Selective Reconstitution and Recovery of Functional γ-Secretase Complex on Budded Baculovirus Particles*\(^{\S}\)

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In vitro reconstitution of functions of membrane proteins is often hampered by aggregation, misfolding, or lack of post-translational modifications of the proteins attributable to overexpression. To overcome this technical obstacle, we have developed a method to express multimeric integral membrane proteins in extracellular (budded) baculovirus particles that are released from Sf9 cells co-infected with multiple transmembrane proteins. We applied this method to the reconstitution of γ-secretase, a membrane protease complex that catalyzes the intramembrane cleavage of β-amyloid precursor protein to release Aβ peptides, the major component of amyloid deposits in Alzheimer brains as well as of Notch. When we co-infected Sf9 cells with human presenilin 1 (PS1), nicastrin, APH-1a, and PEN-2, a high-molecular-weight membrane protein complex that contained PS1 exclusively in its fragment form associated with three other cofactor proteins was reconstituted and recovered in a highly γ-secretase-active state in budded virus particles, whereas nonfunctional PS1 homolog proteins massively contaminated the parental Sf9 cell membranes. The relative γ-secretase activity (per molar PS1 fragments) was concentrated by ~2.5 fold in budded virus particles compared with that in Sf9 membranes. The budded baculovirus system will facilitate structural and functional analyses of γ-secretase, as well as screening of its binding molecules or inhibitors, and will also provide a versatile methodology for the characterization of a variety of membrane protein complexes.

A wide variety of protein expression systems using heterologous organisms have been applied to the structural and functional analyses of multispan membrane proteins. However, overexpression and recovery of membrane proteins in a biologically active state are often hampered by the following problems. First, a significant proportion of overexpressed proteins tend to misfold and aggregate without forming proper tertiary structures. Second, lack of appropriate post-translational modifications, interaction with binding-partner proteins, or proper intracellular transport render the membrane proteins stuck within the early compartments of intracellular membrane trafficking. Furthermore, serious contamination of biologically inactive, nascent, or misfolded proteins is inevitable, which makes the analysis of the functional form of protein difficult.

The infection of recombinant baculovirus into Spodoptera frugiperda insect cells (Sf9) is one of the most widely used methodologies that achieves a high level of expression and yield of recombinant membrane proteins (1). Baculovirus produces budded virus (BV), i.e. particles that contain viral DNA enveloped within Sf9 cell-derived membranes, during its life cycle (Fig. 1). Recently, it has been reported that a seventh membrane-spanning, G protein-coupled receptor was successfully recovered in BV in a functional form complexed with heterotrimetric G proteins and adenylyl cyclase (2, 3). Using this strategy, sterol regulatory element-binding protein-2 (SREBP) and SREBP cleavage-activating protein, which are endoplasmic reticulum-resident membrane proteins in mammalian cells, also were displayed on BV particles with a lesser extent of aggregation and degradation compared with those in cellular membrane fractions (4). However, selective reconstitution of a protein complex composed of multiple integral membrane proteins in a biologically active state has not been achieved yet.

γ-Secretase is a membrane-bound protease complex composed of at least four transmembrane proteins, i.e. presenilin (PS), which is predicted to harbor the catalytic center as an aspartic protease, as well as nicastrin (NCT), APH-1, and PEN-2 as essential transmembrane cofactor proteins (5, 6). γ-Secretase is responsible for the intramembrane cleavage of a subset of type 1, single-span membrane proteins (7), including β-amyloid precursor protein (βAPP), to form amyloid β peptides (Aβ); Aβ is the major component of amyloid deposits in the brains of patients with Alzheimer’s disease and is closely linked to the pathogenesis of Alzheimer’s disease (8). Another important substrate of γ-secretase is Notch, the intracellular domain (ICD) of which is released by γ-secretase and enters the nucleus, thereby transducing intercellular information during tissue development and renewal through transactivation of gene expression (8). Overexpression of the four putative

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1 The abbreviations used are: BV, budded virus; SREBP, sterol regulatory element-binding protein-2; PS, presenilin; NCT, nicastrin; βAPP, amyloid-β precursor protein; Aβ, amyloid β peptide; ICD, intracellular domain; HMW, high molecular weight; CHAIPS0, 3-[3-chloroamidopropyl]dime-thylylammonio]-2-hydroxy-1-propanesulfonate; DAPT, N-(3,5-difluorophenacetyl)-l-alanyll-(S)-phenylglycine t-butyl ester; ELISA, enzyme-linked immuno sorbent assay.

