Presenilin 1 and Presenilin 2 Have Differential Effects on the Stability and Maturation of Nicastrin in Mammalian Brain*

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The presenilins (1, 2) and nicastrin (3) form high molecular mass, multimeric protein complexes involved in the intramembranous proteolysis of several proteins. Post-translational glycosylation and trafficking of nicastrin is necessary for the activity of these complexes. We report here that although there are differences in the post-translational processing of nicastrin in neurons and glia, both of the presenilins are required for the physiological post-translational modification and for the correct subcellular distribution of nicastrin. Absence of presenilin 1 (PS1) is associated with dramatic reductions in the level of mature glycosylated nicastrin and with redistribution of nicastrin away from the cell surface. In contrast, absence of presenilin 2 (PS2) is associated with only modest reductions in the levels of immature nicastrin. It is notable that these differential effects parallel the differential effects of null mutations in PS1 and PS2 on APP and Notch processing. Our data therefore suggest that the differential interactions of PS1 and PS2 with nicastrin reflect different functions for the PS1 and PS2 complexes.

The presenilins (1, 2) and nicastrin (3) form high molecular mass complexes that are involved in the intramembranous proteolysis of several proteins, including β-amyloid precursor protein (APP) and Notch receptor (3–9). The relative roles of the presenilins and nicastrin in these complexes are poorly understood. Nicastrin undergoes a complex, post-translational, metabolic post-translational modification and for the correct subcellular distribution of nicastrin. Absence of presenilin 1 (PS1) is associated with dramatic reductions in the level of mature glycosylated nicastrin and with redistribution of nicastrin away from the cell surface. In contrast, absence of presenilin 2 (PS2) is associated with only modest reductions in the levels of immature nicastrin. It is notable that these differential effects parallel the differential effects of null mutations in PS1 and PS2 on APP and Notch processing. Our data therefore suggest that the differential interactions of PS1 and PS2 with nicastrin reflect different functions for the PS1 and PS2 complexes.

The presenilins were isolated by titruration of enzymatically dissociated cerebellum from 6-day-old mice. The neurons were maintained in minimum essential medium containing Earle’s salts, 2 mM glutamine, 10% fetal bovine serum, 0.6% glucose, 25 mM KCl, 5 units/ml penicillin, and 5 μg/ml streptomycin on poly-d-lysine-coated dishes. To inhibit glial proliferation, 4 μg/ml cytosine arabinofuranoside. After 3 days in vitro, the glial plating medium was replaced with medium in which 15 mM sorbitol was added 18 h post-plating. The cells were used at 7 days in vitro.

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1 The abbreviations used are: APP, β-amyloid precursor protein; PS1, presenilin 1; PS2, presenilin 2; PBS, phosphate-buffered saline; ER, endoplasmic reticulum; Tricine, N-[3-hydroxy-1,1-bis(hydroxymethyl)-ethylglycine.
ventral skin, and tails of 2-day-old mice. The cultures were grown in Dulbecco’s modified Eagle’s medium that contained 2 mM glutamine and 20% fetal bovine serum. The cells were passaged 2–6 times before seeding onto poly-d-lysine-coated coverslips for immunocytochemical studies.

Sample Preparation

Brain tissues and cell cultures were washed in cold PBS and homogenized with ice-cold 1% Nonidet P-40 in Tris-buffered saline (50 mM Tris, pH 7.4, 150 mM NaCl, 2 mM EGTA) containing a complete protease inhibitor mixture (Sigma). Protein concentration analysis was performed by BCA protein assay (Pierce).

Electrophoresis and Immunoblotting

50 µg of total protein from tissue or cell lysates were dissolved in SDS sample buffer, separated on Tris-Tricine gels, and transferred to nitrocellulose membrane (equal loadings were confirmed by Western blotting for actin). The target proteins were visualized by enhanced chemiluminescence (Amersham Biosciences) with antibodies to nicastrin (1:1000 dilution anti-nicastrin C-terminal antibody; Affinity Bioreagents Inc.), to the PS1 N-terminal fragment (1:8000 dilution antibody 14, gift from Dr. S. E. Gandy), to the PS2 N-terminal fragment (1:800 NT14), and to actin (1:2500 anti-actin polyclonal antibody; Sigma). Where necessary, the nitrocellulose membranes were stripped for reuse. The intensities of bands on Western blots were measured by densitometry.

