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Intramembrane Proteolysis by γ-Secretase*

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γ-Secretase mediates the final proteolytic cleavage, which liberates amyloid β-peptide (Aβ), the major component of senile plaques in the brains of Alzheimer disease patients. Therefore, γ-secretase is a prime target for Aβ-lowering therapeutic strategies. γ-Secretase is a protein complex composed of four different subunits, presenilin (PS), APH-1, nicastrin, and PEN-2, which are most likely present in a 1:1:1:1 stoichiometry. PS harbors the catalytically active site, which is critically required for the aspartyl protease activity of γ-secretase. Moreover, numerous familial Alzheimer disease-associated mutations within the PSs increase the production of the aggregation-prone and neurotoxic 42-amino acid Aβ. Nicastrin may serve as a substrate receptor, although this has recently been challenged. PEN-2 is required to stabilize PS within the γ-secretase complex. No particular function has so far been assigned to APH-1. The four components are sufficient and required for γ-secretase activity. At least six different γ-secretase complexes exist that are composed of different variants of PS and APH-1. All γ-secretase complexes can exert pathological Aβ production. Assembly of the γ-secretase complex occurs within the endoplasmic reticulum, and only fully assembled and functional γ-secretase complexes are transported to the plasma membrane. Structural analysis by electron microscopy and chemical cross-linking reveals a water-containing cavity, which allows intramembrane proteolysis. Specific and highly sensitive γ-secretase inhibitors have been developed; however, they interfere with the physiological function of γ-secretase in Notch signaling and thus cause rather significant side effects in human trials. Modulators of γ-secretase, which selectively affect the production of the pathological 42-amino acid Aβ, do not inhibit Notch signaling.

γ-Secretase Releases Amyloid β-Peptide by an Intramembrane Cleavage

AD[3] is the most frequent dementia in the world, affecting millions of people. The amyloid cascade hypothesis, which describes a number of consecutive steps finally leading to synapse dysfunction, synapse loss, and neuronal cell death, is based on the cellular production of Aβ, which initiates the deadly cascade (1). Aβ metabolism is in the center of intense research because Aβ lowering strategies may finally lead to therapeutic treatment or even prevention of AD. The highly amyloidogenic Aβ is released from its precursor, APP, by two sequential proteolytic cleavages mediated by β- and γ-secretases. β-Secretase (BACE1 (β-site APP-cleaving enzyme 1)) (reviewed by Cole and Vassar (64) in the second article of this minireview series) removes the bulk of the ectodomain of APP and leaves behind a small membrane-retained CTF (Fig. 1*A*). Whereas β-secretase cleavage is mediated by a rather conventional aspartyl protease, the second cut is mediated by an unusual protease, γ-secretase. With the identification of APP (2), it became apparent that Aβ has to be liberated from the membrane probably by an intramembrane cleavage event (Fig. 1, *A* and *B*). However, in such a hydrophobic environment, water molecules are either absent or at least very rare. Therefore, biochemists did not believe in physiologically occurring intramembrane cleavage of peptide bonds, and in fact, AD researchers assumed for a long time that Aβ could be generated only upon membrane destruction and subsequent release of the precursor into the brain parenchyma, where conventional proteases would then have access. However, three laboratories independently discovered that Aβ is physiologically produced throughout life by a cellular pathway that must involve intramembrane proteolysis because membrane damage and release of the precursor could be clearly excluded (3–5). Thus, APP processing turned out to be the first example not only for intramembrane cleavage but also for a cellular pathway now termed regulated intramembrane proteolysis. Regulated intramembrane proteolysis describes the sequential processing of an increasing number of single-pass transmembrane proteins, which in some cases is coupled to nuclear signaling (6). In the first step, shedding removes large parts of the substrate's ectodomain. In many cases, the sheddases belong to the family of the ADAM (a disintegrin and metalloprotease) proteases. Other sheddases such as BACE1 may cleave a more restricted panel of substrates (see review article by Cole and Vassar (64)). The remaining stub is then cleaved within its TMD by intramembrane-cleaving proteases termed I-CLiPs (7). In several cases, it is now known that the intramembrane cleavage results in the release of an ICD, which is involved in nuclear signaling and transcriptional regulation (7, 8). However, there is strong evidence that at least the intramembrane-cleaving γ-secretase may also fulfill

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[3] The abbreviations used are: AD, Alzheimer disease; Aβ, amyloid β-peptide; APP, β-amyloid precursor protein; CTF, C-terminal fragment; TMD, transmembrane domain; ICD, intracellular domain; PS, presenilin; SPP, signal peptide peptidase; SPPL, SPP-like; FAD, familial AD; NCT, nicastrin; GSI, γ-secretase inhibitor.

