Cell Surface Presenilin-1 Participates in the γ-Secretase-like Proteolysis of Notch*

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Presenilin-1 (PS1), a polytopic membrane protein primarily localized to the endoplasmic reticulum, is required for efficient proteolysis of both Notch and β-amyloid precursor protein (APP) within their transmembrane domains. The activity that cleaves APP (called γ-secretase) has properties of an aspartyl protease, and mutation of either of the two aspartate residues located in adjacent transmembrane domains of PS1 inhibits γ-secretase processing of APP. We show here that these aspartates are required for Notch processing, since mutation of these residues prevents PS1 from inducing the γ-secretase-like proteolysis of a Notch1 derivative. Thus PS1 might function in Notch cleavage as an aspartyl protease or di-aspartyl protease cofactor.

Notch is a single transmembrane domain cell surface receptor that mediates many cell fate decisions in both vertebrates and invertebrates (1–3). Notch is synthesized as a large precursor (full-length Notch, FLN)† that is cleaved by furin in the trans-Golgi network lumen to generate two fragments that remain associated and form a heterodimeric receptor at the cell surface (4, 5). After receptor binding to a member of the DSL family of ligands on the surface of neighboring cells, the C-terminal, transmembrane-intracellular fragment of Notch (TMIC-N) undergoes further proteolysis by an unidentified protease within its single TM domain (1, 6–8). This ligand-activated cleavage releases the Notch intracellular domain (NICD) from the membrane, allowing it to translocate to the nucleus, where it interacts with members of the CSL family of proteins (1–3). Through these interactions Notch affects the transcriptional activity of target genes and thereby influences cell fate choice.

Genetic analyses revealed that the presenilin (PS) family of proteins interacts with the Notch signaling pathway (9–12). PS are widely expressed polytopic membrane proteins that undergo endoproteolytic processing within their large cytoplasmic loop and accumulate in the ER/Golgi as stable heterodimers (13–16). In humans there are two PS genes, PS1 and PS2, both of which can harbor dominant missense mutations that cause AD (13–16). These mutations all lead to altered proteolysis of the β-amyloid precursor protein (APP), the precursor to Aβ, a 40–43-amino acid peptide deposited in senile plaques in the brains of AD patients (13–16). AD-causing mutations in PS1 and PS2 alter the site preference of γ-secretase, an unidentified activity that cleaves within the single TM domain of APP, leading to increased levels of the longer, more amyloidogenic forms of Aβ (13–16).

Deletion of the PS1 gene specifically reduces the proteolysis of a truncated form of Notch1, mNotch1ΔEm/v (abbreviated ΔE), which undergoes proteolysis constitutively in the absence of ligand (17). Furthermore, ablation of the single Presenilin gene in Drosophila eliminates most or all of the release of NICD from membrane-bound Notch receptor (18, 19). The γ-secretase cleavage of APP is also reduced in PS1–/– neurons (20), and peptidomimetic γ-secretase inhibitors based on the APP cleavage site inhibit both Notch and APP processing with similar efficacy indicating that related enzymes are involved (17). Both Notch and APP form stable complexes with PS1 in transfected mammalian cells, suggesting that PS1 acts directly on the substrates (21, 22). Presenilins have two conserved aspartates residues within adjacent TM domains (Fig. 5A). Exogenous expression of PS1 (23) or PS2 (40) where either aspartate has been mutated dominantly suppresses γ-secretase cleavage of APP in transfected cells. Since γ-secretase has properties of an aspartyl protease (24), it was hypothesized that these aspartyl residues in PS1 form a novel intramembranous protease active site (23). However, Notch cleavage is initiated at the cell surface in response to extracellular ligand binding (1), and Aβ is generated in multiple subcellular compartments in both the secretory and endocytic pathways, whereas PS1 and PS2 are widely reported to be restricted to the ER and Golgi membranes (13–16). These observations are more consistent with PS1 having an indirect role in Notch and...
APP metabolism, as suggested by studies reporting that the Caenorhabditis elegans Notch homologue LIN-12 had altered subcellular distribution in sel-12 (a PS homologue) mutant animals (25), and that PS1-deficient neurons secrete APP at an abnormal rate (26).

To address this discrepancy, we analyzed the effects of mutation of the TM aspartates in PS1 on Notch processing. We report here that these aspartates are required for PS1 to induce ΔE cleavage in PS1-deficient cells. Furthermore, we provide evidence that PS1 forms a complex with ΔE in the ER and that this complex is transported to the cell surface. The TM aspartates in PS1 appear to be specifically involved with ΔE proteolysis and do not affect Notch or PS1 subcellular distribution.