Subcellular Fractionation on Iodixanol Gradient and Glycosidase Treatment

Mouse brain tissue from PS1−/−, PS2−/−, or wild type mice was homogenized with ice-cold homogenization buffer (130 mM KCl, 25 mM NaCl, 1 mM EGTA, 25 mM Tris, pH 7.4) containing a protease inhibitor mixture (Sigma). The postnuclear supernatant was clarified by centrifugation at 1,000 × g for 10 min and at 3,000 × g for 10 min. The resulting supernatant was layered on a step gradient consisting of 1 ml each of 30, 25, 20, 15, 12.5, 10, 7.5, 5, and 2.5% (v/v) iodixanol (Accurate) in homogenization buffer. After centrifugation at 27,000 rpm (SW40 rotor, Beckman) for 32 min, 11 fractions were collected from the top of the gradient. The fractions were analyzed for the presence of nicastrin and protein markers of subcellular organelles by Western blotting. To assess the maturation of nicastrin glycosylation, pooled fractions 9–11 (containing ER markers) and pooled fractions 4–7 (containing Golgi markers) were treated with endoglycosidase H as previously described (10, 26).

Statistical Analyses

All of the experiments were performed at least in triplicate. The Student’s t test for independent groups was used to compare band density on autoradiographs of Western blots. To permit comparison of densitometric results, the intensities of bands on Western blots were measured by densitometry.

Immunocytochemistry

Immunocytochemistry was performed on primary glial cultures prepared from 8-day-old wild type and PS1−/− B6 × 129 mice. The cells were grown on collagen-coated glass coverslips in Dulbecco’s modified Eagle’s medium (Invitrogen) supplemented with 20% fetal calf serum.

RESULTS

To explore how the absence of PS1 affects nicastrin expression, Western blot analyses were performed on brain homogenates prepared from 6-day-old wild type and PS1-deficient (PS1−/−) mice (13, 14) (n = 8 independent wild type and 10 independent PS1−/− mice) (Fig. 1). In brain tissue from wild type mice and from PS1−/− mice, nicastrin was detected both as

| PS1 genotype | +/+ | +/− | −/− | −/− | −/− | +/− |
|--------------|-----|-----|-----|-----|-----|-----|
| Mature NCT   | 55  | 55  | 55  | 55  | 55  | 55  |
| Immature NCT | 34  | 34  | 34  | 34  | 34  | 34  |
| FL-PS1       | 45  | 45  | 45  | 45  | 45  | 45  |
| NTF-PS1      |     |     |     |     |     |     |
| NTF-PS2      |     |     |     |     |     |     |
| Actin        |     |     |     |     |     |     |

Mr. (kDa)

105
55
34
45

FIG. 1. PS1 deficiency inhibits expression and glycosylation of nicastrin in mouse brain. Western blots of 50 µg of total protein from PS1−/− and PS1+− mouse brain lysates were probed with anti-C-termi nal nicastrin polyclonal antibody (Affinity Bioreagents Inc.), anti-PS1-NT antibody (Ab14), anti-PS2 antibody (NT14), or anti-Actin antibody (Sigma). In PS1−/− brain (left two lanes), two isoforms of nicastrin were observed: an immature glycosylated form at ~105 kDa and the mature glycosylated form at ~125 kDa. In PS1−/− brain lysates (center two lanes), the levels of total nicastrin and especially of the mature isoforms are much lower (p < 0.001). Overexpression of a human wild type PS1 transgene in a PS1−/− mouse fully rescues expression of mature and immature forms of nicastrin (right two lanes). Mr., molecular mass; NCT, nicastrin; NTF, N-terminal fragment; FL, full-length; Tg, transgenic.