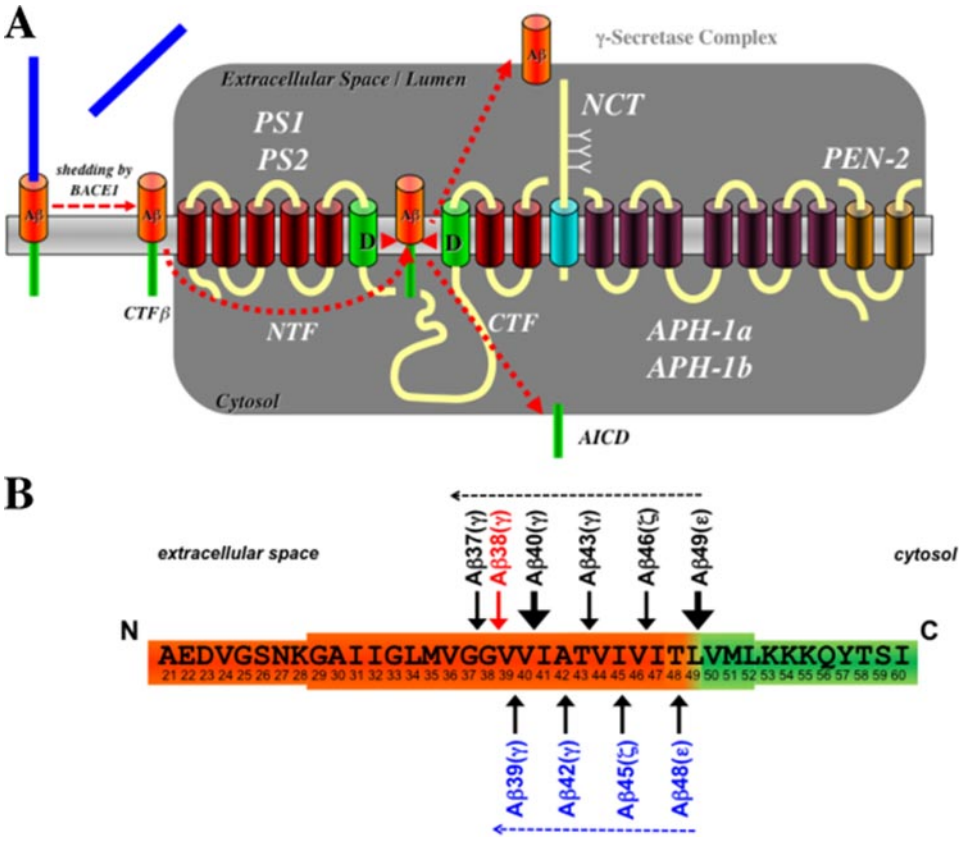

FIGURE 1. *A*, schematic representation of APP processing by β- and γ-secretases. *B*, multiple intramembrane cleavages are mediated by γ-secretase. The known cleavage sites and the resulting processing products are indicated. Major cleavage sites in the APP TMD are indicated by *large arrows*. The Aβ domain is depicted in *orange*, and the APP ICD (*AICD*) in *green*. The TMD is enlarged. *Dashed arrows* indicate the potential direction of the cleavages. For details, see text. *NTF*, N-terminal fragment.

a second and probably major function. In that case, γ-secretase seems to be required for the efficient destruction of membrane-retained protein fragments. This activity has been referred to as the "membrane-proteasome" function of γ-secretase (9). Whereas γ-secretase takes apart membrane stubs, the final degradation of the ICD (see for example, Ref. 10) and the secreted peptides (see for example, Ref. 11) is performed by other proteases.