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Reconstitution of γ-Secretase Activity on Budded Baculovirus

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**Fig. 1. Life cycle of baculovirus and generation of BV.** Baculovirus undergoes a biphasic life cycle in its lepidopteron host. Intracellular copies of viral DNA are targeted into BV or alternatively incorporated within occlusion-derived virus (ODV) within the nucleus.

γ-secretase components in mammalian or Drosophila cells led to the maturation of γ-secretase protein complex, i.e. endoproteolysis of PS to generate fragments (the amino-terminal fragment and the carboxyl-terminal fragment, respectively) and high-molecular-weight (HMW) complex formation, as well as an up-regulation of the proteolytic activity (9–12). Importantly, overexpression of all four components in Saccharomyces cerevisiae, which lacks endogenous γ-secretase activity, successfully reconstituted the γ-secretase activity (13), strongly suggesting that a membrane protein complex composed of the four proteins represents the minimal framework of γ-secretase.

Selective recovery and purification of the active form of γ-secretase complex is essential to its functional and structural characterization, although a majority of exogenously overexpressed PS polypeptides remain inactive in the low-molecular-weight complex that chiefly contains PS holoprotein, presumably because of lack of binding with other essential cofactor proteins (14). Here, we applied the baculovirus BV particle technology to the reconstitution of γ-secretase and succeeded in the selective recovery of the active form of γ-secretase, composed of fragment forms of PS1 complexed with NCT, APH-1a, and PEN-2.

**MATERIALS AND METHODS**

**Construction of Expression Plasmid and BV Particle Preparation**—Full-length cDNAs encoding PS1 in pcDNA3 vector (Invitrogen) (15) and NCT fused with V5/His tags in pEF6-V5/His vector (Invitrogen) (16) were amplified by polymerase chain reaction and inserted into pBlueBac4.5 (Invitrogen). A cDNA encoding human APH-1a was subcloned into a pEF4-myc/His A vector (Invitrogen). We next infected the Sf9 cells with various combinations of recombinant baculoviruses encoding other γ-secretase components (i.e. NCT, APH-1a, and PEN-2, collectively referred to as cofactors) in addition to PS1. Immunoblot analysis showed that PS1 underwent endoproteolysis to generate an amino-terminal fragment and a carboxyl-terminal fragment corresponding to the size of mammalian PS1 fragments only when all four components were infected, although a major proportion of recombinant PS1 polypeptide remained as a holoprotein (Fig. 2A, right lane). In contrast, PS1 fragments were hardly generated upon expression of PS1 plus one or two of the cofactor proteins (Fig. 2A and data not shown). Co-immunoprecipitation studies revealed that any two of the components among PS1, NCT, APH-1a, or PEN-2 were co-immunoprecipitated in Sf9 membranes that expressed the four components by immunoblotting. We then analyzed the BV fractions from Sf9 cells infected with combinations of the four components by immunoblottting. In contrast to Sf9 cell membranes, PS1 polypeptides accumulated almost exclusively in the fragment forms, together with NCT, APH-1a, and PEN-2 in the BV fractions, only when all four components were infected (Fig. 2A, right lane). Co-immu-
noprecipitation studies revealed that amino-terminal fragment and carboxyl-terminal fragment of PS1 were associated as a heterodimer and formed a protein complex with NCT, APH-1, and PEN-2 in a similar manner to that in mammalian cells (Fig. 2B). We next fractionated the Sf9 membranes and BV particles solubilized in CHAPSO by size-exclusion chromatography in 0.25% CHAPSO containing buffer, a condition in which solubilized membranes exhibit γ-secretase activity. Under this condition, PS1 fragments and γ-secretase activities in HeLa membranes were separated in fractions >1 megadalton, as described previously (26). Immunoblot analysis of the eluates of Sf9 cells and BV revealed that fragment forms of PS1 as well as other components were predominantly separated in fractions of HMW ranges >1 megadalton (Fig. 2C). Similar results were obtained by glycerol velocity centrifugation (Fig. 1S). These data suggest that recombinant γ-secretase components undergo complex formation in Sf9 cells infected by baculovirus in a similar manner to that in mammalian cells, and that the HMW complex associated with PS1 fragments, but not a holoprotein, is selectively accumulated on BV particles.

