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Presenilins

Structural Aspects and Posttranslational Events

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Abstract

Most of early-onset forms of Alzheimer's disease (AD) are caused by inherited mutations located on chromosomes 14 and 1, the gene products of which have been recently identified and referred to as presenilins 1 (PS1) and 2 (PS2), respectively. The first phenotypic alterations triggered by mutated PS were reported to be an increased production of the amyloid peptide (Aβ) and, more precisely, its 42 amino-acids long counterpart Aβ42. This overproduction is thought to be responsible for the genesis of the senile plaques that invade the cortical and subcortical areas of these AD-affected brains. The discovery of PSs has triggered numerous studies aimed at better understanding their normal physiology and the dysfunctions brought by the mutations that could explain, at least in part, the neurodegenerative process taking place in this syndrome. In this review, I will focus on the structural aspects of PS and on the various posttranscriptional events they undergo. I will also briefly discuss that current hypotheses concerning their normal functions and the influence of FAD-linked mutations.

Index Entries: Alzheimer's disease; presenilins; βAPP processing; Aβ peptide; mutations; embryogenesis; development; apoptosis; maturation; caspases.

Introduction

Alzheimer's disease (AD) is an age-related syndrom characterized by mnesic alterations and cognitive disorders. Most of AD etiology is sporadic, but about 10% of cases are familial (FAD) and are characterized by a much earlier onset and rapid evolution. These aggressive forms of AD are caused by missense mutations borne by chromosomes 21, 14, and 1 (for reviews *see* refs. *1–4*), the gene products of which were identified as the β-amyloid precursor protein (βAPP) *(5–7)* and presenilins 1 and 2, respectively *(8–10)*. It is important to emphasize that, when mutated, these distinct proteins trigger a common phenotypic alteration of βAPP maturation, i.e., an increased recovery of the amyloid β-peptide, and more particularly

of its 42 amino-acids-long readily aggregable counterpart (Aβ42).

The similar elevation of this pathogenic βAPP catabolite in FAD of various origins labels Aβ as a common denominator occuring early in the etiology of AD, and thereby reinforces the amyloidogenic hypothesis for this neurodegenerative disease. As a corrolary, the elucidation of the physiology of PSs and the understanding of their function(s) should hopefully help us to better understand, at least in part, some etiological aspects of this devastating disease.

Here, I will center my review on the structural aspects concerning PS and the posttranscriptional events they undergo. Finally, I will briefly report on the various putative functions of PS and the possible alterations brought by FAD-linked mutations.

Presenilin Genes, mRNAs and Pathogenic Mutations

Presenilin 1 was first identified by positional cloning *(8)*. The structural organization of the mouse PS1 gene indicates that it spans over 50–75 kb and is organized into 12 exons, the first two corresponding to 5′ untranslated regions *(11,12)*. The longest open reading frame, encoded by exons 3–12, leads to a 467 amino-acid-long protein.

Analysis of PS1 mRNA reveals a major species of about 3 kb and a minor message of about 7 kb *(8)*. Northern blot analysis indicates that these transcripts are ubiquitously expressed in human brain nuclei and peripheral tissues *(8)*. *In situ* hybridization studies indicate that the localization of PS1 mRNA appears similar in human normal and sporadic Alzheimer's disease-affected brains *(13)* and occurs earlier than βAPP mRNA expression in embryonic rat brain *(14)*.

Several splice variants of PS1 have been identified. One of these lacks the sequence encoded by exon 8 that corresponds to one of the putative transmembrane domains of the protein. Another very abundant variant derives from alternative splicing at exon 3, and generates the deletion of a tetrapeptide (Val-Arg-Ser-Gln) that appears to exhibit a consensus phosphorylation site *(8)*, but its functionality remains to be established.

The number of mutations identified on the PS1 sequence continues to increase tremendously. To date, more than 50 missense mutations have been detected (for review *see* ref. *15*), some of them causing AD in a 29-yr-old patient *(16)* or even leading to death in another patient who was only 28 yr old *(17)*. In addition, a mutation corresponding to the abolishment of the splice acceptor site of exon 10 (formerly identified as exon 9) results in the deletion of the sequence encoded by this exon (referred to as ΔE10-PS1 below, *18*).

The gene organization of PS2 is very similar to that of PS1, i.e., 12 exons corresponding to a 24 kb genomic region. Exons 3–12 give rise to an open reading frame encoding a 448 amino-acid-long protein *(19,20)*. Two messenger RNAs of 2.4 and 2.8 kb can be detected, the former being distributed within brain, lung, liver, and placenta, whereas the longer form appears to be more ubiquitously expressed *(10, 21)*. Unlike for PS1, very few mutations on PS2 have been characterized. A mutation has been found in a Volga German kindred that replaces the asparagine residue in position 141 of PS2 for an isoleucine *(21)*. The second substitution is rare and corresponds to the change of methionine 239 for a valine residue *(22)*.

Structure, Topology, and Localization of Presenilins

Hydropathy plots of PS sequences predict a transmembrane protein, the exact structure of which still remains to be discussed. Several groups have postulated an even number of hydrophobic domains (6 or 8) for PS1 *(23, 24)* or *sel-12 (25)*, its *Caenorhabditis elegans* counterpart. This implies that N- and C-terminal PS1 moieties would be directed to the same cell

compartment that is thought to be cytoplasmic *(23–25)*, a hypothesis corroborated by another recent study *(26)*. By contrast, Dewji and Singer *(27)* favor the occurence of a seven-transmembrane domain protein.