**EXPERIMENTAL PROCEDURES**

**Cell Culture and Transfection**—Human embryonic kidney 293 (HEK 293), mouse NIH 3T3 fibroblasts, and stably transfected Chinese hamster ovary cell lines (23) expressing wild-type or mutant PS1 were maintained as described previously (22). Mouse embryonic fibroblasts were derived from PS1−/− or wild-type mice and immortalized with SV40 large T antigen (17). Rat post-natal day 0–3 primary hippocampal cultures were grown in 5% fetal bovine serum and 5% horse serum for 1 week. Human embryonic kidney (HEK) 293 cells were transduced with an adenovirus encoding SV40-immortalized embryonic fibroblasts derived from wild-type (+/+) or PS1 knockout mice (−/−) and harvested at codon 257 (D257A PS1) was expressed, NICD levels were significantly reduced (Fig. 1A) or PS1 knockout mice (−/−) were transfected and analyzed either by anti-myc Western blot (top panel, 7.5% SDS-PAGE), or immunoprecipitated with polyclonal CTF-PS1 antibody followed by Western blot with antibody 13A11 (bottom panel, 10–20% SDS-PAGE). D, quantitation of the above data was performed as in B, averaged from three independent experiments.

**RESULTS**

**Mutation of the Transmembrane Aspartate Residues in PS1 Dominantly Inhibits Notch Processing**—Previous work suggested that presenilins have a similar function in both Notch and APP metabolism (17, 20–22). To test this hypothesis, we examined whether the TM aspartates in PS1 involved in the γ-secretase cleavage of APP (23) also function in Notch processing. Human embryonic kidney (HEK) 293 cells were transiently transfected with mNotch ΔEm/γ (abbreviated ΔE), a truncation of mouse Notch1 that undergoes PS1-regulated TM proteolysis constitutively in the absence of ligand (1, 29). ΔE containing the TM domain, and part of the cytoplasmic tail of Notch1 and has six consecutive myc epitope tags at its C terminus to facilitate detection. Notch processing was detected by the appearance of the Notch intracellular domain (NICD), the C-terminal product of γ-secretase-like cleavage. Transient overexpression of wild-type PS1 had no effect on NICD levels compared with cells expressing endogenous PS1 (Fig. 1A). However when PS1 encoding an aspartate-to-alanine mutation at codon 257 (D257A PS1) was expressed, NICD levels were significantly reduced (Fig. 1B). Similar results were obtained when ΔE was transiently transfected into the stable Chinese hamster ovary cell lines used to document the effect of this
mutation on APP processing (23) (data not shown). Analysis of PS1 expression in these cells by immunoprecipitation followed by Western blot (IP-Western) revealed that overexpression of D257A PS1 had no effect on the accumulation of the N-terminal and C-terminal PS1 endoproteolytic cleavage fragments, which are derived from endogenous PS1 since D257A PS1 does not undergo endoproteolysis (23). Thus, D257A PS1 suppresses \( \Delta E \) processing without altering the levels of endogenous PS1 cleavage fragments.

Mutation of the Transmembrane Aspartates in PS1 Abolishes Its Ability to Induce Notch Cleavage—To determine if the D257A PS1 mutation abolishes PS1 activity, we performed rescue experiments in cells lacking PS1, where \( \Delta E \) cleavage is greatly reduced (17). Co-transfection of wild-type, but not D257A PS1, in PS1 \(-/-\) fibroblasts rescued \( \Delta E \) cleavage (Fig. 1, C and D). PS1 mutated at the other TM aspartate (D385A) also failed to rescue \( \Delta E \) cleavage in PS1 \(-/-\) cells (data not shown). Although the PS1 plasmid led to overexpression in other cell lines (e.g., Fig. 1A), the transfection procedure led to expression of the \( \Delta E \) construct in these same cells (Fig. 1B), exogenous wild-type PS1 protein did not accumulate to high levels in PS1 \(-/-\) cells. PS1 \(-/-\) cells transfected with wild-type PS1 cleave Notch despite expressing nearly undetectable amounts of PS1 (Fig. 1C, lower panel; at maximum exposure low levels of PS1 holoprotein and cleavage fragments were visible; data not shown). A very small amount of PS1 thus induced the cleavage of \( \Delta E \), and mutation of either TM aspartate abolished that ability, even when D257A PS1 accumulated at much higher levels. The absence of PS1 endoproteolysis in D257A or D385A PS1 does not account for loss of activity, because these mutations are also dominant negative in the context of a PS1 mutant that cannot undergo endoproteolysis and is active as a full-length holoprotein (Ref. 23; data not shown). These results demonstrate that mutation of the TM aspartate residues of PS1 eliminate its ability to promote \( \Delta E \) proteolysis, even though very low levels of wild-type PS1 are sufficient to restore \( \Delta E \) cleavage to near wild-type levels.