Cell Surface Biotinylation

Mouse glial cultures were washed three times with ice-cold PBS (pH 8.5; 1 mM MgCl2) and incubated for 30 min on ice with 1 mg/ml EZ-link NHS-biotin (Pierce). The reaction was stopped by washing the cells once and then incubating for 10 min on ice with 50 mM glycine in PBS (pH 8.5; 1 mM MgCl2). The cells were collected in lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Triton X-100, 5 mM EDTA, 1 mM EGTA, protease inhibitors; Sigma) and rocked for 1 h at 4 °C. The lysates were cleared by 21,000 × g centrifugation and assayed for protein content (BCA protein assay; Pierce). The lysate volumes were adjusted to equalize protein concentration, and 1300 µl of each sample was used for affinity purification with 50 µl of Sepharose beads conjugated to neutravidin (Pierce). Following overnight rocking at 4 °C, the beads were washed twice with 1 ml lysis buffer, and two times with Tris buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 4 mM EDTA). The bound proteins were solubilized by heating the beads for 10 min at 65 °C in 2× SDS Tris-glycine sample buffer (Invitrogen) and separated on 10% Tris-glycine SDS-polyacrylamide gels. After transferring the proteins to nitrocellulose membrane, they were probed with the following antibodies: rabbit polyclonal antibodies anti-nicastrin (1:2500) and anti-p cadherin (1:2500; Sigma), mouse monoclonal antibodies anti-calnexin (1:2500; Transduction Laboratories), and anti-glyceraldehyde-3-phosphate dehydrogenase (1:1000; Biodesign).

Metabolic Labeling and Immunoprecipitation

After 6 days in culture, neurons and glia were preincubated for 1 h in methionine-free and cysteine-free Dulbecco’s modified Eagle’s medium. 300 µCi/ml Tran35S-labeling reagent (ICN) was added for 30 min. The media were replaced with unlabeled conditioned medium containing 1 mM methionine and 1 mM cysteine for 1–16 h at 37 °C. The cells were washed in cold PBS and then lysed in 1 ml of buffer (50 mM Tris, pH 7.5, 150 mM NaCl, 2 mM EDTA, 0.5% Triton X-100, and protease inhibitors) on ice for 30 min. The lysates were spun at 13,000 rpm for 5 min, and nicastrin was immunoprecipitated with the polyclonal anti-nicastrin CT antibody and analyzed by 10% Tris-Tricine SDS-polyacrylamide gel electrophoresis (Invitrogen). The gels were dried and exposed to Kodak Biomax film as previously described (26).
PS2 deficiency reduces immature nicastrin levels in mouse brain. Western blots of 50 μg of total protein from PS2+/+ and PS2−/− mouse brain lysates were probed with the same anti-nicastrin, anti-PS1, anti-PS2, and anti-actin antibodies as described in the legend to Fig. 1. Both the overall nicastrin level and the immature nicastrin are less in the PS2−/− mouse than in the PS2+/+ control (p < 0.001), whereas the amount of the mature nicastrin in PS2−/− mice (p > 0.05) is not altered. M., molecular mass; NCT, nicastrin; NTF, N-terminal fragment.

![Diagram of nicastrin expression in PS1 and PS2 genotypes](image)

**Fig. 2.** PS2 deficiency reduces immature nicastrin levels in mouse brain. Western blots of 50 μg of total protein from PS2+/+ and PS2−/− mouse brain lysates were probed with the same anti-nicastrin, anti-PS1, anti-PS2, and anti-actin antibodies as described in the legend to Fig. 1. Both the overall nicastrin level and the immature nicastrin are less in the PS2−/− mouse than in the PS2+/+ control (p < 0.001), whereas the amount of the mature nicastrin in PS2−/− mice (p > 0.05) is not altered. M., molecular mass; NCT, nicastrin; NTF, N-terminal fragment.

a poorly glycosylated, immature form (~105 kDa) and as a highly glycosylated, mature form (~125 kDa). However, when the levels of nicastrin expression in PS1−/− and wild type mouse brain were compared by normalizing to the level of expression in wild type mice (1.00 ± 0.03), nicastrin was expressed at much lower levels in PS1−/− brain (0.52 ± 0.06; p < 0.001) (Fig. 1). In contrast to the results reported in fibroblasts (which displayed either no change or a small increase in immature nicastrin) (11, 12), there was a reduction in the level of immature nicastrin in brain tissue from PS1−/− mice (0.59 ± 0.07) compared with brain tissue from wild type littermates (1.00 ± 0.08; p < 0.001). In agreement with these prior studies, there was a much larger reduction in the level of the mature nicastrin in PS1−/− mouse brain (0.26 ± 0.07) relative to wild type brain (1.00 ± 0.08; p < 0.001). This reduction in nicastrin expression was not related to developmental age, because similar results were observed in 3-month-old mice (data not shown). The reduction was a specific consequence of the absence of PS1 because these changes were fully rescued when either exogenous wild type human PS1 (Fig. 1) or FAD mutant human PS1 (not shown) was introduced by cross-breeding the PS1−/− mice with mice bearing a human PS1 transgene.