Characterization of Reconstituted γ-Secretase Activity in Vitro in Sf9 Cell Membranes or on BV—To characterize the endogenous and reconstituted γ-secretase activities in Sf9 cells, we solubilized the Sf9 membranes with 1% CHAPSO and incubated them with the recombinant C100 substrate in vitro (12, 17). We did not detect any de novo generation of Aβ peptides from the recombinant substrate in mock-infected Sf9 membranes, even in the presence of phosphatidylcholine (Fig. 3A, left), which increases in vitro γ-secretase activity in solubilized mammalian membranes (27). Some γ-secretase activity was observed, but this activity was not sensitive for γ-secretase inhibitors (see below). This was in agreement with the observation that Sf9 cells do not harbor an endogenous γ-secretase activity (24, 25). Overexpression of combinations of any three components did not reconstitute γ-secretase activities to elicit de novo Aβ generation in vitro (data not shown), whereas overexpression of all four components exhibited a dramatic up-regulation of the proteolytic activity in Sf9 cell membranes (Fig. 3A). Interestingly, Aβ42 was the predominantly generated Aβ species relative to Aβ40 when we used Sf9 cells as an enzyme source, and the percentage of Aβ42 as a fraction of de novo generated total Aβ amounted to ~60%. We next analyzed the γ-secretase activity in CHAPSO-solubilized BV particles from multiply infected Sf9 cells and observed a robust Aβ-generating activity in the BV fraction from cells infected with all four components. These activities were ~10-fold higher than that detected in mammalian HeLa cell membranes, the latter harboring the highest level of endogenous γ-secretase activity in mammalian cells (Fig. 3B) (17). Similarly to the results in Sf9 membranes, Aβ42 was again the predominant species in de novo generated Aβ (%Aβ42: mean ± S.E., 65.5 ± 7.1%). Coincubation with γ-secretase inhibitors, L-685,458 or DAPT, abolished the activity reconstituted in BV fractions (Fig. 3C). Notably, reconstituted γ-secretase activity was less sensitive to L-685,458 at 100 nM concentration (65.2% inhibition), which completely abolished the γ-secretase activity in the

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HeLa membrane, whereas DAPT potently inhibited the γ-secretase activity (94.0% inhibition; residual activity was almost equivalent to that in mock-infected BV). These results indicate that γ-secretase activity can be reconstituted in Sf9 cells and BV associated with robust γ-secretase activity by overexpression of all four components of human γ-secretase, as shown previously in other organisms (9–13).

We then examined the γ-secretase activity reconstituted by infection of mutant PS1 carrying D257A or D385A mutations, which is known to be inactive for PS1 endoproteolysis as well as for γ-secretase activity (28), together with the three cofactor proteins. Recombinant PS1/D257A as well as PS1/D385A did not undergo endoproteolysis in Sf9 cells as well as in BV fractions (Fig. 4A), although they formed a >1-megadalton protein complex with other components (Fig. 4B). Reconstituted γ-secretase complex containing recombinant PS1/D257A or PS1/D385A showed almost no Aβ-generating activity, whereas a low level of residual γ-secretase activity that was almost comparable with that in mock-infected BV was observed (Fig. 4C). The identity of this endogenous, γ-secretase-like enzymatic activity should be examined further. Thus, intramembranous aspartates are required for endoproteolysis as well as for the Aβ-generating enzymatic activity in a reconstituted γ-secretase complex in an insect cell system, similarly to that seen in mammalian cells (28).

γ-Secretase cleaves several type I membrane proteins other than βAPP, including Notch, at multiple sites within the transmembrane domain and liberate the ICD (7). To examine whether reconstituted γ-secretase activity in BV cleaves other substrates, we examined the ICD generation from recombinant βAPP or Notch carboxyl-terminal fragments (C100 and N102, respectively (17)) using the BV particles as the source of γ-secretase in vitro. The recombinant substrates were cleaved to generate ICDs by the γ-secretase activity from BV fractions of Sf9 cells infected with all four components, which were sensitive to γ-secretase inhibitors, in a similar manner to those with HeLa-derived γ-secretase (Fig. 5). Finally, we compared the γ-secretase activities in solubilized BV particles and in Sf9 membranes. When normalized by the expression levels of the PS1 amino-terminal fragment, the relative γ-secretase activity in BV particles was increased by ~2.5 fold compared with that of solubilized Sf9 cell membranes, suggesting that the γ-secretase active form of PS1 fragments was enriched in BV fractions (Fig. 6). Thus, we were able to reconstitute the γ-secretase activity.