PS1 Binds \( \Delta E \) before and during \( \Delta E \) Proteolysis—Most studies report that PS1 is restricted to the ER/Golgi (13–16), a finding inconsistent with PS1 having a direct role in \( \Delta E \) cleavage, which occurs in a post-Golgi compartment, most likely at or near the cell surface (1). Furthermore, our previous work showed that PS1 preferentially binds the immature, unprocessed form of Notch1 in transfected mammalian cells, a finding most consistent with PS1 acting on Notch prior to furin cleavage in the trans-Golgi network (22). \( \Delta E \) processing involves two distinct steps: transport to the site of cleavage and proteolysis. Mutation of the TM aspartates could block either and result in reduced NICD levels. We therefore determined when during its maturation \( \Delta E \) binds PS1 using a pulse-chase/co-immunoprecipitation assay. HEK 293 cells transfected with \( \Delta E \) were metabolically labeled for 20 min, followed by chase with unlabeled medium. Lysates were split and subjected to either anti-myc IP (to recover total \( \Delta E \)) or PS1 IP to purify the population of \( \Delta E \) bound to PS1. The ICV construct (6) encodes a polypeptide identical to NICD and was used as a migration control. Our previous work demonstrated that Notch1 is specifically recovered by co-immunoprecipitation with PS1, and that Notch and PS1 do not associate after lysis (22). Thus, this method allows for a determination of whether \( \Delta E \) associates with PS1 prior to or during its proteolytic processing.

Very little NICD accumulated during the 20 min labeling period (time 0), indicating that most newly synthesized \( \Delta E \) had not yet been trafficked beyond the ER/Golgi to the site of proteolysis (Fig. 2). At this time point, uncleaved \( \Delta E \) was co-immunoprecipitated with PS1. We estimate that approxi-
PS1 Regulates the Proteolysis of Notch at Cell Surface

**Fig. 3. Co-immunoprecipitation of biotinylated Notch with PS1.** HEK 293 cells were transiently transfected with FLN6mt and biotinylated (+) or subjected to mock biotinylation (−). Lanes 1–3, SDS extracts were collected and an aliquot (2%) was analyzed by Western blot directly (lysat, lane 1). Bands migrating between FLN and TMIC arise during sample preparation in SDS buffer (22). The remainder of the lysate underwent streptavidin precipitation prior to SDS-PAGE (lanes 2 and 3). TMIC was recovered when cultures were treated with biotin (lane 3), but not when biotin had been omitted (lane 2). Lanes 4–6, the PS1-Notch complex was recovered by anti-PS1 CTF IP, eluted from antibody, and an aliquot (10%) was analyzed directly (lane 4). The remainder underwent streptavidin precipitation followed by SDS-PAGE (lane 6). Lanes 7 and 8, The co-IP procedure was performed with preimmune serum. Notch was detected by anti-myc Western blot (7.5% SDS-PAGE). This filter was reprobed with anti-pan-cadherin (middle panel), and then with anti-β-catenin monoclonal antibody. This experiment is representative of eight independent experiments.

PS1 was transiently transfected into HEK 293 cells, and biotinylated Notch was detected by Western blot (7.5% SDS-PAGE). This filter was reprobed with anti-pan-cadherin (middle panel), and then with anti-β-catenin monoclonal antibody. This experiment is representative of eight independent experiments.