PS1 and PS2 display 67% amino-acid identity. Divergences between the two PS sequences occur mainly at their N-termini and at the level of the sixth hydrophilic loop. Most of the missense mutations detected in PS1 occur at amino acids conserved between the two proteins and are particularly clustered within transmembrane domains, with obvious predominence in the second.

Because cellular and subcellular localizations of proteins often give insights about their putative functions, a series of studies has examined these aspects. Various monoclonal, polyclonal, or affinity-purified antibodies have been generated against PS1 and label neurons in rodent and human brains *(28–30)*. Most of the labeling appears to be associated with neurons *(29,31,32)*, whereas oligodendrocytes, microglia, and astrocytes remain immunonegative *(30)*. In the mouse brain, PS1-like immunoreactivity is mainly associated with perikarya and dendrites, whereas labeling of the axons appears to be very weak *(28)*. This is very similar to the pattern observed with human temporal cortex that is stained mainly at the level of neuronal cell bodies and dendrites *(30)*. Most of these features are also observed in various cell lines. Thus, NT2N and rat hippocampal neurons display PS1-like immunoreactivity on cell bodies and dendrites and little if any on axons *(33,34)*. Similarly, PS1 appears mainly concentrated in the somatodendritic compartment of SH-SY5Y neuroblastoma cells, although lower levels could also be detected in axons *(35)*.

In contrast to PS1, very limited information is available concerning the expression of PS2. An *in situ* hybridization approach indicates similar patterns of PS2 and PS1 expression in human brain, mainly in the neuronal cell population *(31,36)*. Accordingly, a recent study indicated that PS2 colocalizes with PS1 in mouse brain but that, unlike PS1, PS2-like immunoreactivity remains exclusively associated with neuronal cell bodies *(37)*.

At the subcellular level, the available data are not totally consensual. Most studies have reported localization of PS1 *(26,31–33,36,38,39)* and PS2 *(31,32,36,39)* in intracellular membranes of various mammalian cell lines, mainly at the level of the endoplasmic reticulum *(26,33,36,38,39)* and Golgi apparatus *(26,36)*. The distribution in such compartments does not seem to be modified when the subcellular localization of FAD-linked PS is examined *(33,36)*. Two other studies have also reported the association of PS1 *(38,40)* and PS2 *(40)* at the plasma membrane, suggesting the possibility that PS expression at the cell surface could mediate cell-to-cell contacts responsible for adhesion processes *(38)* or transcellular binding *(40,41)*. Finally, a recent study suggested the colocalization of PS with kinetochores occuring at the level of the inner nuclear membrane *(42)*.

The reason for such controversial results is not yet clear but one could envision that the specificity of the immunological probes used in these studies could explain some of these discrepancies. Furthermore, as mature PSs are further processed *(see* Posttranslational Events*)*, it is possible that PS-like immunoreactivity corresponds to the holoproteins but also to some of their derived fragments. In this context, possible variable recognition of these products by the antibodies together with putative cell-specific maturation processes and/or distinct localization of holoproteins and processed fragments could explain distinct phenotypic immunolabeling on intact tissue or cells.

Posttranslational Events

Presenilins do not undergo sulfation, acylation, and sugar incorporation *(26,39)*. Several studies indicate that PS2 holoprotein can be highly phosphorylated in COS and CHO cells *(26,39,43)*. Attemps to identify the candidate kinases responsible for PS2 holoprotein phosphorylation rules out the involvement of the

protein kinase C *(26,39)* and protein kinase A *(26)*. In vitro experiments performed with various purified kinases corroborated these data *(26)* and suggested the possible involvement of casein kinases I and II *(39)*. PS2 incorporates phosphate on serine residues *(26,39)* at positions 7,9, and 19 *(39)*. Interestingly, these residues are located at the N-terminal part of PS2 and are not conserved in the PS1 sequence. This likely explains why the PS1 holoprotein undergoes phosphorylation to a much lesser extent than PS2 *(26,39)*. Another explanation could be that PS1-phosphorylated holoprotein is more susceptible to phosphatase than phosphorylated PS2, as was suggested by the drastic enhancement of PS1 but not PS2 phosphorylation state on okadaic acid treatment of transfected COS cells *(26)*. Biosynthesis and phosphorylation of PS1 are not affected by two of the pathogenic mutations, Ala246-> Glu and Cys410-> Tyr *(26)*. Amino acids of PS2 undergoing phosphorylation in vivo, were recently mapped and identified as aspartyl residues in positions 327 and 330 *(43)*.

Presenilins are efficiently processed proteins. Several studies indicate that an N-terminal product of 28–30 kDa and its 18-kDa C-terminal counterpart are endogenously produced by a yet unknown "presenilinase," are recovered in high amounts, and can accumulate in various mammalian cell lines as well as in transgenic mice *(44–48)*. Recent data also indicate that the N- and C-terminal fragments of PS1 could physically interact *(49)* and form high-molecular-mass heterodimer complexes, the function of which still remains discussed. The high recovery of processed products when compared to holoproteins appears related with longer lifetimes of the former species *(50)*.

The processing cleavage clearly occurs inside the sequence encoded by exon 10 since the ΔE10-PS1 construction resists endoproteolytic cleavage *(45)*. Whether the presence of FAD-linked mutations affects PS processing has been questioned. Okochi and colleagues *(51)* reported a lack of association between a series of PS1 mutations and their susceptibility to processing. This was corroborated by a recent study showing that products of PS1 processing accumulate in the brain of patients bearing the I143T and G384A mutations in a similar extent than in control or sporadic brains *(52)*. Murayama et al. *(53)* later indicated that Cys410->Tyr PS1 resists proteolysis whereas Gly384->Ala or Leu392->Val does not. Accordingly, the mutations Met146->Val and Ala246->Glu were shown to impair processing in PC12 cells *(54)*. The latter data are in apparent contradiction with those obtained with the same mutations in transgenic mice since in this system, processed fragments accumulate to a higher extent than those generated from the wild-type PS1 *(55)*.

