Potential Link between Amyloid β-Protein 42 and C-terminal Fragment γ 49–99 of β-Amyloid Precursor Protein*

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Toru Sato‡§, Naoshi Dohmae‡, Yue Qi‡, Nobuto Kakuda‡, Hiroaki Misonou‡, Rie Mitsumori‡, Hiroko Maruyama¶, Edward H. Koo‡, Christian Haass**‡‡, Koji Takio‡, Maho Morishima-Kawashima‡, Shoichi Ishiura‡, and Yasuo Ihara‡§§

From the ‡Department of Neuropathology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan, the §Department of Life Science, Graduate School of Arts and Science, University of Tokyo, 3-8-1 Komaba, Meguro-ku, Tokyo 153-8902, Japan, the ¶Biomolecular Characterization Division, Characterization Center, The Institute of Physical and Chemical Research, Wako, Saitama 351-0198, Japan, the **Department of Neurosciences, University of California, San Diego, La Jolla, California 92039, and the §§Adolf-Butenandt-Institute, Department of Biochemistry, Laboratory for Alzheimer’s and Parkinson’s Disease Research, Ludwig-Maximilians-University, D-80336 Munich, Germany

A novel cleavage of β-amyloid precursor protein (APP), referred to as ε-cleavage, occurs downstream of the γ-cleavage and generates predominantly a C-terminal fragment (CTFγ) that begins at Val-50, according to amyloid β-protein (Aβ) numbering. Whether this cleavage occurs independently of, or is coordinated with, γ-cleavage is unknown. Using a cell-free system, we show here that, although Aβ42 and CTFγ 50–99 were the predominant species produced by membranes prepared from cells overexpressing wild-type (wt) APP and wt presenilin (PS) 1 or 2, the production of CTFγ 49–99, which begins at Leu-49, was remarkably enhanced in membranes from cells overexpressing mutant (mt) APP or mtPS1/2 that increases the production of Aβ42. Furthermore, a γ-secretase inhibitor, which suppresses Aβ40 production and paradoxically enhances Aβ42 production at low concentrations, caused the proportion of CTFγ 50–99 to decrease and that of CTFγ 49–99 to increase significantly. These results strongly suggest a link between the production of Aβ42 and CTFγ 49–99 and provide an important insight into the mechanisms of altered γ-cleavage caused by mtAPP and mtPS1/2.

Senile plaques, one of the neuropathological hallmarks of Alzheimer’s disease (AD), are composed primarily of amyloid β-protein (Aβ) (1). Two major Aβ species consisting of 40 and 42 residues are generated mainly in neurons and constitutively secreted. A shorter one, Aβ40, is predominant, and a longer one, Aβ42, is a minor species (<10%) among secreted Aβ species. Aβ is produced from β-amyloid precursor protein (APP) through sequential cleavage by proteases referred to as β- and γ-secretases. γ-Secretase was identified as a type I membrane aspartic protease β-site APP-cleaving enzyme (BACE) (2), but the identity of γ-secretase has remained unknown. γ-Secretase cleaves APP in the middle of the transmembrane domain, releasing Aβ and its counterpart, C-terminal fragment γ of APP (CTFγ). Most recent studies have shown that γ-secretase forms a large complex composed of presenilin (PS) 1 or 2, nicastrin, PEN-2, and APH-1, and the activity of γ-secretase is now known to depend on these proteins (3–7).

One of the Aβ species, Aβ42, has a much higher aggregation potential (8, 9) and is believed to be initially deposited in senile plaques (10). It is reasonable to postulate that Aβ42 accumulation in the brain is the very initial event in the development of AD including sporadic AD. Indeed, all mutations of PS1/2 and some mutations of APP that cause familial AD result in increased Aβ42 production (11).