When nicastrin expression was examined in brain homogenates from PS2−/− mice, it was discovered that the overall levels of nicastrin (0.62 ± 0.04; n = 12) were also slightly reduced in comparison with those in wild type mice (1.00 ± 0.07; n = 10; p = 0.001) (Fig. 2). The reductions observed in PS2−/− mice were almost entirely due to the loss of immature forms of nicastrin (0.57 ± 0.07) (p < 0.001) (Fig. 2). The amount of mature nicastrin was not significantly different between wild type (1.00 ± 0.17) and PS2−/− mice (0.80 ± 0.11; p > 0.05) (Fig. 2). Direct comparison of the effects of null mutations in PS1 and PS2 on nicastrin biology revealed a dramatic difference in their effects on the stabilization of mature nicastrin isoforms (PS1−/−:0.26 ± 0.07; PS2−/−:0.80 ± 0.11; p < 0.001) but equivalent effects on immature nicastrin isoforms, which were reduced to about half the level in wild type mice (PS1−/−, 0.59 ± 0.07; PS2−/−, 0.57 ± 0.07; p = not significant).

We have shown previously that glycosylation and trafficking of nicastrin to the Golgi is essential for the functional activity of the presenilins (9, 10, 15). To investigate whether the absence of PS1 and/or PS2 might alter the processing and intracellular distribution of nicastrin, we used iodixanol gradient fractionation to separate Golgi and ER-derived membranes (Fig. 3) (9). In agreement with our previously published data (10), nicastrin from wild type mouse brain underwent a typical ER-to-Golgi maturation, with the majority of mature species localized to the Golgi (Fig. 3). The distribution of mature and immature nicastrin species was similar in PS2−/− brain tissue, although the overall abundance of nicastrin was reduced in PS2−/− mouse brain (Fig. 3). In contrast, in PS1−/− mouse brain, both the abundance and the distribution of nicastrin isoforms were grossly disturbed. The higher molecular mass, endoglycosidase H glycosidase-resistant, mature forms of nicastrin were distributed broadly throughout the gradient. NCT, nicastrin; m, mature; im, immature.

**Fig. 3.** Absence of PS1 affects intracellular distribution of nicastrin. Cell lysates from PS1−/−, PS2−/−, or wild type murine brains were fractionated on iodixanol gradients, and the fractions were resolved by SDS-PAGE and investigated by Western blotting. Fractions 8–11 contain ER membranes (marker protein: calnexin). Fractions 4–7 are enriched in Golgi membranes (marker protein: GM130). In brain homogenates from wild type mice and from PS2−/− mice, the majority of maturely glycosylated nicastrin is located in the Golgi compartment. In PS1−/− homogenates, the abundance of both mature and immature forms of nicastrin is dramatically reduced, and low levels of the mature form are distributed broadly throughout the gradient. NCT, nicastrin; m, mature; im, immature.

To confirm these results, the subcellular distribution of nicastrin was also examined by confocal microscopy using both permeabilized and nonpermeabilized glia. In glia from wild type mice, nicastrin immunoreactivity appeared as distinct granular structures that were distributed from ER to Golgi (Fig. 4A, upper rows) and as a diffuse punctate pattern on the cell surface (Fig. 4A, lower rows; TrkB was used as a positive control). The intracellular anti-nicastrin staining colocalized mostly with the ER protein Bip (Fig. 4E, upper rows) but also overlapped with structures that contained markers for the Golgi (GM130; Fig. 4C, upper rows). In contrast, and in agreement with the biochemical studies described above, nicastrin immunofluorescence in glial cells obtained from PS1−/− mice was substantially reduced (Fig. 4B, upper rows). Furthermore, in agreement with the biochemical studies in PS1−/− glial cells (described below) and in PS1−/− fibroblasts described previously (11), nicastrin immunoreactivity at the cell surface was massively reduced or absent in PS1−/− cells (Fig. 4B, upper rows). The subcellular distribution of nicastrin immunoreactivity in PS2−/− cells showed no obvious change (Fig. 4B, lower rows, D, and F). The localization of nicastrin in subcellular
domains that do not contain robust levels of presenilins even in wild type cells is concordant with previous biochemical fractionation studies showing that a significant portion of nicastrin exists in high molecular mass, membrane-bound fractions that contain little or no presenilin immunoreactivity (3, 10). Elsewhere, we show that these other fractions contain APH-1 (16).