It is interesting to note that the cleavage of PS1 gives rise to a C-terminal fragment that, unlike PS1, behaves as a protein kinase C *(56,57)* and protein kinase A *(56)* substrate. Whether the selective phosphorylation of this product can be envisioned as a clue to postulate that it corresponds to the functionally occuring PS1-related species remains to be established.

Recent studies demonstrated that PS1 and PS2 undergo additional alternative cleavages at Asp345/Ser346 and Asp329/Ser330, two sites reminiscent of sequences targeted by caspase-like proteases *(58–60)*. The C-terminal PS1 fragment also behaves as a caspase substrate *(60)*. Involvement of caspase 3 or a close congener was proposed based on the use of specific caspase inhibitors, mutational analysis, and hydrolysis of PS by recombinant caspase 3 *(58,60)*. More recently, van de Craen et al. *(61)* reported on the susceptibility of PS1 and PS2 to cleavages by a series of caspase activities and demonstrated that caspases 8 and 3 displayed the highest catalytic activities toward both PS1 and PS2. Caspase-mediated cleavages appear to be exacerbated by Asn141->Ile PS2 mutation in H4 cells *(59)*, whereas a series FAD-linked PS1 mutations do not influence susceptibility to caspase cleavage *(61)*.

To be complete, it must be noted here that against the current view on the processing of PS, one recent report suggests that the

observed PS fragments are artifactual and are caused by drastic protein extraction and analysis procedures, inducing cell trauma and increased susceptibility to proteolysis *(62)*.

Downstream to the above posttranslational events, both PS1 *(63–66)* and PS2 *(46,67)* are ultimately catabolized by the multicatalytic proteasomal complex. This agrees well with the observation that both proteins can undergo ubiquitination *(46,63,67)*. FAD-linked mutations on PS1 *(63)* and PS2 *(67)* do not modify the rates of degradation by the proteasome. It should be noted that the proteasomal complex also regulates the intracellular concentrations of the C-terminal PS1/PS2 fragments *(67,68)*.

General Considerations on the Functions of Presenilins

Genetic evidences suggested a role of PS in cell signaling. Thus, *sel-12*, the *C. elegans* counterpart of PS, interacts with *lin-12*, the Notch analog present in this nematode, thereby modulating the *lin-12*-mediated cell signaling *(69)*. Interestingly, wild-type PS1 can functionally rescue for *sel-12* deficiency, whereas mutated PS1 displays drastically reduced ability to substitute for *sel-12* mutant phenotype *(70)*. In agreement with these observations, PS1 was recently shown to physically interact with Notch *(71)*. Notch is a single transmembrane domain protein that, after cell-surface activation, is cleaved at its C-terminus, giving rise to a biologically active intracellular fragment. This short domain is translocated into the nucleus, modulating the expression of some of the genes committed in cell fate decisions. It was recently reported that this intracellular cleavage is abolished when PS1 gene is invalidated in mammalian cells *(72)* and that this impairs Notch activity in *Drosophila (73,74)*. Interestingly, PS1 also interacts with βAPP and the PS1 gene knockout drastically reduces the production of Aβ by preventing the γ-secretase cleavage *(75)*. These data suggest that PS1 either corresponds to the genuine βAPP/Notch-cleaving activity or is involved in the chaperoning of these proteins to a cell compartment displaying these cleaving enzymes. Both hypotheses still stand since Wolfe et al. recently reported on the loss of γ-secretase activity after mutating PS1 at aspartyl residues reminiscent of those found in acidic proteases *(76)*. On the other hand, the involvement of PS1 in the intracellular trafficking of a series of proteins has been recently reported *(77,78)*. Interestingly, this function appears to be altered by FAD-linked mutations *(78)*.

PS1 also interacts with β-catenin and other proteins belonging to the Armadillo proteins family *(78–82)*. This interaction is affected by FAD-linked mutations and perturbates the commitment of cells to apoptosis *(81)*. The relationship between PSs and apoptotic processes is somewhat controversial because it appears that PS1 and PS2 could have distinct influence on this paradigm. Briefly, PS2 appears mainly "proapoptotic," whereas PS1 was reported to be either "antiapoptotic" or able to modulate cell susceptibility to apoptotic stimuli. The pro- or antiapoptotic control by PSs could be caused by some of their catabolites derived from secondary cleavages by "presenilinase" or caspases (for reviews *see refs. 83–86*).

Finally, PSs interact with a series of other proteins, including the G-protein Go *(87)*, rab11, a GTPase activity *(88)*, a calcium-binding protein recently named calsenilin *(89)*, and glycogen synthase kinase-3β *(90)*. The above very brief considerations on PSs functions have been recently reviewed in more detail *(86)*.

Conclusion

It appears from the above statements that PSs are likely multifunctional proteins undergoing numerous posttranslational events and involved in the control of various normal functions. When mutated, these proteins likely contribute, at least in part, to some of the familial forms of Alzheimer's disease. The virtually

complete abolishment of Aβ formation after knocking out PS gene could have been seen optimistically as the identification of a putative therapeutic target for "fighting" AD. This hope has been short-lived because one can now consider, with respect to the normal function of PS in the cleavage and/or trafficking of various proteins involved in cell signaling, that abolishing PS activities could have disastrous side effects on cell physiology. It remains therefore of fundamental interest to definitely establish whether PSs act upstream to secretases or as genuine secretases. In the former case, targetting secretases downstream to PSs action would remain a possible track to slow down or arrest the exacerbation of Aβ production occuring in AD, whatever its sporadic or genetic etiology.