Recently, we and other groups found that APP is cleaved by PS-dependent γ-secretase, not only in the middle of the transmembrane domain (γ-cleavage) but also near the cytoplasmic membrane boundary (ε-cleavage) (12–15). The major product of the latter process is a CTF of APP that begins at Val-50. This cleavage site is a few residues inside the membrane from the cytoplasmic/membrane boundary and is similar to site 3 cleavage of Notch (16). Since production of CTFγ is inhibited by a dominant negative mutant of PS1 (17), ε-cleavage is PS-dependent as well as γ-cleavage (13–15). Furthermore, ε-cleavage is also inhibited with γ-secretase inhibitors, which are known to selectively bind to PS1/2 (12–15). However, it has remained unknown how ε-cleavage relates to the generation of distinct Aβ species or whether this step is essential to generate Aβ. We therefore examined whether there is a link between CTFγ and Aβ production using a cell-free system. Taking advantage of familial AD mutations of APP and PS1/2 and a γ-secretase inhibitor, we show here that, when Aβ40 is predominantly produced, CTFγ 50–99 is the major product of ε-cleavage, and when a large amount of Aβ42 is produced, CTFγ 49–99 is predominantly produced. Thus, ε-cleavage may be linked to the specificity of γ-cleavage.

EXPERIMENTAL PROCEDURES

APP- and PS1/2-overexpressing Cells—Chinese hamster ovary (CHO) cells transfected with cDNA encoding wild-type (wt) APP751 (7WD10 cells) and mutant (mt) APP751 (V717F) were described previ-
We used a cell-free Aβ production system consisting of a negative mutant of PS2, D366A (21), produced negligible amounts of Aβ40/42 and a trace amount of CTFγ (Fig. 1, A and B). This mutant accumulated large amounts of CTFβ/γ, the immediate substrates for γ-secretase (Fig. 1, A and B). Thus, this cell-free Aβ production system reflects the Aβ secretion profile observed in *in vitro* culture of each cell line (data not shown). Cell-free production of Aβ and CTFγ took very similar time courses, with both gradually declining after 10–20 min (19) (data not shown). A specific γ-secretase inhibitor, DFK-167 (22), suppressed Aβ40 production but paradoxically enhanced Aβ42 production at low concentrations in the membranes from wtPS2 cells, as previously reported (22, 23) (Fig. 1, C and D).

We wondered whether production of Aβ40 and Aβ42 is correlated with particular species of CTFγ. The generated CTFγ was separated into soluble and membrane-bound forms. A larger proportion of CTFγ was released into soluble fraction during incubation, and the remaining proportion of CTFγ was left membrane-bound (see Table I). As a first step to purification of CTFγ, highly efficient immunoprecipitation with C4 (see “Experimental Procedures”) was employed. CTFγ immunoprecipitated from the soluble and membrane-bound fractions was subjected to mass spectrometric analysis; several species of CTFγ were identified in each of the membranes (Fig. 1E), the results of which were consistent with our previous report (12). The largest peak in wtAPP and wtPS2 membranes represented CTFγ 50–99. Notably, whereas CTFγ 49–99 was a minor signal in wtAPP and wtPS2 membranes, it became a major signal in mtAPP (V717F and mtPS2 (N141I) membranes. This raises the possibility that the extent of Aβ40 production is related to that of CTFγ 50–99 production, and that of Aβ42 production is related to that of CTFγ 49–99 production.