γ-Secretase Is a G*X*GD-type Aspartyl Protease Activity

γ-Secretase is the founding member of the intramembrane-cleaving aspartyl proteases. Although γ-secretase was the first proposed protease activity, which mediates intramembrane cleavage, its identification took a long time, and structural data are still available only to a very limited extent (12). There is a good reason for this "slowed" progress as compared with other intramembrane-cleaving proteases because γ-secretase turned out to be a complicated complex composed of four essential subunits (13), the precise interactions of which are technically very difficult to investigate. One of the subunits, PS, contains the two catalytically active aspartate residues (14), which are located within TMD6 and TMD7 (Fig. 1*A*). The N-terminal catalytically active site of PS is embedded in a conserved YD motif, whereas the C-terminal active-site domain contains the equally conserved G*X*GD motif (15), which now serves as the family-characterizing name of the *GXGD-type aspartyl proteases* (16). Besides γ-secretase/PS, two families of related proteases, which also belong to the G*X*GD-type aspartyl proteases, have been identified. These include the procaryotic type 4 prepilin peptidases and SPP, as well as the SPP homologs, the SPPL proteases (see also the related data on SPP/SPPL by Fluhrer *et al.*[4]). The catalytic subunit of γ-secretase, PS, is the best studied G*X*GD-type I-CLiP (17) and represents the prototype of this novel class of aspartyl proteases (16). PS is a polytopic membrane protein consisting of nine TMDs and is endoproteolytically cleaved into an ~30-kDa N-terminal and ~20-kDa C-terminal fragment (Fig. 1*A*) (18). This cleavage occurs within the large cytoplasmic loop between TMD6 and TMD7 within a short hydrophobic domain that is believed to dive into the membrane and is very likely an autoproteolytic event (13), although this has not been formally proven. Endoproteolysis is not an absolute prerequisite for γ-secretase activity (19) as initially suggested (14), but it may rather be required to maximize stable conformation of the two active-site aspartates in TMD6 and TMD7 of PS.

PS was initially identified as a protein genetically linked to AD pathogenesis by the discovery that mutations in the two homologs of PS in humans, PS1 and PS2, are associated with FAD (summarized in Refs. 8 and 17). These FAD mutations affect the cleavage specificity of γ-secretase by causing an increase of the 42-amino acid variant of Aβ (Fig. 1*B*), very similar to that of a subset of FAD mutations identified earlier in the C-terminal part of the APP TMD close to the γ-secretase cleavage sites (20, 21). Thus, mutations in both the protease and its substrate cause an alteration in γ-secretase cleavage specificity and give rise to enhanced production of the disease-causing Aβ42. Mutations in PS1 are the predominant cause of FAD, as more than 150 FAD mutations have been identified in this gene compared with only few mutations in the PS2 gene. This is likely due to its lower neuronal expression (22) and/or specific activity (23, 24) compared with PS1. The greater impact of PS1 than PS2 on γ-secretase activity is also reflected by the finding that the PS1 knock-out allows only very little residual γ-secretase activity, which is completely lost in the PS1/PS2 double knock-out (25–28). γ-Secretase has a number of substrates other than APP, which are all type I transmembrane proteins that undergo shedding prior to the cleavage by γ-secretase (16). The cell differentiation regulator protein Notch is appar-

[4] R. Fluhrer, H. Steiner, and C. Haass, submitted for publication.

ently the most important physiological substrate of γ-secretase, as genetic ablations of PS and other γ-secretase subunits (see below) are associated with severe Notch phenotypes (29).

Apart from the catalytic subunit PS, three other integral membrane proteins, NCT, APH-1 (anterior pharynx-defective-1), and PEN-2 (presenilin enhancer-2), are essential γ-secretase complex subunits (30, 31). NCT is an ~100-kDa type I membrane glycoprotein with a large ectodomain and a short cytoplasmic domain (32) and serves as a γ-secretase substrate receptor (33). γ-Secretase substrates are initially recognized by NCT, which is believed to identify the free N terminus of a γ-secretase substrate (33). The initial recognition of substrates by NCT requires the bulk of the substrate ectodomain to be removed by shedding. Shedded substrates of γ-secretase then typically retain 20–30 amino acids of their ectodomain (34). However, the model that NCT functions as a substrate receptor (33) that selects substrates of appropriate length has recently been challenged (35). Following the initial binding, the substrate moves to another, second binding site, the docking site, prior to substrate cleavage. Pharmacological mapping of the docking site to the PS N-terminal/C-terminal fragment interface in very close proximity to the active site might implicate the G*X*GD active-site motif as part of this site (36). The functional role of the docking site is still unclear, but it is possible that this site contributes to or serves as a final substrate identification/selection site. Indeed, this is suggested by the recent observation that residue *X* of the G*X*GD active-site motif (Leu^{383} in PS1) is critical for APP/Notch substrate selectivity (37).