References

1. Tanzi R. E., St. George-Hyslop P., and Gusella J. F. (1991) Molecular genetics of Alzheimer disease amyloid. *J. Biol. Chem.* **266,** 20579–20582.
2. Tanzi R. E., Kovacs D. M., Kim T-W., Moir R. D., Guenette S. Y., and Wasco W. (1996) The gene defects responsible for familial Alzheimer's disease. *Neurobiol. Disease* **3,** 159–168.
3. Mullan M and Crawford F. (1993) Genetic and molecular advances in Alzheimer's disease. *Trends Neurosci.* **16,** 398–403.
4. Schellenberg G. D. (1995) Genetic dissection of Alzheimer disease, a heterogeneous disorder. *Proc. Natl. Acad. Sci. USA* **92,** 8552–8559.
5. Kang J., Lemaire H-G., Unterbeck A., Salbaum J. M., Masters C. L., Grzeschik K-H., Multhaup G, Beyreuther K., and Müller-Hill B. (1987) The precursor of Alzheimer's disease amyloid A4 protein resembles a cell-surface receptor. *Nature* **325,** 733–736.
6. Tanzi R. E., Gusella J. F., Watkins P. C., Bruns G. A. P., St. George-Hyslop P., Van Keuren M. L., Patterson D., Pagan S., Kurnit D. M., Neve R. L. (1987) Amyloid β protein gene: cDNA, mRNA distribution, and genetic linkage near the Alzheimer locus. *Science* **235,** 880–884.
7. Goldgaber D., Lerman M. I., McBride O. W., Saffiotti U., and Gajdusek D. C. (1987) Characterization and chromosomal localization of a cDNA encoding brain amyloid of Alzheimer's disease. *Science* **235,** 887–880.
8. Sherrington R., Rogaev E. I., Liang Y., Rogaeva E. A., Levesque G, Ikeda M, Chi H, Lin C., Li G., Holman K., Tsuda T., Mar L., Foncin J. F., Bruni A. C., Montesi M. P., Sorbi S., Rainero I., Pinessi L., Nee L., Chumakov I., Pollen D., Brookes A., Sanseau P., Polinski R. J., Wasco W., Da Silva HAR, Haines J. L., Pericak-Vance M. A., Tanzi R. E., Roses A. D., Fraser P. E., Rommens J. M., and St George-Hyslop P. H. (1995) Cloning of a gene bearing missense mutations in early-onset familial Alzheimer's disease. *Nature* **375,** 754–760.
9. Levy-Lahad E., Wasco W., Poorkaj P., Romano D. M., Oshima J., Pettingell W. H., Yu C., Jondro P. D., Schmidt S. D., Wang K., Crowley A. C., Fu Y. H., Guenette S. Y., Galas D., Nemens E., Wisjman E. M., Bird T. D., Schellenberg G. D., and Tanzi R. (1995) Candidate gene for the chromosome 1 familial Alzheimer's disease locus. *Science* **269,** 973–977.
10. Rogaev E. I., Sherrington R., Rogaeva E. A., Levesque G., Ikeda M., Liang Y., Chi H., Lin C., Holman K., Tsuda T., Mar L., Sorbi S., Nacmias B., Piacentini S., Amaducci L., Chumakov I., Cohen D., Lannfelt L., Fraser P. E., Rommens J. M., and St Georges-Hyslop P. H. (1995) Familial Alzheimer's disease in kindreds with missense mutations in a gene on chromosome 1 related to the Alzheimer's disease type 3 gene. *Nature* **376,** 775–778.
11. Alzheimer's Disease Collaborative Group (1996) The structure of the presenilin 1 (S182) gene and identification of six novel mutations in early onset A. D. families. *Nature Genet.* **11,** 219–222.
12. Mitsuda N., Roses A. D., and Vitek M. P. (1997) Transcriptional regulation of the mouse presenilin-1 gene. *J. Biol. Chem.* **272,** 23,489–23,497.
13. Nishiyama K., Murayama S., Suzuki T., Mitsui Y., Sakaki Y., and Kanazawa I. (1996) Presenilin 1 mRNA expression in hippocampi of sporadic Alzheimer's disease patients. *Neurosci. Res.* **26,** 75–78.
14. Tanimukai H., Sato K., Kudo T., Kashiwagi Y., Tohyama M., and Takeda M. (1999) Regional distribution of presenilin-1 messenger RNA in the embryonic rat brain: comparison with β-amyloid precursor protein messenger RNA localization. *Neuroscience* **90,** 27–39.
15. Hardy J. (1997) Amyloid, the presenilins and Alzheimer's disease. *Trends Neurosci.* **20,** 154–159.
16. Campion D., Brice A., Dumanchin C., Puel M., Baulac M., De La Sayette V., Hannequin D.,

Duyckaerts C., Michon A., Martin C., Moreau V., Penet C., Martinez M., Clerget-Darpoux F., Agid Y., and Frebourg T. (1996) A novel presenilin 1 mutation resulting in familial Alzheimer's disease with an onset age of 29 years. *Neuroreport* **7,** 1582–1584.