Production of Aβ42 and CTFγ 49–99 Is Increased in Membranes from CHO Cells Expressing mtPS1—To further confirm such increased production of CTFγ 49–99, we examined membrane fractions prepared from wt or mtPS1 (M146L, M233T, and G384A)-overexpressing cells. By incubation for 20 min, wtPS1 membranes produced Aβ40 predominantly, a situation that is similar to that of wtAPP and wtPS2 membranes (Fig. 2, A and B). On the other hand, mtPS1 membranes produced larger amounts of Aβ42, although the ratios of Aβ42 to Aβ40 differed among mutations (Fig. 2A). Concomitantly produced CTFγ was immunoprecipitated and subjected to mass spectrometric analysis (Fig. 2C). In the wtPS1 membranes, the largest signal was CTFγ 50–99. In contrast, in the mtPS1 membranes, the peaks for CTFγ 49–99 were found to be significantly larger. In M233T and G384A membranes in which Aβ42 was produced predominantly (Fig. 2A), the peaks for CTFγ 49–99 were much higher (Fig. 2C). In contrast, in M146L membranes, the peak for CTFγ 49–99 was almost at the same level as that in wtPS1 (Fig. 2C), and the Aβ42 production was only slightly increased (Fig. 2A).

Membranes from HEK293 Cells Expressing mtAPP Show an Increased Production of Aβ42 and CTFγ 49–99—To determine whether these differences were due to an idiosyncrasy of CHO cells, we examined the membrane fractions from wt, V717G, and V717F APP-overexpressing HEK293 cells. As shown in Fig. 3, A and B, whereas Aβ40 was predominantly produced in the membranes from wtAPP-overexpressing cells, the proportion of Aβ42 production was significantly increased in membranes from V717G and V717F APP-overexpressing cells. The CTFγ immunoprecipitated from membranes of HEK293 cells was similarly analyzed by mass spectrometry. The major species was CTFγ 50–99 in the membrane of wtAPP-overexpressing cells (Fig. 3C). On the other hand, the major peaks in the membranes from V717G- and V717F-overexpressing HEK293 cells were CTFγ 49–99, which is similar to mtAPP-overexpressing membranes.

**RESULTS**

| Production of Aβ42 and CTFγ 49–99 Is Increased in Membranes from CHO Cells Expressing mtAPP or mtPS2—To learn whether there is any relationship between Aβ40/42 and CTFγ, we used a cell-free Aβ or CTFγ production system consisting of membranes prepared from various stable transfectants. When membranes prepared from wtAPP-overexpressing or wtPS2-overexpressing cells were incubated, Aβ40 was a predominant species produced (Fig. 1A). In contrast, when the incubated membranes were from mtAPP (V717F) and mtPS2 (N141I) cells, the proportion of Aβ42 was increased specifically and predominantly (Fig. 1A). Besides Aβ40/42, the membranes also produced comparable amounts of CTFγ, a counterpart of Aβ (Fig. 1B). Membranes from the cells overexpressing a dominant |
FIG. 1. Production of Aβ and CTFγ in membranes from various cell lines. The membrane fractions prepared from various cell lines (overexpressing wAPP, mtAPP (V717F), wtPS2, mtPS2 (N141I PS2), and γ-secretase dominant negative mutant (D366A PS2)) were incubated and subjected to Western blotting for Aβ (A) and CTFγ (B), as described under “Experimental Procedures.” Similar levels of Aβ40 and increased levels of Aβ42 production were observed in mtAPP (V717F) membranes, as compared with wtAPP membranes. The extents of Aβ production cannot be directly compared between these cells, because the expression level of APP is higher in mtAPP cells than in wtAPP cells (data not shown). An arrowhead indicates CTFαβ, which cross-reacted with BC05. The ratio of Aβ40 (open bar) and Aβ42 (closed bar) relative to the total Aβ (Aβ40 + Aβ42) is provided in a lower panel in A. The asterisks indicate significant decrease or increase in the proportion (Student’s t test) as compared with that in wtAPP or wtPS2 membrane, respectively (*, p < 0.001; ***, p < 0.00001). C, the membrane fractions from wtPS2-overexpressing cell lines were incubated for 20 min in the presence of 1% Me2SO (control) or various concentrations of DFK-167. The amounts of total Aβ (assessed by 6E10) and CTFγ (left) and of Aβ40 and Aβ42 produced (right) relative to those produced in the absence of inhibitor are plotted. The asterisks indicate significant differences (Student’s t test) against 0 μM (*, p < 0.01; **, p < 0.0005). D, the levels (left panel) and
CTFγ was quantified with an amino acid sequencer, starting from the same amount of the membrane protein (40 mg). The amounts of CTFγ 50–99 and 49–99 were assessed by the yields of Val-50 at the first and second cycles, respectively. Similar values were obtained when assessed by the yields of Met-51 (data not shown). The left columns show representative values for CTFγ in soluble and membrane-bound fractions and total CTFγ. The proportions of soluble (n = 3; mean ± S.D.), membrane-bound (n = 2), and total (n = 2) CTFγ 49–99 and 50–99 represent the averages of three or two independent experiments, respectively (right column). The proportions of CTFγ 49–99 in the soluble fractions are significantly (p < 0.0005) increased in mtAPP cells and in mtPS2 cells. The asterisks indicate significant decrease and increase (Student’s t test) relative to nontreated sample (*, p < 0.05).