The other two complex components, the ~20-kDa seven-TMD protein APH-1 and the smallest subunit, the ~10-kDa hairpin PEN-2 protein, are highly hydrophobic subunits (30, 31). PEN-2 is required for the stabilization of the PS fragments in the complex (38, 39), whereas the function of APH-1 is currently unclear. Two homologs of APH-1, APH-1a and APH-1b, have been identified in humans. APH-1a exists in a long (APH-1aL) and short (APH-1aS) splice variant differing in the C terminus of the protein. Because neither the catalytic subunits PS1 and PS2 nor APH-1a (including splice variants) and APH-1b occur in the same complexes (40, 41), a minimal set of at least six (not taking PS splice variants into account) distinct γ-secretase complexes exist in human cells. Whether these complexes differ in specificity for the many γ-secretase substrates has been only poorly investigated so far. Subtle differences have been reported for APH-1a- and APH-1b-containing complexes (42). PS1 complexes have a higher specific activity for APP compared with PS2 complexes (23, 24, 43). Complexes containing either PS1 or PS2 FAD mutant variants in combination with any of the APH-1 species preserve pathogenic activity, *i.e.* Aβ42-increasing activity (43). This suggests that no discrete pathogenic γ-secretase complexes exist.

Assembly and Stoichiometry of the γ-Secretase Complex

The four subunits of γ-secretase assemble into a functional complex in the early compartments of the secretory pathway (reviewed in Ref. 44). NCT and APH-1 form an initial assembly intermediate, which stabilizes the PS holoprotein. Finally, PEN-2 assembles into this ternary complex and triggers endoproteolysis of PS. When assembly is completed, the complex travels to its functional sites at the plasma membrane and the late compartments of the secretory pathway. Complex formation is tightly regulated and depends on the availability of the individual subunits, which is apparently maintained by a balanced expression of the subunits. The subunits, which are in excess over the others and thus lack their stabilizing partners, are rapidly degraded. Once assembled into the full complex or into an assembly intermediate, the subunits become highly stable. Apart from degradation, complex formation is also controlled by the presence of the auxiliary protein Rer1, which helps to retain unassembled PEN-2 (45) or NCT within the endoplasmic reticulum (46). Several TMDs in the individual subunits, as well as the C termini of PEN-2 and PS, are essential domains and provide interaction sites crucial for γ-secretase assembly (44). Native gel electrophoresis suggests that γ-secretase has a molecular mass of ~500 kDa, which is approximately twice the sum of the molecular masses of the four essential subunits (~200–250 kDa) (8, 47, 48). Although quantitation of the four subunits in an active γ-secretase complex suggests a 1:1:1:1 stoichiometry (48), evidence for PS dimerization has also been provided (49). In addition, the complex may contain additive nonessential regulatory subunits such as TMP21 (50) and CD147 (51) (see below).

A Water-containing Cavity

Because of the tremendous difficulty in obtaining high amounts of pure crystallizable γ-secretase, high resolution structural information is not yet available for this I-CLiP. However, the first structural studies by Lazarov *et al.* (12) using electron microscopy recently showed a large spherical structure of γ-secretase with the interesting feature of two small central openings, one oriented to the extracellular space and the other to the cytosol. These small openings in the particle might represent exit sites of an internal water-containing cavity for the cleavage products. Consistent with a water-containing cavity are two recent studies showing that the active-site region in TMD6 and TMD7 of PS is water-accessible (52, 53). Very recently, it was demonstrated that TMD9 and the hydrophobic domain in the large cytoplasmic loop of PS (between TMD6 and TMD7) are dynamic parts of the water-containing cavity; moreover, the conserved PAL motif at the cytoplasmic edge of TMD9 directly contributes to the catalytic center because it can be cross-linked to the active site located within TMD6 (54).

Stepwise Intramembrane Cleavage by γ-Secretase

Once the substrate has accessed the catalytic site, it is cleaved within its TMD at two major topologically distinct sites termed the ε- and γ-sites (Fig. 1*B*) (summarized in Ref. 20). The current model suggests that the cleavage occurs in a stepwise manner, with γ-secretase cutting the APP CTF first at the ε-site, which is close to the cytoplasmic border of the membrane. This cleavage releases the APP ICD from the membrane and leaves a long Aβ species in the membrane. Further cleavages then occur roughly every third amino acid down the α-helical TMD via the ζ- to the γ-site until the peptide is short enough to be released from the membrane (Fig. 1*B*). The cleavages at the individual sites are heterogeneous and give rise to roughly two different product lines, Aβ49 (ε)-Aβ46 (ζ)-Aβ43 (γ)-Aβ40 (γ) and Aβ37 (γ),

whereas the other product line generates Aβ48 (ε)-Aβ45 (ζ)-Aβ42 (γ)-Aβ39 (γ) (Fig. 1*B*) (55). The latter line of products can indeed be detected in some but not all PS FAD mutants investigated so far (55). However, the precise mechanism of sequential processing is still unclear. Moreover, the two product lines described above do not explain the generation of Aβ38. Interestingly, dimerization of the APP TMD has been suggested to be another important mechanistic determinant for the generation of Aβ42. Dimerization of the APP CTF is mediated by G*XXX*G dimerization motifs, which, when disrupted, leads to a rather selectively reduced production of Aβ42 without significantly affecting the generation of Aβ40 (56).