**Transfected HEK 293 cells**

- **biotinylation**
- **mock biotinylation**
- **Streptavidin precipitation**
- **SDS lysis**
- **co-IP supernatant**
- **PS1 CTF immunoprecipitation**
- **elution into 2% SDS at 95°C**
- **SDS-PAGE**

**Lanes 1–3**

- **Lanes 4–6**

**Lanes 7 & 8**

**Starting Material:**
- **Total lysate (−)**
- **α-PS1 IP (−)**
- **preimmune IP (−)**
- **α-PS1 IP (+)**
- **preimmune IP (+)**

**Results:**

- **FLN**
- **TMIC**
- **β-catenin**
- **Notch**

**HEK 293 cells transfected with ΔE and either PS1 or D257A PS1 and, after biotin treatment, biotinylated proteins co-immunoprecipitated with PS1 (IP pellet) and free from PS1 (IP supernatant) were recovered separately by streptavidin. While D257A PS1 reduced the levels of NICD, it had no significant effect on the amounts of cell surface ΔE, either free or bound to PS1. We then quantitated the amount of NICD generated, the amount of total ΔE bound to PS1, the amount of biotinylated ΔE bound to PS1, and the total amount of biotinylated ΔE when either PS1 or D257A PS1 were co-expressed. When these values were normalized to ΔE levels in the lysate, a significant reduction in the amount of NICD produced was revealed, while the other parameters showed no significant difference (Fig. 4C). Thus, D257A PS1 reduces NICD without detectably interfering with Notch-PS1 complex formation or the total amount of Notch that reaches the cell surface. In addition, since a large percentage of cell surface Notch was recovered with PS1 antibodies, the Notch-PS1 complex appears to be efficiently transported to the cell surface (see also Fig. 3). However, the presence of some free ΔE at the cell surface suggests that Notch does not require PS1 for trafficking to that compartment, consistent with observations in Drosophila mutants lacking presenilin (18, 19).

**Biotinylation of Cell Surface PS1**—These data suggest that some PS1 is present in the plasma membrane. PS1 has recently been reported to be present in the cell surface of Jurkat cells after adhesion to a collagen matrix (32), and PS1 immunoreactivity has been detected at the cell surface (33) or within vesicles adjacent to the plasma membrane by immunoelectron microscopy (34). Furthermore, Drosophila presenilin is clearly present at or near the cell surface in several cell types (35). However, other studies in mammalian cells report an exclusive ER/Golgi localization using immunofluorescence techniques (13–16). To unequivocally demonstrate the presence of endogenous PS1 at the cell surface of mammalian cells, we used a sensitive biotinylation assay. Analysis of the predicted structure of PS1 suggests that seven lysine and arginine residues would extend into the extracellular space and be accessible to biotin if PS1 were in the plasma membrane (Fig. 5A). Rat primary hippocampal cultures were biotinylated, followed by PS1 immunoprecipitation. After PS1 was eluted and re-precip-
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Under conditions in which we detected biotinylated cadherins and PS1, there was no detectable biotinylation of either cytoplasmic protein. Both of these control proteins contain numerous arginine and lysine residues, and when the glycine quenching agent was omitted from the wash procedure following biotin treatment, both negative control proteins were extensively biotinylated, demonstrating that they are sensitive indicators of intracellular biotinylation (data not shown). The PS1 holoprotein and its cleavage fragments are biotinylated in HEK293 cells expressing endogenous levels of PS1 or overexpressing full-length PS1 (Fig. 5C), and biotinylated endogenous PS1 was detected in mouse NIH3T3 fibroblasts and wild-type SV40-immortalized mouse embryonic fibroblasts (data not shown). Thus a small percentage of endogenous PS1 is expressed at the cell surface in all cell types tested.

D257A PS1 Reaches the Cell Surface and Does Not Suppress the Cell Surface Expression of Endogenous PS1—Since the PS1 endoproteolytic cleavage products are probably the active form of the molecule (36), we addressed the possibility that the D257A mutation dominantly suppresses Notch cleavage by competing with endogenous PS1 for transport to the cell surface. HEK 293 cells transfected with either PS1 or D257A PS1 were biotinylated. In cells transfected with wild-type or D257A PS1, virtually all full-length PS1 is exogenous (Fig. 5C). Since D257A PS1 is not cleaved to generate endoproteolytic fragments, the NTF-PS1 expressed in cells transfected with that plasmid are derived entirely from endogenous PS1. No difference was seen in the levels of biotinylated full-length PS1 or D257A PS1. Moreover, the D257A PS1 protein had no detectable effect on the cell surface expression of endogenous NTF-PS1 (Fig. 6). Taken together with previous subcellular fractionation experiments (23), mutation of the TM aspartates does not grossly alter PS1 subcellular distribution.