Table I

| Cell Lines | Molecular species | Yield | Proportions |
|------------|------------------|-------|-------------|
|            |                  | pmol  | %           | %           | %           | %           |
|            |                  |       | Soluble     | Membrane-bound | Total | Soluble | Membrane-bound | Total |
| wtAPP      | CTFγ 50–99       | 4.40  | 2.36        | 6.77         |        | 70.4 ± 4.3 | 74.9         | 71.3  |
|            | CTFγ 49–99       | 2.19  | 0.69        | 2.89         |        | 29.6 ± 4.3 | 25.1         | 28.7  |
| mtAPP (V717F) | CTFγ 50–99 | 1.13  | 1.55        | 2.68         |        | 21.9 ± 4.1 | 34.8         | 27.8  |
|            | CTFγ 49–99       | 4.45  | 2.91        | 7.36         |        | 78.1 ± 4.1 | 65.2         | 72.2  |
| wtPS2      | CTFγ 50–99       | 8.78  | 1.20        | 9.98         |        | 81.5 ± 3.7 | 72.3         | 80.7  |
|            | CTFγ 49–99       | 1.47  | 0.39        | 1.86         |        | 18.5 ± 3.7 | 27.7         | 19.3  |
| mtPS2 + DFK-167 | CTFγ 50–99 | 4.29  | 1.23        | 5.52         |        | 74.8 ± 0.9 | 72.8         | 74.6  |
|            | CTFγ 49–99       | 1.37  | 0.48        | 1.85         |        | 25.2 ± 0.9 | 27.2         | 25.4  |
| mtPS2 (N141I) | CTFγ 50–99     | 2.10  | 1.03        | 3.13         |        | 43.0 ± 2.8 | 64.8         | 49.7  |
|            | CTFγ 49–99       | 2.48  | 0.53        | 3.01         |        | 57.0 ± 2.8 | 35.2         | 50.3  |

Fig. 2. Production of Aβ and CTFγ in membranes from wt or mtPS1 cell lines and identity of CTFγ species. The membrane fractions prepared from wt and mtPS1 (M146L, M233T, and G384A) cell lines were incubated and subjected to Western blotting for Aβ (A) and CTFγ (B). An arrowhead indicates CTFγ/β, which cross-reacted with BC05. Aβ40 and Aβ42 were quantified by luminescent intensities. Bars, S.D. (n = 3). The asterisks indicate significant differences (Student’s t test) relative to wtPS1 membrane (*, p < 0.005; **, p < 0.0005; ***, p < 0.0001). C, the CTFγ immunoprecipitated from the soluble fraction of each cell membrane was subjected to mass spectrometric analysis. Representative spectra for CTFγ from the membranes of wtPS1, M146L, M233T, and G384A cell lines are shown.