γ-Secretase as a Therapeutic Target

Inhibition of γ-secretase activity is an important approach for therapeutic treatment of AD, and GSI identification and development are in an advanced state (57). GSIs targeting the active site of γ-secretase in PS interfere with the cleavage of other physiologically important substrates such as Notch, by blocking the generation of the Notch ICD. GSIs thereby affect normal cell differentiation and are associated with severe side effects (58). The development of APP/Notch-selective inhibitors is thus a major focus of the pharmaceutical industry and academic institutions. A subset of nonsteroidal anti-inflammatory drugs has been shown to selectively decrease cleavage at the γ-42 site and to enhance cleavage at the γ-38 site without affecting cleavage at the γ-40 site (59). Although a number of compounds that modulate γ-secretase cleavage have been identified, Aβ38 and Aβ42 production is not generally coupled (60). Most important, however, nonsteroidal anti-inflammatory drugs do not affect cleavage at the ε-site and thus do not affect the γ-secretase-mediated release of the ICD from Notch (59). Modulation of γ-secretase cleavage specificity at the γ-sites might represent a safer way to inhibit Aβ42 production, and the development of potent modulators is on the way. However, a so far unresolved problem is that the currently known inhibitors and modulators are in many cases ineffective in inhibiting the pathological activity of FAD mutant PS (60, 61), suggesting structural changes of mutant γ-secretase complexes, rendering them resistant to inhibitor/modulator binding.

Another interesting observation that might be exploited for drug targeting in the future is the selective modulation of ε-site *versus* γ-site cleavage of APP by γ-secretase interactors such as TMP21 (50). Although the mechanism is so far unclear, deficiency in TMP21 causes a selective increase of γ-secretase cleavage at the γ-site without affecting the ε-site. This negative mode of modulation of cleavage at the γ-site has also been observed for CD147 (51), another putative regulatory γ-secretase subunit. However, it should also be noted that, more recently, CD147 has been implicated in the degradation of secreted Aβ and may thus not be directly involved in modulating γ-secretase activity (62). Interestingly, mutations in the extracellular juxtamembrane domain of APP have recently been shown to affect γ-site cleavage without affecting γ-secretase cleavage at the ε-site (63). Thus, there are also sequence determinants affecting γ-site *versus* ε-site cleavage within the substrate. Clearly, further studies are required to clarify the mode of action of these modulatory γ-secretase interactors. Nevertheless, γ-secretase-related research progressed tremendously over the last years and enabled chemists for the first time to develop and investigate sensitive inhibitors/modulators, which in the long run may turn out to pave the way to an amyloid-lowering therapy.