![Diagram](image_url)

**Fig. 4. Profound reduction of cell surface and Golgi nicastrin immunofluorescence in PS1-deficient mouse glial cells.** Mouse cerebellar glial cells were fixed with 4% paraformaldehyde. Permeabilized cells (A, upper rows, and C–E) and unpermeabilized cells (A, lower rows, and B) were incubated with anti-nicastrin antibody and anti-TrkB, a marker for cell surface (A, B), with anti-GM130, a marker of the Golgi (C, D) or anti-KDEL, a marker of the ER (Bip; E, F) antibody (the far right panels on A were also stained with Bip as a positive control for permeabilized cells). Nicastrin (red) immunoreactivity in wild type cells was detected as a punctate pattern at cell surface (A, lower rows) and as a granular pattern in ER and Golgi, where it colocalized in varying degrees with Bip (E) and GM130 (C). In PS1-deficient cells, nicastrin immunoreactivity (red) was profoundly reduced at the cell surface (B, upper rows), slightly colocalized with GM130 (C, lower rows), and exhibited a rough granular pattern mainly in ER (E, lower rows). PS2-deficient cells had the same immunoreactive pattern as wild type cells (B, lower rows, D, and F). White squares indicate enlarged areas in A and B. NCT, nicastrin; wt, wild type.
Presenilins Differentially Affect Nicastrin Maturation

Fig. 5. Nicastrin trafficking to the cell surface requires PS1. Western blots of neuravidin-purified biotinylated cell surface proteins revealed nicastrin and cell surface marker cadherin but not ER marker calnexin. Maturely glycosylated nicastrin (M) was exclusively precipitated by neuravidin in wild type and in PS2-deficient glia, whereas in PS1-deficient glia, nicastrin precipitation was severely reduced and was predominantly the immaturely glycosylated form (Imm). Lys-PreAP lanes were loaded with 15 μg (18 μl) of total protein, and Bound lanes were loaded with 22 μl of sample (45% of precipitated proteins from the immunoprecipitation volume of 1300 μl). Wt, wild type; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

These perturbations in cell surface labeling of nicastrin were independently corroborated by covalently labeling glial cell surface proteins with a membrane impermeant biotinylating reagent. In wild type cells, only maturely glycosylated nicastrin was affinity purified by neuravidin beads following biotinylation, and this fraction represented ~3% of total cellular nicastrin (Fig. 5). In contrast, recovery of biotinylated nicastrin was substantially reduced in PS1−/− cells and consisted of both mature and immature forms of nicastrin. Cadherin, a positive control protein that reaches the cell surface, and calnexin, a negative control protein that is confined to ER membranes, were equivalently recovered in wild type and PS1−/− cells. This result is also in good agreement with the altered distribution of mature and immature forms of nicastrin on biochemical subcellular fractionation (Fig. 3). Taken together, these results suggest that the changes in subcellular distribution of nicastrin in PS1−/− cells are not due to nonspecific changes in protein trafficking.

To examine the dynamic processing of nicastrin in presenilin-deficient cells, pulse-chase labeling studies were performed in both granule cells and glia purified from wild type, PS1−/−, or PS2−/− mouse brains (Fig. 6). In neurons from wild type mice, mature forms of nicastrin could be detected ~2 h following metabolic labeling, although there was significant persistence of immature forms up to 16 h (in agreement with the Western blotting data from whole brain homogenates) (compare with Figs. 1 and 2). In PS2−/− granule cells, mature forms of nicastrin were also detectable at 2 h following labeling. However, in contrast to wild type neurons, the intensity of the immature band decayed slightly at ~2 h, such that the relative intensities of the signals from immature and mature forms of nicastrin were approximately equal at 16 h after chase. This is in agreement with the static observations in whole brain lysates and suggests that, in the absence of PS2, immature nicastrin is slightly destabilized. In granule cells from PS1−/− brains, the amount of nicastrin was significantly reduced and could only be visualized after prolonged exposure (~30 days). Not only were the levels reduced, but the appearance of mature forms of nicastrin was delayed until 4–8 h. Analysis of the pulse-chase data from cultured glia revealed a similar pattern of delayed and reduced maturation of nicastrin in PS1−/− cells and destabilization of immature nicastrin in PS2−/− cells.