CHO cells (see Fig. 1E). Interestingly, all cell lines of HEK293 cells appeared to produce significantly larger amounts of CTFγ 52–99 as compared with CHO cells, an observation that agrees with previous reports (14, 24). From these results, it is likely that, although e-cleavage may show some variability among cell lines, CTFγ 50–99 is a predominant species in wtAPP and wtPS1/2 membranes in which Aβ40 was predominantly produced, and CTFγ 49–99 production is increased in mtAPP and mtPS1/2 membranes, in which Aβ42 production was increased.

Quantification of CTFγ Species Using an Amino Acid Sequencer—Peak heights for several CTFγ species on mass spectrometric profiles may not be quantitative. Thus, we sought to

proportions (right panel) of produced Aβ40 and Aβ42 in the presence of 10 μM DFK-167. The asterisk indicates significant increase or decrease (Student’s t test) relative to the levels of Aβ40/42 and their proportions at 0 μM (*, p < 0.05; **, p < 0.0005). Bars, S.D. (n = 3) in A, C, and D, E, the CTFγ immunoprecipitated from the soluble fraction was subjected to mass spectrometric analysis. Representative spectra for CTFγ obtained from the membranes of wtAPP, mtAPP (V717F), wtPS2, and mtPS2 (N141I) cell lines are shown. The results obtained from the membrane-bound fraction were very similar to those from the soluble fraction in each cell line (data not shown).
quantify precisely each molecular species of CTFγ produced using an amino acid sequencer. The CTFγ species produced in membranes from four cell lines as shown in Fig. 1 were carefully analyzed. C4-immunoprecipitated CTFγ was fractionated by gel filtration, followed by RP-HPLC (Fig. 4A). The fractions corresponding to peaks 1 and 2 (see Fig. 4A) were subjected to amino acid sequence analysis. Five species of CTFγ were identified (see Fig. 4E); peak 1 contained four molecular species (CTFγ 52–99, 51–99, and 50–99 and a trace amount of 49–99), whereas peak 2 contained two molecular species (CTFγ 49–99 and 48–99). Among them, CTFγ 50–99 and 49–99 accounted for >90% of CTFγ in each cell line, and the remaining species (CTFγ 52–99, 51–99, and 48–99) were at detectable but negligible levels. Other species (e.g., CTFγ 53–99, 54–99, etc.) were undetectable by sequence analysis. The accurate proportion of CTFγ 49–99/50–99 for each cell line is provided in Table I.

The proportions of CTFγ 49–99 were 28.7 and 19.3% for wtAPP and wtPS2 membranes, respectively (Table I and Fig. 4B). Both membranes produced Aβ40 predominantly (Fig. 1A). In contrast, the proportions of CTFγ 49–99 were increased to 72.2 and 50.3% for the membranes of mtAPP (V717F) and mtPS2 (N141I) cells, respectively, both of which produced increased amounts of Aβ42 (Fig. 1A). These data were consistent with the results of mass spectrometric analysis, indicating that the evaluation by mass spectrometry is very informative in the present study.

Proportion of CTFγ 50–99 Is Decreased at a Low Concentration of DFK-167—To further verify that the increase in the proportion of CTFγ 49–99 is independent of the cell type, we next examined the effect of DFK-167, an inhibitor that at low concentrations specifically inhibited the production of Aβ40 and paradoxically increased Aβ42 production (Fig. 1D), on the proportion of CTFγ species produced by the membranes (Table I). The proportions of CTFγ 50–99 and CTFγ 49–99 in the soluble fraction of 10 μM inhibitor-treated wtPS2 membrane were significantly (p < 0.05) changed to 74.8 ± 0.9% from 81.5 ± 3.7% and to 25.2 ± 0.9% from 18.5 ± 3.7%, respectively, concomitantly with the proportion of Aβ40 produced decreasing to ~60% from more than 80% and Aβ42 produced increasing to ~40% from less than 20% (Figs. 1D and 4B). These results strongly suggest that Aβ40 and Aβ42 production is related to that of CTFγ 50–99 and CTFγ 49–99, respectively (Fig. 4C).