REFERENCES

1. Hardy, J., and Selkoe, D. J. (2002) *Science* **297,** 353–356
2. Kang, J., Lemaire, H. G., Unterbeck, A., Salbaum, J. M., Masters, C. L., Grzeschik, K. H., Multhaup, G., Beyreuther, K., and Muller-Hill, B. (1987) *Nature* **325,** 733–736
3. Busciglio, J., Gabuzda, D. H., Matsudaira, P., and Yankner, B. A. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90,** 2092–2096
4. Shoji, M., Golde, T. E., Ghiso, J., Cheung, T. T., Estus, S., Shaffer, L. M., Cai, X. D., McKay, D. M., Tintner, R., Frangione, B., and Younkin, S. G. (1992) *Science* **258,** 126–129
5. Haass, C., Schlossmacher, M. G., Hung, A. Y., Vigo-Pelfrey, C., Mellon, A., Ostaszewski, B. L., Lieberburg, I., Koo, E. H., Schenk, D., Teplow, D. B., and Selkoe, D. J. (1992) *Nature* **359,** 322–325
6. Brown, M. S., Ye, J., Rawson, R. B., and Goldstein, J. L. (2000) *Cell* **100,** 391–398
7. Wolfe, M. S., and Kopan, R. (2004) *Science* **305,** 1119–1123
8. Haass, C. (2004) *EMBO J.* **23,** 483–488
9. Kopan, R., and Ilagan, M. X. (2004) *Nat. Rev. Mol. Cell Biol.* **5,** 499–504
10. Edbauer, D., Willem, M., Lammich, S., Steiner, H., and Haass, C. (2002) *J. Biol. Chem.* **277,** 13389–13393
11. Iwata, N., Tsubuki, S., Takaki, Y., Shirotani, K., Lu, B., Gerard, N. P., Gerard, C., Hama, E., Lee, H. J., and Saido, T. C. (2001) *Science* **292,** 1550–1552
12. Lazarov, V. K., Fraering, P. C., Ye, W., Wolfe, M. S., Selkoe, D. J., and Li, H. (2006) *Proc. Natl. Acad. Sci. U. S. A.* **103,** 6889–6894
13. Edbauer, D., Winkler, E., Regula, J. T., Pesold, B., Steiner, H., and Haass, C. (2003) *Nat. Cell Biol.* **5,** 486–488
14. Wolfe, M. S., Xia, W., Ostaszewski, B. L., Diehl, T. S., Kimberly, W. T., and Selkoe, D. J. (1999) *Nature* **398,** 513–517
15. Steiner, H., Kostka, M., Romig, H., Basset, G., Pesold, B., Hardy, J., Capell, A., Meyn, L., Grim, M. G., Baumeister, R., Fechteler, K., and Haass, C. (2000) *Nat. Cell Biol.* **2,** 848–851
16. Haass, C., and Steiner, H. (2002) *Trends Cell Biol.* **12,** 556–562
17. Steiner, H. (2008) *Curr. Alzheimer Res.* **5,** 147–157
18. Thinakaran, G., Borchelt, D. R., Lee, M. K., Slunt, H. H., Spitzer, L., Kim, G., Ratovitsky, T., Davenport, F., Nordstedt, C., Seeger, M., Hardy, J., Levey, A. I., Gandy, S. E., Jenkins, N. A., Copeland, N. G., Price, D. L., and Sisodia, S. S. (1996) *Neuron* **17,** 181–190
19. Steiner, H., Romig, H., Pesold, B., Philipp, U., Baader, M., Citron, M., Loetscher, H., Jacobsen, H., and Haass, C. (1999) *Biochemistry* **38,** 14600–14605
20. Haass, C., and Selkoe, D. J. (2007) *Nat. Rev. Mol. Cell Biol.* **8,** 101–112
21. Scheuner, D., Eckman, C., Jensen, M., Song, X., Citron, M., Suzuki, N., Bird, T. D., Hardy, J., Hutton, M., Kukull, W., Larson, E., Levy-Lahad, E., Viitanen, M., Peskind, E., Poorkaj, P., Schellenberg, G., Tanzi, R., Wasco, W., Lannfelt, L., Selkoe, D., and Younkin, S. (1996) *Nat. Med.* **2,** 864–870
22. Sherrington, R., Froelich, S., Sorbi, S., Campion, D., Chi, H., Rogaeva, E. A., Levesque, G., Rogaev, E. I., Lin, C., Liang, Y., Ikeda, M., Mar, L., Brice, A., Agid, Y., Percy, M. E., Clerget-Darpoux, F., Piacentini, S., Marcon, G., Nacmias, B., Amaducci, L., Frebourg, T., Lannfelt, L., Rommens, J. M., and St George-Hyslop, P. H. (1996) *Hum. Mol. Genet.* **5,** 985–988
23. Lai, M. T., Chen, E., Crouthamel, 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
24. Bentahir, M., Nyabi, O., Verhamme, J., Tolia, A., Horre, K., Wiltfang, J., Esselmann, H., and De Strooper, B. (2006) *J. Neurochem.* **96,** 732–742
25. Herreman, A., Serneels, L., Annaert, W., Collen, D., Schoonjans, L., and De Strooper, B. (2000) *Nat. Cell Biol.* **2,** 461–462
26. Herreman, A., Hartmann, D., Annaert, W., Saftig, P., Craessaerts, K., Sern-