17. Wisniewski T., Dowjat W. K., Buxbaum J. D., Khorkova O., Efthimiopoulos S., Kulczycki J., Lojkowska W., Wegiel J., Wisniewski H. M., and Frangione B. (1998) A novel polish presenilin-1 mutation (P117L) is associated with Alzheimer's disease and leads to death as early as the age of 28 years. *NeuroReport* **9,** 217–221.
18. Perez-Tur J., Froelich S., Prihar G., Crook R., Baker M., Duff K., Wragg M., Busfield F., Lendon C., Clark R. F., Roques P., Fuldner R. A., Johnston J., Cowburn R., Forsell C., Axelman K., Lilius L., Houlden H., Karran E., Roberts G. W., Rossor M., Adams M. D., Hardy J., Goate A., Lannfelt L., and Hutton M. (1995) A mutation in Alzheimer's disease destroying a splice acceptor site in the presenilin-1 gene. *NeuroReport* **7,** 297–301.
19. Levy-Lahad E., Poorkaj P., Wang K., Fu Y. H., Oshima J., Mulligan J., and Schellenberg G. D. (1996) Genomic structure and expression of STM2, the chromosome 1 familial Alzheimer disease gene. *Genomics* **34,** 198–204.
20. Prihar G., Fuldner R. A., Perez-Tur J., Lincoln S., Duff K., Crook R., Hardy J., Philips C. A., Venter C., Talbot C., Clark R. F., Goate A., Li J., Potter H., Karran E., Roberts G. W., Hutton M., and Adams M. D. (1996) Structure and alternative splicing of the presenilin-2 gene. *NeuroReport* **7,** 1680–1684.
21. Levy-Lahad E., Wijsman E. M., Nemens E., Anderson L., Goddard A. B., Weber J. L., Bird T. D., and Schellenberg G. D. (1995) A familial Alzheimer's disease locus on chromosome 1. *Science* **269,** 970–973.
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. (1996) Alzheimer's disease associated with mutations in presenilin 2 is rare and variably penetrant. *Hum. Mol. Genet.* **5,** 985–988.
23. Doan A., Thinakaran G., Borchelt D. R., Slunt H. H., Ratovitsky T., Podlisny M., Selkoe D. J., Seeger M., Gandy S. E., Price D. L., and Sisodia S. S. (1996) Protein topology of presenilin 1. *Neuron* **17,** 1023–1030.
24. Lehmann S., Chiesa R., and Harris D. A. (1997) Evidence for a six-transmembrane domain structure of presenilin 1. *J. Biol. Chem.* **272,** 12,047–12,051.
25. Li X. and Greenwald I. (1996) Membrane topology of the C-elegans SEL-12 presenilin. *J. Biol. Chem.* **17,** 1015–1021.
26. De Strooper B., Beullens M., Contreras B., Levesque L., Craessaerts K., Cordell B., Moechars D., Bollen M., Fraser P., St. George-Hyslop P., and Van Leuven F. (1997) Phosphorylation, subcellular localization, and membrane orientation of the Alzheimer's disease-associated presenilins. *J. Biol. Chem.* **272,** 3590–3598.
27. Dewji N. N. and Singer S. J. (1997) The seven-transmembrane spanning topography of the Alzheimer disease-related presenilin proteins in the plasma membranes of cultured cells. *Proc. Natl. Acad. Sci. USA* **94,** 14,025–14,030.
28. Elder G. A., Tezapsidis N., Carter J., Shioi J., Bouras C., Li H-C., Johnston J. M., Efthimiopoulos S., Friedrich V. L., and Robakis N. K. (1996) Identification and neuron specific expression of the S182/presenilin 1 protein in human and rodent brains. *J. Neurosci. Res.* **45,** 308–320.
29. Moussaoui S., Czech C., Pradier L., Blanchard V., Bonici B., Gohin M., Imperato A., and Revah F. (1996) Immunohistochemical analysis of presenilin-1 expression in the mouse brain. *FEBS Letts.* **383,** 219–222.
30. Kim K. S., Wegiel J., Sapienza V., Chen J., Hong H., and Wisniewski H. M. (1997) Immunoreactivity of presenilin-1 in human, rat and mouse brain. *Brain Res.* **757,** 159–163.
31. Huynh D. P., Vinters H. V., Ho D. H. D., Ho V. V., and Pulst S-M. (1997) Neuronal expresion and intracellular localization of presenilins in normal and Alzheimer's disease. *J. Neuropathol. Exp. Neurol.* **56,** 1009–1017.
32. Culvenor J. G., Maher F., Evin G., Malchiodi-Albedi F., Cappai R., Underwood J. R., Davis J. B., Karran E. H., Roberts G. W., Beyreuther K., and Masters C. L. (1997) Alzheimer's disease-associated presenilin 1 in neuronal cells: evidence for localization to the endoplasmic reticulum-golgi intermediate compartment. *J. Neurosci. Res.* **49,** 719–731.
33. Cook D. G., Sung J. C., Golde T. E., Felsenstein K. M., Wojczyk B. S., Tanzi R. E., Trojanowski J. Q., Lee V. M-Y., and Doms R. W. (1996) Expres-