**Fig. 3. Characterization of reconstituted γ-secretase activity.** A, levels of de novo generated Aβ40 (□) and Aβ42 (■)(n = 2) generated from recombinant C100 by in vitro coincubation with solubilized Sf9 membranes infected with PS1, NCT-V5/His, APH-1a-myc/His, or His-PEN-2. B, levels of Aβ (n = 3, mean ± S.E.) generated from recombinant C100 by in vitro coincubation with solubilized BV particles infected with various combinations of γ-secretase components. HeLa cell membrane fraction was used as a control. C, effect of γ-secretase inhibitors. Recombinant substrates were coincubated with solubilized BV or HeLa membrane in the presence of DAPT (100 μM) or L-685,458 (100 nM). Both inhibitors inhibited the reconstituted γ-secretase activity.
complex in BV membranes. This complex contained the fragment forms of PS1 and was enriched in proteolytic activity.

DISCUSSION

In this study, we selectively reconstituted and recovered human γ-secretase complex in an active form, using BV particles. The baculovirus/insect cell system is one of the most widely used methodologies for overexpression of recombinant proteins, including multispan integral membrane proteins (reviewed by Massotte (1)). We used this system for the reconstitution of γ-secretase complex by gel filtration. Note that mutant PS1 holoproteins were detected within fractions with molecular masses over 1 megadalton. γ-secretase activity of reconstituted aspartate-mutant γ-secretase complex (n = 3, mean ± S.E.). Mutation in either of the aspartates virtually abolished the γ-secretase activity in vitro. wt, wild type.

Recent reports that G-protein-coupled receptors can be reconstituted in a functional form, recruiting endogenous Gα subunit or adenylyl cyclase as interactors in the extracellular BV particles released from infected Sf9 cells (2), prompted us to apply this strategy to the effective reconstitution of γ-secretase composed of multiple integral membrane proteins. Surprisingly, BV fractions from Sf9 cells infected with all four components almost exclusively contained the γ-secretase complex harboring PS1 fragments, with minimal contamination of nascent or misfolded PS1 holoproteins. Reconstituted γ-secretase
on BV again reproduced similar biochemical and enzymatic properties to those in mammalian cells (i.e., HMW protein complex formation, inhibition by γ-secretase inhibitors, requirement of the transmembrane aspartates, and cleavage of both βAPP and Notch). The mechanism whereby reconstituted γ-secretase in SF9 cells or BV shifted the de novo generation of Aβ to Aβ42 species remains unclear, but it is highly reminiscent of the overproduction of Aβ42 by expression of wild-type Drosophila PS in mammalian cells, which predominantly generates Aβ40 in Drosophila cells (23); the three-dimensional structure of the catalytic pocket of γ-secretase, which determines the positions of the preferred γ-secretase cleavage site, may be altered depending on the cell species or the environment surrounding the enzyme complex. Moreover, very high γ-secretase activity (~10 fold that of mammalian HeLa cells) was recovered in BV. Notably, the relative γ-secretase activity standardized by the levels of PS1 fragments also was higher than that in the SF9 cell membrane. This supports the notion that a limited proportion of HMW protein complex composed of the four components is γ-secretase-active, as suggested by the limited recovery of active γ-secretase complex using activity-dependent purification (29, 30), and that some additional factors or conditions are necessary for the full activation of γ-secretase (31).

The mechanism whereby “active form” γ-secretase complex containing PS1 fragments is selectively accumulated on BV is unknown. One possibility would be that the BV envelope may preferentially recruit membranes derived from plasma membrane or late intracellular membrane compartments, which are enriched in an active form of γ-secretase (32-34). However, the observation that endoplasmic reticulum-resident membrane proteins, such as SREBP or SREBP cleavage-activating protein, can be recovered on BV as well (4) may argue against this view. Folding, complex formation, and functions of integral membrane proteins may be properly achieved on BV, based on some unknown mechanism specific to the life cycle of baculovirus. Intriguingly, the major phospholipid consisting of BV envelope is phosphatidylserine, whereas phosphatidylcholine and phosphatidyethanolamine are the major phospholipids in host SF9 cells (35-37). Recently, palmitoylation of GP64 protein, which is highly abundant and necessary for efficient budding and production of BV as well as its entry into host cells, was reported (38). However, GP64 was not detected in lipid rafts in SF9 cells, and its palmitoylation did not affect the apparent exclusion of GP64 from lipid rafts, although lipid rafts have been shown to play roles in the infection cycles of several viruses (e.g., HIV-1 and influenza virus) (39). These results may suggest that specific membrane domains other than rafts may be involved in baculovirus budding as well as in the effective formation of a functional protein complex in SF9 cells.