eels, L., Umans, L., Schrijvers, V., Checler, F., Vanderstichele, H., Baekelandt, V., Dressel, R., Cupers, P., Huylebroeck, D., Zwijsen, A., Van Leuven, F., and De Strooper, B. (1999) *Proc. Natl. Acad. Sci. U. S. A.* **96,** 11872–11877
27. De Strooper, B., Saftig, P., Craessaerts, K., Vanderstichele, H., Guhde, G., Annaert, W., Von Figura, K., and Van Leuven, F. (1998) *Nature* **391,** 387–390
28. Zhang, Z., Nadeau, P., Song, W., Donoviel, D., Yuan, M., Bernstein, A., and Yankner, B. A. (2000) *Nat. Cell Biol.* **2,** 463–465
29. Selkoe, D., and Kopan, R. (2003) *Annu. Rev. Neurosci.* **26,** 565–597
30. Francis, R., McGrath, G., Zhang, J., Ruddy, D. A., Sym, M., Apfeld, J., Nicoll, M., Maxwell, M., Hai, B., Ellis, M. C., Parks, A. L., Xu, W., Li, J., Gurney, M., Myers, R. L., Himes, C. S., Hiebsch, R. D., Ruble, C., Nye, J. S., and Curtis, D. (2002) *Dev. Cell* **3,** 85–97
31. Goutte, C., Tsunozaki, M., Hale, V. A., and Priess, J. R. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 775–779
32. Yu, G., Nishimura, M., Arawaka, S., Levitan, D., Zhang, L., Tandon, A., Song, Y. Q., Rogaeva, E., Chen, F., Kawarai, T., Supala, A., Levesque, L., Yu, H., Yang, D. S., Holmes, E., Milman, P., Liang, Y., Zhang, D. M., Xu, D. H., Sato, C., Rogaev, E., Smith, M., Janus, C., Zhang, Y., Aebersold, R., Farrer, L. S., Sorbi, S., Bruni, A., Fraser, P., and St George-Hyslop, P. (2000) *Nature* **407,** 48–54
33. Shah, S., Lee, S. F., Tabuchi, K., Hao, Y. H., Yu, C., LaPlant, Q., Ball, H., Dann, C. E., III, Sudhof, T., and Yu, G. (2005) *Cell* **122,** 435–447
34. Struhl, G., and Adachi, A. (2000) *Mol. Cell* **6,** 625–636
35. Chávez-Gutiérrez, L., Tolia, A., Maes, E., Li, T., Wong, P. C., and De Strooper, B. (2008) *J. Biol. Chem.* **283,** 20096–20105
36. Kornilova, A. Y., Bihel, F., Das, C., and Wolfe, M. S. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102,** 3230–3235
37. Yamasaki, A., Eimer, S., Okochi, M., Smialowska, A., Kaether, C., Baumeister, R., Haass, C., and Steiner, H. (2006) *J. Neurosci.* **26,** 3821–3828
38. Prokop, S., Shirotani, K., Edbauer, D., Haass, C., and Steiner, H. (2004) *J. Biol. Chem.* **279,** 23255–23261
39. Hasegawa, H., Sanjo, N., Chen, F., Gu, Y. J., Shier, C., Petit, A., Kawarai, T., Katayama, T., Schmidt, S. D., Mathews, P. M., Schmitt-Ulms, G., Fraser, P. E., and St George-Hyslop, P. (2004) *J. Biol. Chem.* **279,** 46455–46463
40. Shirotani, K., Edbauer, D., Prokop, S., Haass, C., and Steiner, H. (2004) *J. Biol. Chem.* **279,** 41340–41345
41. Hebert, S. S., Serneels, L., Dejaegere, T., Horre, K., Dabrowski, M., Baert, V., Annaert, W., Hartmann, D., and De Strooper, B. (2004) *Neurobiol. Dis.* **17,** 260–272
42. Serneels, L., Dejaegere, T., Craessaerts, K., Horre, K., Jorissen, E., Tousseyn, T., Hebert, S., Coolen, M., Martens, G., Zwijsen, A., Annaert, W., Hartmann, D., and De Strooper, B. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102,** 1719–1724
43. Shirotani, K., Tomioka, M., Kremmer, E., Haass, C., and Steiner, H. (2007) *Neurobiol. Dis.* **27,** 102–107
44. Kaether, C., Haass, C., and Steiner, H. (2006) *Neurodegener. Dis.* **3,** 187–312