DISCUSSION

The presenilin family of proteins is required for Notch signaling (9–12). Genetic and biochemical studies have demonstrated that presenilin affect the proteolytic release of the NICD from the Notch receptor (17–19). However, the function of the presenilins is not yet known, making it is unclear how they regulate Notch proteolysis. Since Notch cleavage occurs at or near the cell surface in response to extracellular ligand (6), and PS1 accumulates in the ER/Golgi where it binds immature, full-length Notch in transfected mammalian cells (22), it seemed likely that PS1 affects some aspect of receptor maturation or trafficking indirectly leading to altered proteolysis. In this report we show that PS1 is not restricted to the ER but is constitutively expressed at the cell surface in a number of mammalian cell types (Fig. 5). Furthermore, PS1 forms a complex with Notch soon after synthesis, and this complex is efficiently transported to the cell surface, where Notch cleavage is initiated (Figs. 2 and 3).

PS1 has recently been proposed to be an aspartyl protease, since mutation of either of the two transmembrane aspartyl residues in PS1 prevents PS1 endoproteolysis and dominantly suppresses the aspartyl-protease-like γ-secretase processing of APP (23). We show here that these transmembrane aspartyl residues are also involved in the proteolytic release of NICD from Notch, and are required for PS1 activity in a Notch cleavage assay. Thus, it is conceivable that presenilins are aspartyl proteases, or di-aspartyl protease cofactors, that function in both NICD release and APP metabolism. This hypothesis is supported by the observation that peptidomimetic inhibitors of γ-secretase based on the APP cleavage site inhibit Notch processing with highly similar efficacy, indicating that identical/related protease(s) are involved in both events. The fact that Notch and APP show no sequence similarities around the sites

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**FIG. 4. Lack of effect of D257A PS1 on Notch-PS1 complex formation and on Notch trafficking. A, HEK 293 cells transiently transfected with ΔE, PS1, or PS1 D257A were lysed in co-IP buffer and analyzed directly (lysat), 10%, or immunoprecipitated with anti-myc. PS1 was detected with antibody NTI (4–15% SDS-PAGE). B, HEK 293 cells were transiently transfected with PS1 or D257A PS1 and treated with (+) or without (−) biotin. An aliquot of lysate was analyzed directly (lysat), 5%; the remainder was immunoprecipitated with the polyclonal CTF-PS1 antiserum. Proteins co-immunoprecipitated with PS1 were eluted, and an aliquot (10%) was analyzed directly (PS1 IP). This elute and the supernatant from the PS1 IP were then precipitated with streptavidin. Notch was detected by anti-myc Western blot (7.5% PS1 were eluted, and an aliquot (10%) was analyzed directly (PS1 IP). These values were divided by the band intensity for total cell surface ΔE bound to PS1, the amount of ΔE recovered with PS1 divided by ΔE; total cell surface ΔE, total biotinylated ΔE divided by ΔE; cell surface ΔE bound to PS1, the amount of biotinylated ΔE recovered with PS1 divided by ΔE. Error bar represents S.E., and * signifies p < 0.01(Student's t test).

Biotinylated, biotinylated PS1-NTF was recovered from cells treated with biotin, but not mock-treated controls (Fig. 5B). Biotinylated PS1-NTF was also recovered but less efficiently, possibly due to having one lysine residue compared with the NTF, which has six arginine or lysine residues (Fig. 5A).

To ensure that we were not detecting intracellular PS1 that had become labeled with biotin after lysis, we analyzed two combinations of the molecule (36), we addressed the possibility that the D257A mutation dominantly suppresses Notch cleavage by competing with endogenous PS1 for transport to the cell surface. HEK 293 cells transfected with either PS1 or D257A PS1 were biotinylated. In cells transfected with wild-type or D257A PS1, virtually all full-length PS1 is exogenous (Fig. 5C). Since D257A PS1 is not cleaved to generate endoproteolytic fragments, the NTF-PS1 expressed in cells transfected with that plasmid are derived entirely from endogenous PS1. No difference was seen in the levels of biotinylated full-length PS1 or D257A PS1. Moreover, the D257A PS1 protein had no detectable effect on the cell surface expression of endogenous NTF-PS1 (Fig. 6). Taken together with previous subcellular fractionation experiments (23), mutation of the TM aspartates does not grossly alter PS1 subcellular distribution.

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of cleavage suggests that this novel enzyme(s) has little primary sequence specificity.