We and others have reconstituted the γ-secretase complex by overexpression of the four components in mammalian, Drosophila, or yeast cells (10-13). However, the enzymatic activity in BV was by far higher than those in mammalian or Drosophila cells (Fig. 3B and data not shown). Considering the previous observations on the effective incorporation of endogenous proteins into the functional protein complex (e.g., exogenous G protein coupled receptor and endogenous G protein) observed in BV (2), it is possible that the γ-secretase complex reconstituted in BV incorporated relevant binding partner proteins that were endogenously present in BV, resulting in the formation of a “hyperactive” enzyme complex. Approximately 30 proteins have been identified by SDS-PAGE and Coomassie blue staining in the BV envelope (35). Recently, proteomic analysis of the occlusion-derived form of baculovirus was reported, although similar data on BV have not been publicized yet (40). Thus, it would be possible to identify the missing interacting partners of the PS complex in SF9 proteins based on proteomic analysis of the γ-secretase reconstituted on BV. Other possible applications of the reconstituted γ-secretase on BV may include production of active form-specific or blocking antibodies against γ-secretase and generation of an immobilized γ-secretase as a biochip sensor that may be applicable to large-scale screening of physiological interactors or low-molecular-weight compounds for drug discovery (4).

A number of integral membrane proteins with important functions, including ion channels or receptor proteins, form hetero-multimeric protein complexes (1). Purification of a large quantity of protein complex in a biologically active state is indispensable to the biochemical, functional, and especially structural studies of these complexes. The purification procedures presented in this report for recombinant membrane proteins in an active form in BV fractions, recovered from multiply infected SF9 cells, should provide a versatile methodology for the characterization of various integral membrane protein

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4 T. Hamakubo, Y. Urano, R. Fukuda, and T. Kodama, unpublished result.
complexes. It should also open up the way to one of the ultimate goals in γ-secretase research—to crystallize the complex and unravel its three-dimensional structure in an active state.

