal secretion of APP. The normal levels of APP cleavage products seen in cells expressing mutant PS1 constructs suggest that the familial Alzheimer mutations in PS1 do not cause a full loss of PS1 function. Other studies suggest the same conclusion. For instance, mutant PS1 constructs are able to rescue the developmental lethality seen in PS1 knockout mice (although they are unable to correct cytoskeletal defects) (30). PS1 mutant constructs are also able to partially rescue the egg laying deficits seen in Sel12 mutants in the nematode (31, 32). Thus, multiple lines of evidence indicate that the mutations in PS1 that cause familial AD do not cause a full loss of function phenotype.

The function of PS2 is also unknown. PS2 has been shown to bind APP, and several studies suggest that PS2 regulates apoptosis (24) (26, 27). A surprising outcome of our investigation is the observation that PS2 can compensate for the loss of PS1 in the PS1 −/− cells. Overexpressing PS2 in PS1 −/− cells increases secretion of APP, reduces basal production of APP C-terminal fragments, and restores appropriate coupling of APP processing to PKC. These experiments indicate that overexpressed PS2 has enough functional similarity to PS1 to restore the loss of APP processing in cells lacking PS1. One other study has examined the effects of PS2 on APP processing in cells expressing PS1; however, the effects of PS2 on these cells was not as strong as the effects that we observed (33). That study (33) examined the actions of the PS2 C-terminal fragment, Alg-3 but failed to observe any effects of Alg3 on APP processing. In contrast, we observed that transfecting Alg3 into PS1 −/− cells increases levels of APP C-terminal fragments. This increase suggests that Alg3 is inhibiting endogenous PS2 function, which is consistent with its effects on apoptosis (25, 27, 34). The ability of PS2 and Alg3 to alter APP processing in PS1 −/− cells suggests that PS2 is capable of functioning like PS1 in regulating APP cleavage. The smaller effects of PS2 and Alg3 on APP cleavage in cells expressing PS1 suggests that PS1 might mask some of the actions of PS2. As with many other redundant biological systems, PS2 might act as a backup for PS1.

Our data add to an increasing number of studies showing that PS1 and PS2 regulate APP processing. There are several possible mechanisms that are consistent with a broader role in APP processing. PS1 and PS2 might directly regulate the activity of other secretases, such as α and γ secretase. Another possibility is that presenilins do not directly regulate the APP secretases but regulate the compartmentalization of APP or its secretases. In this latter case, loss of PS1 could lead to a different distribution of APP within the microenvironment of the endoplasmic reticulum, which would alter its availability to the proteins necessary for the catabolism of APP, APPs, and APP C-terminal fragments. Our data also suggest that PS1 and PS2 are sensitive to the activity of PKC and ERK that PS1 is necessary to allow APP processing to respond to changes in the activity of PKC and ERK. The signal transduction coupling imparted by presenilins could result either from control of the
compartimentalization of APP, control of the interaction of APP with secretases, or control of the activity of secretases. The observation that PS1 and PS2 have a role in the signal transduction coupling of APP processing adds to the increasing number of reports indicating that presenilins interact with signal transduction proteins quite extensively and are themselves substrates of kinases (35–38). The potential physiological effects of such interactions have generally been unclear, but the requirement of PS1 for coupling of APP processing to signal transduction suggests that this interaction of PS1 with signal transduction enzymes might serve to regulate APP processing.

The importance of PS1 and PS2 in regulating APP processing and the ability of loss of PS1 or transfecting Alg3 to inhibit APP processing suggest that inhibiting PS1 or PS2 in normal cells might reduce Alzheimer's disease (AD) pathology. In fact, it appears that the presenilins participate in the developmental lethality associated with lack of PS1, and PS1 inhibition on adult physiology is not known. APP does not require for normal processing of NOTCH, glycosylation of the Trk B receptor is altered in cells lacking PS1, and there is a possibility that apoptosis might also be reduced (17, 41–45). Trk B receptor is altered in cells lacking PS1, and there is a possibility that apoptosis might also be reduced (17, 41–45).