sion and analysis of presenilin 1 in a human neuronal system: localization in cell bodies and dendrites. *Proc. Natl. Acad. Sci. USA* **93,** 9223–9228.
34. Capell A., Saffrich R., Olivo J-C., Meyn L., Walter J., Grünberg J., Mathews P., Nixon R., Dotti C., and Haass C. (1997) Cellular expression and proteolytic processing of presenilin proteins is developmentally regulated during neuronal differentiation. *J. Neurochem.* **69,** 2432–2440.
35. Busciglio J., Hartmann H., Lorenzo A., Wong C., Baumann K., Sommer B., Staufenbiel M., and Yankner B. A. (1997) Neuronal localization of presenilin-1 and association with amyloid plaques and neurofibrillary tangles in Alzheimer's disease. *J. Neurosci.* **17,** 5101–5107.
36. Kovacs D. M., Fausett H. J., K. J. P., Kim T. W., Moir R. D., Merriam D. E., Hollister R. D., Hallmark O. G., Mancini R., Felsenstein K. M., Hyman B. T., Tanzi R. E., and Wasco W. (1996) Alzheimer-associated presenilins 1 and 2: neuronal expression in brain and localization to intracellular membranes in mammalian cells. *Nature. Med.* **2,** 224–229.
37. Blanchard V., Czech C., Bonici B., Clavel N., Gohin M., Dalet K., Revah F., Pradier L., Imperato A., and Moussaoui S. (1997) Immunohistochemical analysis of presenilin 2 expression in the mouse brain: distribution pattern and colocalization with presenilin 1 protein. *Brain Res.* **758,** 209–217.
38. Takashima A., Sato M., Mercken M., Tanaka S., Honda T., Sato K., Murayama M., Noguchi K., Nakazato Y., and Takahashi H. (1996) Localization of Alzheimer-associated presenilin 1 in transfected COS-7 cells. *Biochem. Biophys. Res. Commun.* **227,** 423–426.
39. Walter J., Capell A., Grünberg J., Pesold B., Schindzielorz A., Prior R., Podlisny M. B., Fraser P., St. George Hyslop P., Selkoe D. J., and Haass C. (1996) The Alzheimer's disease-associated presenilins are differentially phosphorylated proteins located predominantly within the endoplasmic reticulum. *Mol. Med.* **2,** 673–691.
40. Dewji N. N. and Singer S. J. (1997) Cell surface expression of the Alzheimer disease-related presenilin proteins. *Proc. Natl. Acad. Sci. USA* **94,** 9926–9931.
41. Dewji N. N. and Singer S. J. (1998) Specific intercellular binding of the β-amyloid precursor protein to the presenilins induces intercellular signalling: its significance for Alzheimer's disease. *Proc. Natl. Acad. Sci. USA* **93,** 15,055–15,060.
42. Li J., Xu M., Zhou H., and Ma J., Potter H. (1997) Alzheimer presenilins in the nuclear membrane, interphase kinetochores, and centrosomes suggest a role in chromosome seggregation. *Cell* **90,** 917–927.
43. Walter J., Schindzielorz A., Grünberg J., and Haass C. (1999) Phosphorylation of presenilin-2 regulates its cleavage by caspases and retards progression of apoptosis. *Proc. Natl. Acad. Sci. USA* **96,** 1391–1396.
44. Ward R. V., Davis J. B., Gray C. W., Barton A. J. L., Bresciani L. G., Caivano M., Murphy V. F., Duff K., Hutton M., Hardy J., Roberts G. W., and Karran E. H. (1996) Presenilin-1 is processed into two major cleavage products in neuronal cell lines. *Neurodegeneration* **5,** 293–298.
45. 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. (1996) Endoproteolysis of presenilin 1 and accumulation of processed derivatives in vivo. *Neuron* **17,** 181–190.
46. Kim T. W., Pettingell W. H., Hallmark O. G., Moir R. D., Wasco W., and Tanzi R. E. (1997) Endoproteolytic cleavage and proteasomal degradation of presenilin 2 in transfected cells. *J. Biol. Chem.* **272,** 11,006–11,010.
47. Baumann K., Paganetti P. A., Sturchler-Pierrat C., Wong C., Hartmann H., Cescato R., Frey P., Yankner B. A., Sommer B., and Staufenbiel M. (1997) Distinct processing of endogenous and overexpressed recombinant presenilin 1. *Neurobiol. Aging* **18,** 181–189.
48. Shirotani K., Takahashi K., Ozawa K., Kunishita T., and Tabira T. (1997) Determination of a cleavage site of presenilin 2 protein in stably transfected SH-SY5Y human neuroblastoma cell lines. *Biochem. Biophys. Res. Commun.* **240,** 728–731.
49. Capell A., Grünberg J., Pesold B., Diehlmann A., Citron M., Nixon R., Beyreuther K., Selkoe D. J., and Haass C. (1998) The proteolytic fragments of the Alzheimer's disease-associated presenilin-1 form heterodimers and occur as a 100–150-kDa molecular mass. *J. Biol. Chem.* **273,** 3205–3211.
50. Ratovitski T., Slunt H. H., Thinakaran G., Price D. L., Sisodia S. S., and Borchelt D. R. (1997)