These studies suggest that, in both PS1-deficient neurons and glia, nicastrin fails to undergo appropriate glycosylation maturation. In contrast, in PS2-deficient neurons and glia, the maturation of nicastrin is much less affected, with the principal changes being a subtle reduction in the signal intensity for immature isoforms of nicastrin in PS2−/− cells at 4 and 8 h compared with wild type cells.

DISCUSSION

When considered together with other data, our experiments reveal that PS1 and PS2 are required for the correct stabilization, glycosylation, and trafficking of nicastrin. These results are in good agreement with the conclusion that both nicastrin and the presenilins are required to form stable, high molecular mass complexes (11, 12). However, close inspection of our results also reveals that PS1 and PS2 do not have equivalent roles in the maturation of nicastrin. In PS1-deficient mice, there was a much greater reduction in the levels of all forms of nicastrin, and there was an especially marked reduction in the maturely glycosylated forms. In contrast, in PS2-deficient mouse brains, there was a less pronounced reduction in overall nicastrin levels, and this reduction was predominantly in the immaturely glycosylated forms. Furthermore, the magnitude of the reductions in immature nicastrin was similar in PS1−/− and PS2−/− murine brain.

These results suggest that the stabilization of the high molecular mass, terminally glycosylated forms of nicastrin in brain is primarily dependent on PS1. The roles of PS1 and PS2 in the stabilization of the immaturely glycosylated forms of nicastrin are more complex and may depend on other factors, such as APH-1, which we have shown to bind to immature forms of nicastrin and the presenilins (16). Indeed, the factors stabilizing immature nicastrin may be cell type-dependent in view of these differences in the metabolic fates of immature isoforms of nicastrin in glia and neurons (Fig. 6). Although both PS1 and PS2 are likely to be involved in the initial maturation and trafficking of immature nicastrin, their activities are not fully redundant. Thus, in the absence of PS2, PS1 is still able to capture sufficient immature nicastrin to generate normal levels of mature nicastrin. In contrast, PS2 by itself is unable to fully support the complete maturation of nicastrin.

The differential effects of PS1 and PS2 deficiency on nicastrin processing parallel the different effects of PS1 and PS2 deficiency on APP and Notch processing. In PS1−/− cells, there
are significant reductions in the amounts of mature glycosylated nicastrin (this study), and these reductions are accompanied by significant parallel reductions in the activities of both γ-secretase (S) and S3-secretase (T). In contrast, in PS2−/− cells, the reductions in mature nicastrin levels (this study) and in γ-secretase/S3-secretase activity are both relatively minor (17–19). This raises the possibility that these events are mechanistically linked in one of two ways. First, it is conceivable that the presenilins play the same role in the proteolytic processing of nicastrin, APP, and Notch. However, to our knowledge, there is currently no evidence that nicastrin undergoes proteolytic cleavage (in contrast to APP (5), Notch (4), and ErbB4 (20)). The alternate possibility is that the activities of the presenilins could be mediated through their overlapping but nonidentical effects on nicastrin processing.

The precise details of the interaction between each of the presenilins and nicastrin remain to be fully worked out. It is tempting to speculate that both PS1 and PS2 facilitate the entry of immature nicastrin into compartments for glycosylation, after which nicastrin enters the mature, functionally active presenilin complexes. PS1 might also be necessary for the stabilization of these mature glycosylated forms of nicastrin and for their correct translocation to the subcellular sites of γ-secretase and S3 site cleavage of APP and Notch. Although our results are compatible with a role for the presenilins in regulating intracellular trafficking of membrane proteins, our data do not prove that this stabilization/trafficking activity is an authentic functional property of the presenilins (which have also been postulated to have catalytic activity). Nevertheless, previous experiments in invertebrates have suggested that PS1 may be involved in intracellular trafficking of nicastrin (11, 21–23). Indeed, another presenilin homologue in Caenorhabditis elegans called spe4 is involved in the intracellular compartmentalization of the major sperm protein (24). However, an important corollary of our results is that the absence of any component of the nicastrin-presenilin complex has effects on the other components of the complex. This necessarily makes the assignment of specific functional properties to any given member of the complex problematic.

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