Additive Effects of mtAPP and mtPS1 on Aβ and CTFγ Species—An increase in CTFγ 49–99 production accompanying a decrease in CTFγ 50–99 production was observed in mtAPP-, mtPS1-, and mtPS2-overexpressing cells. To examine whether the combination of mtAPP and mtPS1 has an additional effect on the produced CTFγ species, we established stable transfectants co-expressing mtAPP (V717F; hereafter VF) and wt or mtPS1 (M233T or G384A). The ratio of Aβ40/42 produced in wtPS1 membranes (VF/wtPS1) was almost the same as that observed in V717F-only membranes (Fig. 5, A and B). On the other hand, the proportion of Aβ42 production was significantly increased in mtPS1 membranes (VF/mtPS1) as compared with VF/wtPS1. This additive effect of mtAPP and mtPS1 on Aβ42 production was consistent with the previous report (25). Interestingly, the membranes from VF/ G384A cells produced much smaller amounts of Aβ and CTFγ and accumulated CTFαβ (Fig. 5, A and B). It is possible that this mtPS1-associated γ-secretase has a lower affinity and/or inefficient cleavage for V717F substrate.

We next analyzed CTFγ species produced in these membranes by mass spectrometry. The major species was CTFγ
FIG. 4. Purification of CTF by RP-HPLC and proportion of CTF 49–99/CTF 50–99. A, pooled CTF after gel filtration was further purified by RP-HPLC. Representative RP-HPLC profiles show that the relative heights of peaks 1 and 2 differed among cell lines. Note that peaks 1 and 2 appear to consist of multiple peaks. Peaks indicated by asterisks were buffer-derived. B, the proportions of CTF 49–99 (closed bar) and 50–99 (open bar) produced by the membranes from wtAPP, mtAPP (V717F), wPS2, and mPS2 (N141I) cells are shown. An increase in the proportion of CTF 49–99 and a decrease in the proportion of CTF 50–99 by DFK-167 treatment at 10 μM were significant in the soluble fraction (see Table I). C, the two major sites each for γ- and ε-cleavage of APP according to Aβ numbering (top) are illustrated. Aβ40 and Aβ42 production is related to that of CTF 50–99 and CTF 49–99, respectively.

FIG. 5. Production of Aβ and CTF by the membranes from mtAPP/mtPS1 double transfectants and identity of CTF species. The membrane fractions prepared from transfectants expressing mtAPP (V717F) alone and double transfectants expressing mtAPP and wt or mtPS1 (M233T and G384A) were incubated and subjected to Western blotting for Aβ (A) and CTF (B). An arrowhead indicates CTFαβ. Aβ40 and Aβ42 were quantified by luminescent intensities. Bars, S.D. (n = 3). The asterisks indicate significant differences (Student’s t test) relative to the proportions of Aβ40/42 in VF/wtPS1 membrane (*, p < 0.05; **, p < 0.005). C, the CTF immunoprecipitated from the soluble fraction of each cell membranes was subjected to mass spectrometric analysis. Representative spectra for CTF in the membranes from VF/wt, M233T, or G384A PS1 double transfectants are shown.

49–99 in the VF/wtPS1 membranes, similarly to V717F membranes (Fig. 5C). On the other hand, the peaks for CTF 50–99 were found to be smaller in both VF/M233T and VF/G384A membranes. In these membranes, the peaks of CTF 50–99 were also remarkably smaller as compared with mtPS1-only membranes (Figs. 2C and 5C). Because mass spectrometric analyses are not so quantitative, we could not accurately assess whether or how much CTF 49–99 in these membranes was increased as compared with that in VF/wtPS1 membrane. However, these results strongly suggest that
mtAPP and mtPS1 have an additive effect on CTFγ species produced.