Endoproteolytic processing and stabilization of wild-type and mutant presenilin. *J. Biol. Chem.* **272,** 24,536–24,541.
51. Okochi M, Ishii K., Usami M., Sahara N., Kametani F., Tanaka K., Frazer P. E., Ikeda M., Saunders A. M., Hendriks L., Shoji S-I., Nee L. E., Martin J-J., Van Broeckhoven C., St. George-Hyslop P., Roses A. D., and Mori H. (1997) Proteolytic processing of presenilin-1 (PS-1) is not associated with Alzheimer's disease with or without PS-1 mutations. *FEBS Lett.* **418,** 162–166.
52. Hendriks L., Thinakaran G., Harris C. L., De Jonghe C., Martin J. J., Sisodia S. S., and Van Broeckoven C. (1997) Processing of presenilin 1 in brains of patients with Alzheimer's disease and controls. *NeuroReport* **8,** 1717–1721.
53. Murayama O., Honda T., Mercken M., Murayama M., Yasutake K., Nihonmatsu N., Nakazato Y., Michel G., Song S., Sato K., Takahashi H., and Takashima A. (1997) Different effects of Alzheimer-associated mutations of presenilin 1 on its processing. *Neurosci. Lett.* **229,** 61–64.
54. Mercken M., Takahashi H., Honda T., Sato K., Murayama M., Nakazato Y., Noguchi K., Imahori K., and Takashima A. (1996) Characterization of human presenilin 1 using N-terminal specific monoclonal antibodies: evidence that Alzheimer mutations affect proteolytic processing. *FEBS Lett.* **389,** 297–303.
55. Lee M. K., Borchelt D. R., Kim G., Thinakaran G., Slunt H. H., Ratovitski T., Martin L. J., Kittur A., Gandy S., Levey A. I., Jenkins N., Copeland N., Price D. L., and Sisodia S. S. (1997) Hyperaccumulation of FAD-linked presenilin 1 variants in vivo. *Nature Med.* **3,** 756–760.
56. Seeger M., Nordstedt C., Petanceska S., Kovacs D. M., Gouras G. K., Hahne S., Fraser P., Levesque L., Czernik A. J., St-George-Hyslop P., Sisodia S. S., Thinakaran G., Tanzi R. E., Greengard P., and Gandy S. (1997) Evidence for phosphorylation and oligomeric assembly of presenilin 1. *Proc. Natl. Acad. Sci. USA* **94,** 5090–5094.
57. Walter J., Grünberg J., Capell A., Pesold B., Schindzielorz A., Citron M., Mendla K., StGeorge-Hyslop P., Multhaup G., Selkoe D. J., and Haass C. (1997) Proteolytic processing of the Alzheimer disease associated presenilin-1 generates an in vivo substrate for protein kinase C. *Proc. Natl. Acad. Sci. USA* **94,** 5349–5354.
58. Loetscher H., Deuschle U., Brockhaus M., Reinhard D., Nelboeck P., Mous J., Grünberg J., Haass C., and Jacobsen H. (1997) Presenilins are processed by caspase-like proteases. *J. Biol. Chem.* **272,** 20,655–20,659.
59. Kim T-W., Pettingell W. H., Jung Y-K., Kovacs D. M., and Tanzi R. E. (1997) Alternative cleavage of Alzheimer-associated presenilins during apoptosis by a caspase-3 family protease. *Science* **277,** 373–376.
60. Grünberg J., Walter J., Loetscher H., Deuschle U., Jacobsen H., and Haass C. (1998) Alzheimer's disease associated presenilin-1 holoprotein and its 18–20 kDa C-terminal fragment are death substrates for proteases of the caspase family. *Biochemistry* **37,** 2263–2270.
61. van de Craen M., de Jonghe C., van den Brande I., Declercq W., van Gassen G., van Criekinge W., Vanderhoeven I., Fiers W., van Broeckhoven C., Hendricks L., and Vandenabeele P. (1999) Identification of caspases that cleave presenilin-1 and presenilin-2. Five presenilin-1 (PS1) mutations do not alter the sensitivity of PS1 to caspases. *Neurosci. Lett.* **260,** 149–154.
62. Dewji N. N., Do C., and Singer S. J. (1997) On the spurious endoproteolytic processing of the presenilin proteins in cultured cells and tissues. *Proc. Natl. Acad. Sci. USA* **94,** 14,031–14,036.
63. Marambaud P., Ancolio K., Lopez-Perez E., and Checler F. (1998) Proteasome inhibitors prevent the degradation of familial Alzheimer's disease-linked presenilin 1 and trigger increased Aβ42 secretion by human cells. *Mol. Med.* **4,** 146–156.
64. Honda T., Yasutake K., Nihonmatsu N., Mercken M., Takahashi H., Murayama O., Sato K., Omori A., Tsubuki S., Saido T. C., and Takashima A. (1999) Dual roles of proteasome in the metabolism of presenilin 1. *J. Neurochem.* **72,** 255–261.
65. Steiner H., Capell A., Pesold B., Citron M., Kloetzel P. M., Selkoe D. J., Romig H., Mendla K., and Haass C. (1998) Expression of Alzheimer's disease-associated presenilin-1 is controlled by proteolytic degradation and complex formation. *J. Biol. Chem.* **273,** 32,322–32,331.
66. Fraser P. E., Levesque G., Yu G., Mills L. R., Thirwell J., Frantseva M., Gandy S. E., Seeger M., Carlen P. L., and St George-Hyslop P. (1998) Presenilin 1 is actively degraded by the 26S proteasome. *Neurobiol. Aging* **19,** S19–S21.
67. Marambaud P., Alves da Costa C., Ancolio K., and Checler F. (1998) Alzheimer's disease-linked mutation of presenilin 2 (N141I-PS2) drastically lowers APPα secretion: control by