In order for PS1 to be directly involved in Notch and APP proteolysis as a protease or protease co-factor, it would have to be present when these substrates are cleaved. Immunohistochemical studies reveal that in mammals (37, 38) and Drosophila (18, 19, 35), presenilins are widely expressed throughout development and show broad overlap with Notch and APP expression. At a subcellular level, however, most studies report that PS1 is restricted to the ER/Golgi using immunofluorescence techniques (13–16), whereas Notch cleavage is induced at the cell surface after binding to extracellular ligand. The immunohistochemistry performed here (Fig. 5) and elsewhere (32), indicate that a small percentage of PS1, perhaps 1–10%, is present at the cell surface. This low level of cell surface PS1 is not inconsistent with immunofluorescence studies, which might not detect fluorescent signal near the cell surface that is 1–10% as intense as the signal from the ER/Golgi. This result is in agreement with immunoelectron microscopy experiments where PS1 was detected at the cell surface (33), and from immunohistochemical studies in Drosophila, which detect presenilin at or near the cell surface in several tissues (35). Thus, PS1 has a broader subcellular distribution than previously appreciated and most likely functions in multiple subcellular locations.

PS1 is likely to play a similar role in both APP metabolism and Notch signaling (17). PS1 preferentially binds immature APP and Notch within the ER/Golgi in transfected mammalian cells (21, 22). However, when we examined the presenilin-Notch complex in Drosophila cells endogenously expressing both proteins, the mature, TMIC form of the receptor was preferentially bound to presenilin, demonstrating that these proteins can interact following furin processing in vivo (22). Based on this observation, it is possible that PS1 also interacts predominantly with mature, post-Golgi forms of APP at physiological expression levels. This point is critical for understanding the role of the presenilins in APP metabolism, since Aβ is generated in multiple vesicular locations in both the secretory and endocytic pathways (14). Unlike the TM processing of Notch, there is no current understanding of the functional significance of APP cleavage by γ-secretase. As far as is known, there is no extracellular ligand-mediated stimulus for γ-secretase cleavage of APP, and Aβ appears to be generated in multiple subcellular sites, where as Notch cleavage is restricted to a site near the cell surface. The mechanisms that prevent the premature PS1-dependent cleavage of Notch (or its constitutively active derivative ΔE) during secretion are unknown, but might be absent from APP proteolysis, which appears to be less tightly regulated.

Expression of D257A PS1 dominantly suppresses both Notch processing and APP cleavage, but has no apparent effect on the levels of endogenous PS1 endoproteolytic cleavage fragments (Fig. 1A) or their ability to reach the cell surface (Fig. 6). Since this mutation produces an inactive molecule, its dominant effect suggests that it either competes with endogenous PS1 for factors that are rate-limiting for substrate cleavage, or that PS1 functions as a multimeric complex that is inactivated by the incorporation of these mutant proteins.

Our results suggest that Notch is co-transported with PS1 to...
the cell surface, and that the trafficking of this complex is efficient. Even though ~5% of total cellular ΔE is co-immunoprecipitated with PS1, we find that approximately half of the biotinylated ΔE is recovered with PS1 at the cell surface (Fig. 4B). Similar results were observed when FLN6mt, which encodes a nearly full-length form of Notch1 including the entire ectodomain, was examined in these assays (Fig. 3 and data not shown). These results might suggest that the formation of the PS1-Notch complex affects the trafficking of either or both proteins. However, we found that a substantial percentage of cell surface Notch is not recovered with PS1 (Fig. 4B). Furthermore, cell surface PS1 was endogenously expressed in HEK293 cells, in the absence of detectable levels of (endogenous) cell surface Notch1. In addition, we found no reliable difference in the levels of biotinylated FLN6mt or ΔE when PS1-deficient cells were compared with wild-type controls (data not shown). These results suggest that both proteins can reach the cell surface independently and do not require the other for transport, a conclusion supported by studies of Notch subcellular localization in Drosophila presenilin mutants (18, 19). It was recently shown that biotinylated, presumably cell surface PS1 interacts with filamin (32, 39), an actin-binding protein, following substrate-induced redistribution of intracellular PS1 to the cell surface of Jurkat cells. Perhaps filamin or other cytoskeletal interacting proteins that bind PS1 facilitate trafficking of the Notch-PS1 complex.

In summary, we propose that PS1 forms a complex with Notch in the ER, but that this complex is not competent to cleave Notch until it is transported to the cell surface where it becomes de-repressed following ligand binding to Notch. The TM aspartates are specifically required for proteolytic competence, possibly as the active site of an unprecedented intramembranous aspartyl protease. Alternatively, PS1 may participate in proteolysis by acting as a required di-aspartyl cofactor or regulatory subunit for an as yet unidentified protease.

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