**DISCUSSION**

It has long been claimed that mutations of APP clustered close to the C terminus of Aβ alter the cleavage specificity of γ-secretase, leading to increased Aβ42 production. Accordingly, we thought that such APP mutations would preferentially affect γ-cleavage without substantial effects on ε-cleavage that occurs near the cytoplasmic membrane boundary. However, most unexpectedly, as shown in Figs. 1E and 3C, mtAPP (V171F) had a remarkable effect on the γ-cleavage and generated a large amount of CTFγ 49–99 (e.g. 72.2%). This large increase in the production of long CTFγ was far more than increased proportion of Aβ42 produced (e.g. 39.5 ± 0.98% for V171F in CHO cells) (Fig. 1A). As is the case, the extent of Aβ42 production is not proportionate to the extent of long CTFγ production. For example, mtPS2 (N141I) generated Aβ42 predominantly (81.6 ± 2.1%), whereas the proportion of CTFγ 49–99 was 50.3%, and similar results can be seen among other mutations of PS1 (Fig. 2). Furthermore, the presence of 10 µM DFK-167 increased production of Aβ42 by about 2-fold, and the extent of long Aβ42 (1–3.0-fold). Thus, the proportion of Aβ40 and Aβ42 does not faithfully reflect that of CTFγ 50–99 and 49–99.

In contrast to CHO cells, HEK293 cell membranes produced a significant amount of another CTFγ, CTFγ 52–99 (Fig. 3C). Moreover, CTFγ 52–99 was significantly increased in mtAPP membranes. Even in CHO cells, CTFγ 52–99 appears to be very slightly increased in the membranes from mtAPP and mtPS1/2 cells (Figs. 1E, 2C, and 2C). Thus, the production of CTFγ 52–99 may also be related to that of Aβ42, although further study is required to confirm this view.

A number of recent reports have argued for a reciprocal relationship between γ-cleavage and Notch site 3 cleavage. Several PS1 mutations that increase the production of Aβ42 were found to reduce Notch site 3 cleavage (24, 26–29) and production of CTFγ as well (24, 29). These results are consistent with the present quantitative data based on sequencing, showing that the total amounts of CTFγ produced in the mtPS2 membranes are reduced to ~50% of those of wtPS2 membranes (Table 1). Thus, mtPS2 causes not only a reduction in the extent of γ-cleavage but also an alteration in the proportion of CTFγ species (i.e. an increase of long CTFγ). This contrasts with mtAPP, which does not affect the extent of ε-cleavage (24) but produces an unexpectedly large amount of CTFγ 49–99 (Fig. 1E, 3C, and 4C). It is therefore reasonable to speculate that this particular characteristic underlies the mechanisms for increased Aβ42 production by each mtAPP and mtPS1/2 membranes. The differing characteristics of mtPS1/2 and mtAPP might further complicate the relationship between γ- and ε-cleavage.

Thus far identified substrates of γ-secretase are cleaved at or near the cytoplasmic membrane boundary, and are some are also in the middle of the transmembrane domain (16, 30–33). This suggests that γ-cleavage and ε-cleavage are universal phenomena in a particular subset of type I membrane proteins. Further, a potential link between Aβ42 and CTFγ 49–99 raises further questions. Which cleavage, γ- or ε-cleavage, comes first, and how does one cleavage affect the other? It is possible to detect a particular CTFγ longer than CTFγ 48–99 by either mass spectrometric analysis or sequencing. One possible interpretation is that CTFβ is first cleaved at the ε-site, and thus cleaved products (Aβ1–48 and 1–49) undergo γ-cleavage, and Aβ40/42 are secreted. Previous studies reported the existence of long Aβ1–46 by mass spectrometric analysis (34, 35). However, in our hands, longer Aβ species were undetectable, although we cannot rule out the possibility that the steady-state levels of such intermediates in the lysates are below the detection limit. It is also possible that γ- and ε-cleavage occur simultaneously or nearly so along the CTFβ molecule, leaving a small hydrophobic membrane peptide that must be difficult to isolate and detect.

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