the proteasome. *Biochem. Biophys. Res. Commun.* **252,** 134–138.
68. Alves da Costa C., Ancolio K., and Checler F. (1999) C-terminal maturation fragments of presenilin 1 and 2 control secretion of APPα and Aβ by human cells and are degraded by the proteasome. *Mol. Med.* **5,** 160–168.
69. Levitan D. and Greenwald I. (1995) Facilitation of *lin-12*-mediated signalling by *sel-12,* a *Caenorhabditis elegans S182* Alzheimer's disease gene. *Nature* **377,** 351–354.
70. Levitan D., Doyle T. G., Brousseau D., Lee M. K., Thinakaran G., Slunt H. H., Sisodia S. S., and Greenwald I. (1996) Assessment of normal and mutant human presenilin function in *Caenorhabditis elegans. Proc. Natl. Acad. Sci. USA* **93,** 14,940–14,944.
71. Ray W. J., Yao M., Nowotny P., Mumm J., Zhang W., Wu J., Kopan R., and Goate A. (1999) Evidence for a physical interaction between presenilin and Notch. *Proc. Natl. Acad. Sci. USA* **96,** 3263–3268.
72. De strooper B., Annaert W., Cupers P., Saftig P., Craessaerts K., Mumm J. S., Schroeter E. H., Schrijvers V., Wolfe M. S., Ray W. J., Goate A., and Kopan R. (1999) A presenilin-1-dependent γ-secretase-like protease mediates release of Notch intracellular domain. *Nature* **398,** 518–522.
73. Ye Y., Lukinova N., and Fortini M. E. (1999) Neurogenic phenotypes and altered Notch processing in drosophila presenilin mutants. *Nature* **398,** 525–529.
74. Struhl G. and Greenwald I. (1999) Presenilin is required for activity and nuclear access of Notch in drosophila. *Nature* **398,** 522–525.
75. De Strooper B., Saftig P., Craessaerts K., Vanderstichele H., Guhde G., Von Figura K., and Van Leuven F. (1998) Deficiency of presenilin 1 inhibits the normal cleavage of amyloid precursor protein. *Nature* **391,** 387–390.
76. Wolfe M. S., Xia W., Ostaszewski B. L., Diehl T. S., Kimberly W. T., and Selkoe D. J. (1999) Two transmembrane aspartates in presenilin-1 required for presenilin endoproteolysis and γ-secretase activity. *Nature* **398,** 513–517.
77. Naruse S., Thinakaran G., Luo J-J., Kusiak J. W., T. T., Iwatsubo T., Qian X., Ginty D. D., Price D. L., Borchelt D. R., Wong P. C., and Sisodia S. S. (1998) Effects of PS1 deficiency on membrane protein trafficking in neurons. *Neuron* **21,** 1213–1221.
78. Nishimura M., Yu G., Levesque G., Zhang D. M., Ruel L., Chen F., Milman P., Holmes E., Liang Y., Kawarai T., Jo E., Supala A., Rogaeva E., Xu D-M., Janus C., Levesque L., Bi Q., Duthie M., Rozmahel R., Mattila K., Lannfelt L., Westaway D., Mount H. T. J., Woodgett J., Fraser P. E., and St George-Hyslop P. (1999) Presenilin mutations associated with Alzheimer's disease cause defective intracellular trafficking of β-catenin, a component of the presenilin protein complex. *Nature Med.* **5,** 164–169.
79. Yu G., Chen F., Levesque G., Nishimura M., Zhang D-M., Levesque L., Rogaeva E., Xu D., Liang Y., Duthie M., St George-Hyslop P. H., and Fraser P. E. (1998) The presenilin 1 protein is component of a high molecular weight intracellular complex that contains β-catenin. *J. Biol. Chem.* **273,** 16,470–16,475.
80. Tesco G., Kim T-W., Dielhmann A., Beyreuther K., and Tanzi R. (1998) Abrogation of the presenilin 1/β-catenin interaction and preservation of the heterodimeric presenilin 1 complex following caspase activation. *J. Biol. Chem.* **273,**33,909–33,914.
81. Zhang Z., Hartmann H., Do V. M., Abramowski D., Sturchler-Pierrat C., Staufenbiel M., Sommer B., van de wetering M., Clevers H., Saftig P., De Strooper B., He X., and Yankner B. A. (1998) Destabilization of β-catenin by mutations in presenilin-1potentiates neuronal apoptosis. *Nature* **395,** 698–702.
82. Levesque G., Yu G., Nishimura M., Zhang D. M., Levesque L., Yu H., Xu D., Liang Y., Rogaeve E., Ikeda M., Duthie M., Murgolo N., Wang L., Vander Vere P., Bayne M. L., Strader C. D., Rommens J. M., Fraser P. E., and St. George-Hyslop P. (1999) Presenilins interact with armadillo proteins including neural-specific plakophilin-related protein and β-catenin. *J. Neurochem.* **72,** 999–1008.
83. Haass C. (1997) Presenilins, genes for life and death. *Neurons* **18,**687–690.
84. Hutton M. and Hardy J. (1997) The presenilins and Alzheimer's disease. *Human Mol. Genet.* **6,** 1639–1646.
85. Mattson M. P., Gue Q., Furukawa K., and Pedersen W. A. (1998) Presenilins, the endoplasmic reticulum, and neuronal apoptosis in Alzheimer's disease. *J. Neurochem.* **70,** 1–14.
86. Checler F. (1999) Presenilins: multifunctional proteins involved in Alzheimer's disease Pathology. *LIFE,* in press.
87. Smine A., Xu X., Nishiyama K., Katada T., Gambetti P., Yadav S. P., Wu X., Shi Y-C., Yasuhara S., Homburger V., and Okamoto T. (1998) Regula-

tion of brain G-protein Go by Alzheimer's disease gene presenilin-1. *J. Biol. Chem.* **273,** 16,281–16,288.
88. Dumanchin C., Czech C., Campion D., Cuif M-H., Poyot T., Charbonnier F., Goud B., Pradier L., and Frebourg T. (1999) Presenilins interact with Rab11, a small GTPase involved in the regulation of vesicular transport. *Hum. Mol. Genet.* **8,** 1263–1269.
89. Buxbaum J. D., Choi E-K., Luo Y., Lilliehook C., Crowley A. C., Merriam D. E., and Wasco W. (1998) Calsenilin: A calcium-binding protein that interacts with the presenilins and regulates the levels of a presenilin fragment. *Nature Med.* **4,** 1177–1181.
90. Takashima A., Murayama M., Murayama O., Kohno T., Honda T., Yasutake K., Nihonmatsu N., Mercken M., Yamaguchi H., Sugihara S., and Wolozin B. (1998) Presenilin1 associates with glycogen synthase kinase-3β and its substrate tau. *Proc. Natl. Acad. Sci. USA* **95,** 9637–9641.