45. Kaether, C., Scheuermann, J., Fassler, M., Zilow, S., Shirotani, K., Valkova, C., Novak, B., Kacmar, S., Steiner, H., and Haass, C. (2007) *EMBO Rep.* **8,** 743–748
46. Spasic, D., Raemaekers, T., Dillen, K., Declerck, I., Baert, V., Serneels, L., Fullekrug, J., and Annaert, W. (2007) *J. Cell Biol.* **176,** 629–640
47. Edbauer, D., Winkler, E., Haass, C., and Steiner, H. (2002) *Proc. Natl. Acad. Sci. U. S. A.* **99,** 8666–8671
48. Sato, T., Diehl, T. S., Narayanan, S., Funamoto, S., Ihara, Y., De Strooper, B., Steiner, H., Haass, C., and Wolfe, M. S. (2007) *J. Biol. Chem.* **282,** 33985–33993
49. Schroeter, E. H., Ilagan, M. X., Brunkan, A. L., Hecimovic, S., Li, Y.-M., Xu, M., Lewis, H. D., Saxena, M. T., De Strooper, B., Coonrod, A., Tomita, T., Iwatsubo, T., Moore, C. L., Goate, A., Wolfe, M. S., Shearman, M., and Kopan, R. (2003) *Proc. Natl. Acad. Sci. U. S. A.* **100,** 13075–13080
50. Chen, F., Hasegawa, H., Schmitt-Ulms, G., Kawarai, T., Bohm, C., Katayama, T., Gu, Y., Sanjo, N., Glista, M., Rogaeva, E., Wakutani, Y., Pardossi-Piquard, R., Ruan, X., Tandon, A., Checler, F., Marambaud, P., Hansen, K., Westaway, D., St George-Hyslop, P., and Fraser, P. (2006) *Nature* **440,** 1208–1212
51. Zhou, S., Zhou, H., Walian, P. J., and Jap, B. K. (2005) *Proc. Natl. Acad. Sci. U. S. A.* **102,** 7499–7504
52. Sato, C., Morohashi, Y., Tomita, T., and Iwatsubo, T. (2006) *J. Neurosci.* **26,** 12081–12088
53. Tolia, A., Chavez-Gutierrez, L., and De Strooper, B. (2006) *J. Biol. Chem.* **281,** 27633–27642
54. Tolia, A., Horré, K., and De Strooper, B. (2008) *J. Biol. Chem.* **283,** 19793–19803
55. Qi-Takahara, Y., Morishima-Kawashima, M., Tanimura, Y., Dolios, G., Hirotani, N., Horikoshi, Y., Kametani, F., Maeda, M., Saido, T. C., Wang, R., and Ihara, Y. (2005) *J. Neurosci.* **25,** 436–445
56. Munter, L. M., Voigt, P., Harmeier, A., Kaden, D., Gottschalk, K. E., Weise, C., Pipkorn, R., Schaefer, M., Langosch, D., and Multhaup, G. (2007) *EMBO J.* **26,** 1702–1712
57. Churcher, I., and Beher, D. (2005) *Curr. Pharm. Des.* **11,** 3363–3382
58. Geling, A., Steiner, H., Willem, M., Bally-Cuif, L., and Haass, C. (2002) *EMBO Rep.* **3,** 688–694
59. Weggen, S., Eriksen, J. L., Das, P., Sagi, S. A., Wang, R., Pietrzik, C. U., Findlay, K. A., Smith, T. E., Murphy, M. P., Bulter, T., Kang, D. E., Marquez-Sterling, N., Golde, T. E., and Koo, E. H. (2001) *Nature* **414,** 212–216
60. Page, R. M., Baumann, K., Tomioka, M., Perez-Revuelta, B. I., Fukumori, A., Jacobsen, H., Flohr, A., Luebbers, T., Ozmen, L., Steiner, H., and Haass, C. (2008) *J. Biol. Chem.* **283,** 677–683
61. Czirr, E., Leuchtenberger, S., Dorner-Ciossek, C., Schneider, A., Jucker, M., Koo, E. H., Pietrzik, C. U., Baumann, K., and Weggen, S. (2007) *J. Biol. Chem.* **282,** 24504–24513
62. Vetrivel, K. S., Zhang, X., Meckler, X., Cheng, H., Lee, S., Gong, P., Lopes, K. O., Chen, Y., Iwata, N., Yin, K.-J., Lee, J.-M., Parent, A. T., Saido, T. C., Li, Y.-M., Sisodia, S. S., and Thinakaran, G. (2008) *J. Biol. Chem.* **283,** 19489–19498
63. Ren, Z., Schenk, D., Basi, G. S., and Shapiro, I. P. (2007) *J. Biol. Chem.* **282,** 35350–35360
64. Cole, S. L., and Vassar, R. (2008) *J. Biol. Chem.* **283,** 29621–29625

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