References

1. Rogge, E. I., Sherrington, R., Roggeva, E. A., Tevesque, G., Iedea, M., Liang, Y., Chi, H., Lin, C., Holman, K., Tsuda, T., Mar, L., Sorbi, S., Nacmias, B., Piacentini, S., Amaducci, L., Chumakov, I., Cohen, D., Lannfelt, L., Fraser, P. E., Rommens, J. M., and St. George-Hyslop, P. H. (1995) Nature 376, 775–778.
2. Levy-Lahad, E., Wasco, W., Poskajk, P., Romano, D. M., Oshima, J., Pettingell, G. W., Yu, C. E., Jondro, R. P., Schmidt, S. D., Wang, K., Crowley, A. C., Fu, Y.-H., Guenette, S. Y., Galas, D., Nemenu, E. Wijman, E. M., Bird, T. D., Schellenberg, G. D., and Tani, R. E. (1995) Science 269, 973–977.
3. Clark, R. Hutton, M. Fuldner, R., Froelich, S., Karran, E., Talbot, C., Crook, T. D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Vitaiten, M., Poskajk, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., and Younkin, S. (1996) Nature 384, 867–870.
4. Citron, M., Westaway, D., Xia, W., Carleson, G., Diehl, T., Tevesque, G., Johnson-Wood, K., Lee, M., Seubert, P., Davis, A., Khodolenko, D., Motter, R., Sherrington, R., Perry, B., Yao, H., Strome, R., Lieberburg, I., Rommens, J., Kim, S., Schenk, D., Fraser, P., St. George-Hyslop, P., and Selkoe, D. J. (1997) Nat. Med. 3, 67–72.
5. Jarrett, J., Berger, E., and Lansbury, P. (1993) Biochemistry 32, 4693–4697.
6. Selkoe, D. J. (1996) Cold Spring Harbor Symp. Quant. Biol. 61, 587–596.
7. Wolfe, M. P., Hickman, L. J., Eckman, C. B., Ujno, S. N., Wang, B., and Golde, T. E. (1999) J. Biol. Chem. 274, 11914–11923.
8. Esch, F., Keim, P. S., Seibert, E. C., Blacher, R. W., Culwell, A. R., Oltersdorff, T., Mcguire, D., and Ward, P. J. (1990) Science 248, 1129–1124.
9. Singleton, S. S., Koo, E. H., and Beverskotted, K. U., Vocêbe, A., Rpques, P., Cline, R., Philips, C., Venter, J., Forsell, L., Axelsen, K., Lantos, P., Lannfelt, L., Rosser, M., Roberts, G., Adams, M., Hardy, J., and Goate, A. (1995) Nature Genetics 11, 219–222.
10. Sisodia, S. S., Koo, E. H., and Beverskotted, K. U., Vocêbe, A., Rpques, P., Cline, R., Philips, C., Venter, J., Forsell, L., Axelsen, K., Lantos, P., Lannfelt, L., Rosser, M., Roberts, G., Adams, M., Hardy, J., and Goate, A. (1995) Nature Genetics 11, 219–222.
11. Buxbaum, J. D., and Greengard, P. (1996) Ann. N. Y. Acad. Sci. 777, 327–331.
12. Nitsch, R., Black, B., Wurtman, R., and Growdon, J. (1992) Science 256, 304–307.
13. Buxbaum, J. D., Gandy, S. E., Cicotti, P., Ehrlich, M. E., Czernik, A. J., Fraccaso, R. P., Ramahadran, T. V., Unbecket, A. J., and Greengard, P. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 6003–6006.
14. Black, E. N., Nitsch, R. M., Linweve, E., Kunz, G. M., Jr., Breu, J., Eldar, H., and Wurtman, R. J. (1993) J. Biol. Chem. 268, 21097–21101.
15. Xia, W., Zhang, J., Perez, R., Koo, E. H., and Selkoe, D. J. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 8206–8213.
16. De Strouwer, B., Safigt, P., Craesselaerts, K., Vanderstichele, H., Guilde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1997) Nature 391, 387–390.
17. Naruse, S., Shinakarakan, G., Luo, J., Kuiskai, J., Tomita, T., Iwatsubo, T., Qian, X., Ginty, D., Price, D., Borchelt, D., Wong, P., and Sisodia, S. (1998) Neuron 21, 1215–1221.
18. Wolfe, M., Xia, W., Ostaszewski, B., Diehl, T., Kimberly, W., and Selkoe, D. (1999) Nature 398, 513–517.
19. Suzuki, N., Cheung, T., Cai, A., Okada, A., Otros, E., Eckman, C., Golde, T., and Younkin, S. (1994) Science 266, 1338–1340.
20. Berekich, B. E., Thirakaran, G., Wong, P. C., Sisodia, S. S., and Nye, J. S. (1999) Curr. Biol. 9, 1493–1498.
21. Wong, P., Zheng, H., Chen, H., Becher, M., Sirinathsinghi, D., Trumbauer, M., Chen, H., Price, D., Van der Ploeg, L., and Sisodia, S. (1997) Nature 387, 288–292.
22. Mats, I., Charest, D., Lam, F., Beverskotted, K. U., Nacmias, B., and Reiner, P. (1997) J. Neurosci. 17, 9451–9452.
23. Slack, B. E., Breu, J., Muchnicki, L., and Wurtman, R. J. (1997) Biochem. J. 327, 245–249.
24. Weidemann, A., Paliga, K., Durrwang, U., Czech, C., Ashen, G., Masters, C. L., and Younkin, S. (1994) Science 264, 1215–1221.
25. Vito, G., Goates, A., Ruppel, B., and Beverskotted, K. U., Vocêbe, A., Rpques, P., Cline, R., Philips, C., Venter, J., Forsell, L., Axelsen, K., Lantos, P., Lannfelt, L., Rosser, M., Roberts, G., Adams, M., Hardy, J., and Goate, A. (1995) Nature Genetics 11, 219–222.
26. Schenker, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T. D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Vitaiten, M., Poskajk, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., and Younkin, S. (1996) Nature Med. 2, 864–870.
27. Infection of PS1 → cells with Alg3, the cells were incubated in serum-free medium for 2 h, and lysates of the cells were then immunoblotted with CT15. Transfection of PS1 → cells with Alg33 increases levels of APP C-terminal fragments (CTF, lower arrow) but does not change levels of holo-APP (APP, upper arrow).
Regulation of APP Processing by PS1 and PS2