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REFERENCES

1. Massotte, D. (2003) Biochim. Biophys. Acta 1610, 77–89
2. Leisel, T. P., Ansanay, H., St-Ôge, S., Gay, B., Boulanger, P., Stroberg, A. D., Marullo, S., and Bouvier, M. (1997) Nat. Biotech. 15, 1300–1304
3. Masuda, K., Itoh, H., Sakihama, T., Akiyama, C., Takahashi, K., Fukuda, R., Yokomizo, T., Shimizu, T., Kodama, T., and Hamakubo, T. (2003) J. Biol. Chem. 278, 24552–24562
4. Urano, Y., Yamaguchi, M., Fukuda, R., Masuda, K., Takahashi, K., Uchiyama, Y., Iwanari, H., Jiang, B.Y., Naito, M., Kodama, T., and Hamakubo, T. (2003) Biochem. Biophys. Res. Commun. 308, 191–196
5. Wolfe, M. S. (2002) Nat. Rev. Drug Discov. 1, 859–866
6. De Strooper, B. (2003) Neuron 38, 9–12
7. Haass, C., and Steiner, H. (2002) Trends Cell Biol. 12, 556–562
8. Selkoe, D. J., and Kopan, R. (2003) Annu. Rev. Neurosci. 26, 565–597
9. Hu, Y., and Fortini, M. E. (2003) J. Cell Biol. 161, 685–690
10. Kin, S. H., Iseuchi, T., Yu, C., and Sisodia, S. S. (2003) J. Biol. Chem. 278, 33992-34002
11. Kimberly, W. T., LaViolette, M. J., Osztaszewski, B. L., Ye, W., Wolfe, M. S., and Selkoe, D. J. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 6382–6387
12. Takasugi, N., Tomita, T., Hayashi, I., Tsurowska, M., Niimura, M., Takahashi, Y., Thinakaran, G., and Iwatsubo, T. (2003) Nature 422, 438–441
13. Edsbaer, D., Winkler, E., Regula, J. T., Pesold, B., Steiner, H., and Haass, C. (2003) Nat. Cell Biol. 5, 486–488
14. Thinakaran, G., Harris, C. L., Ratovitski, T., Davenport, F., Slunt, H. H., Price, D. L., Borchelt, D. R., and Sisodia, S. S. (1997) J. Biol. Chem. 272, 29415–29422
15. Tomita, T., Maruyama, K., Saito, T. C., Kume, H., Shinozaki, K., Tokuhiro, S., Capell, A., Walter, J., Grenberg, J., Haass, C., Iwatsubo, T., and Obata, K. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 2025–2030
16. Tomita, T., Katayama, R., Takikawa, T., and Iwatsubo, T. (2002) FEBS Lett. 530, 117–121
17. Takahashi, Y., Hayashi, I., Tominari, Y., Rikimaru, K., Morohashi, Y., Kan, T., Natsume, H., Fukuyama, T., Tomita, T., and Iwatsubo, T. (2003) J. Biol. Chem. 278, 18664–18670
18. Kan, T., Tominari, Y., Morohashi, Y., Natsugari, H., Tomita, T., Iwatsubo, T., and Fukuyama, T. (2003) Chem. Commun. 7, 2244–2245
19. Tomita, T., Watabiki, T., Takikawa, R., Morohashi, Y., Takasugi, N., Kopan, R., De Strooper, B., and Iwatsubo, T. (2001) J. Biol. Chem. 276, 33273–33281
20. Tomita, T., Takikawa, R., Koyama, M., Morohashi, Y., Takasugi, N., Saito, T. C., Maruyama, K., and Iwatsubo, T. (1999) J. Neurosci. 19, 10627–10634
21. Tomita, T., Tokuhiro, S., Hashimoto, T., Akas, K., Saito, T. C., Maruyama, K., and Iwatsubo, T. (1998) J. Biol. Chem. 273, 21153–21160
22. Morohashi, Y., Hamano, N., Haya, S., Tatsukawa, T., Watabiki, T., Takasugi, N., Imaizumi, Y., Tomita, T., and Iwatsubo, T. (2002) J. Biol. Chem. 277, 14965–14975
23. Takasugi, N., Takahashi, Y., Morohashi, Y., Tomita, T., and Iwatsubo, T. (2002) J. Biol. Chem. 277, 50198–50205
24. October, J. N., Esselmann, R., Taison, B., Menager, J., Czech, C., and Mercen, L. (2000) J. Biol. Chem. 275, 1025–1028
25. Pitsi, D., Kienlen-Campard, P., and Octave, J. N. (2002) J. Neurochem. 83, 380–389
26. Li, Y. M., Lai, M. T., Xu, M., Huang, Q., DiMuzio-Mower, J., Sardana, M. K., Shi, X. P., Yin, K. C., Shafer, J. A., and Gardell, S. J. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 6138–6143
27. Kimberly, W. T., Esler, W. P., Ye, W., Ostaszewski, B. L., Gao, J., Diehl, T., Selkoe, D. J., and Wolfe, M. S. (2003) Biochemistry 42, 137–144
28. Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999) Nature 399, 513–517
29. Beher, D., Fricke, M., Nadin, A., Clarke, E. E., Wrigley, J. D., Li, Y. M., Culvenor, J. G., Masters, C. L., Harrison, T., and Shearman, M. S. (2003) Biochemistry 42, 8133–8142
30. Lai, M. T., Chen, E., Crosthanel, M. C., DiMuzio-Mower, J., Xu, M., Huang, Q., Price, E., Register, R. B., Shi, X. P., Donoviel, D. B., Bernstein, A., Hazuda, D., Gardell, S. J., and Li, Y. M. (2003) J. Biol. Chem. 278, 22475–22481
31. Iwatsubo, T. (2004) Mol. Psychiatry 9, 8–10
32. Xia, W., Zhang, J., Ostaszewski, B. L., Kimberly, W. T., Seubert, P., Koo, E. H., Shen, J., and Selkoe, D. J. (1998) Biochemistry 37, 16460–16471
33. Iwata, H., Tomita, T., Maruyama, K., and Iwatsubo, T. (2001) J. Biol. Chem. 276, 21678–21685
34. Kaether, C., Lannich, S., Edsbaer, D., Ertl, M., Riedtorf, J., Capell, A., Steiner, H., and Haass, C. (2002) J. Cell Biol. 155, 551–561
35. Brauner, S. C., and Summers, M. D. (1994) Virology 202, 315–328
36. Yeh, L. H., Bajpai, R. K., and Sun, G. Y. (1997) Lipids 32, 481–487
37. Marheineke, K., Grunewald, S., Christie, W., and Reissner, H. (1998) FEBS Lett. 441, 49–52
38. Zhang, S. X., Han, Y., and Blussard, G. (2003) J. Virol. 77, 6265–6273
39. Manes, S., del Real, G., and Martinez-A, C. (2003) Nat. Rev. Immunol. 3, 557–568
40. Brauner, S. C., Russell, W. K., Rosas-Aceita, G., Russell, D., and Summers, M. D. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 9797–9802