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Review

The structure and functions of the presenilins

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Abstract. The presenilins (PSs) were new proteins discovered in 1995 to be involved, among other functions, in the molecular mechanisms leading to Alzheimer's disease. These proteins have been the subject of many investigations since then to elucidate their molecular structures and functions. Until now, the conclusions about PS structure have been discordant, but the 8-TM structure has been accepted by the Alzheimer's community, with the evidence for the 7-TM structure largely ignored. Here the evidence is reviewed for the 6-TM, 7-TM, 8-TM and other proposed models of PS topography and possibilities offered for the differences in interpretation of the various sets of data. The conclusion is that at this stage, the 7-TM model for cell surface PS is most likely the correct one.

Key words. Alzheimer's disease; presenilin; β-amyloid precursor protein (β-APP); topography or topology of integral membrane proteins; reporter group; immunofluorescence microscopy.

Introduction

The presenilin proteins PS-1 and PS-2 are polytopic integral membrane proteins of 467 and 448 amino acids, respectively, with corresponding molecular weights of 57 and 55 kDa. The PS-1 gene is on chromosome 14 (14q24.3) [1] and was initially discovered by genetic linkage studies in families in which Alzheimer's disease (AD) was manifested as an autosomal dominant inherited trait (FAD). The closely related PS-2 gene on chromosome 1 (1q42.2) was identified subsequently because of its close sequence similarity (67% homology) to PS-1 [2]. Both PS-1 and PS-2 are organized into ten exons that display tissue-specific alternate splicing [3–6]. The biological functions of these splice variants are not understood, but the intact forms were shown to be associated with the γ-secretase activity that generates the Aβ fragments of β-APP (see below).

Cellular localization of PS

The question of the localization of PS in a cell is of primary importance for understanding PS interactions and functions. We had initially reported, from immunofluorescence experiments [7] with cultured cells transfected with PS-1 and PS-2 cDNAs, that the PS proteins were in part expressible at the cell surface membrane. However, from the outset, a number of laboratories reported that in their transfected cell culture systems, PS was confined to the cell interior, primarily in the endoplasmic reticulum (ER) and in the Golgi apparatus [8–14]. There have also been reports of their localization in nuclear membranes [15]. With the exception of one other report of their cell surface expression [16], the entire field came to agree that the PS proteins had an exclusively intracellular localization. We investigated this matter directly in 1997 [17]. We showed by imunofluorescence experiments, using two polyclonal anti-peptide antibodies specific for (i) residues 345–354 in the large-loop region of PS-1 [1] and (ii) residues 24–35 in the N-terminal domain of PS-2 [2], that live cultured DAMI cells and differentiated human

NT2N neurons could be specifically immunolabeled for their endogenous as well as transfected PS. In contrast, the intracellular protein tubulin in these cells could not be immunolabeled unless the cells were first fixed and permeabilized. Our results therefore firmly established that the presenilins are in part expressed at the surfaces of cells with the major portion being expressed in the cell interior, as would be expected for most integral membrane proteins in a cell at steady state. Investigators in the field possibly did not initially detect the PS proteins at the cell surface in their immunofluorescence experiments [8–14] because they did not take steps to discriminate experimentally between the relatively small amounts of PS expressed at the cell surface, and the much larger intracellular pool, as we did in our immunofluorescence experiments [17, 18] by using of *live* cultured cells for the surface-expressed protein. Even more difficult is to establish the cell surface expression of membrane proteins for cells within intact tissues, and this may require high-resolution immunoelectron microscopic analysis. After several years of dispute, our finding of PS at the cell surface has now been confirmed by several groups [19–22], including, most recently [23], by the use of labeling with proposed transition state analogs of PS.

Structure: topographical considerations

Knowledge of the native three-dimensional structure of PS in cell membranes is of primary importance for understanding PS interactions and functions at atomic resolution. This would currently be accomplished by a high-resolution X-ray crystallographic analysis of appropriate crystals of PS in a suitable detergent. In the absence of such an analysis, limited but still useful information can be obtained by a variety of methods that can yield

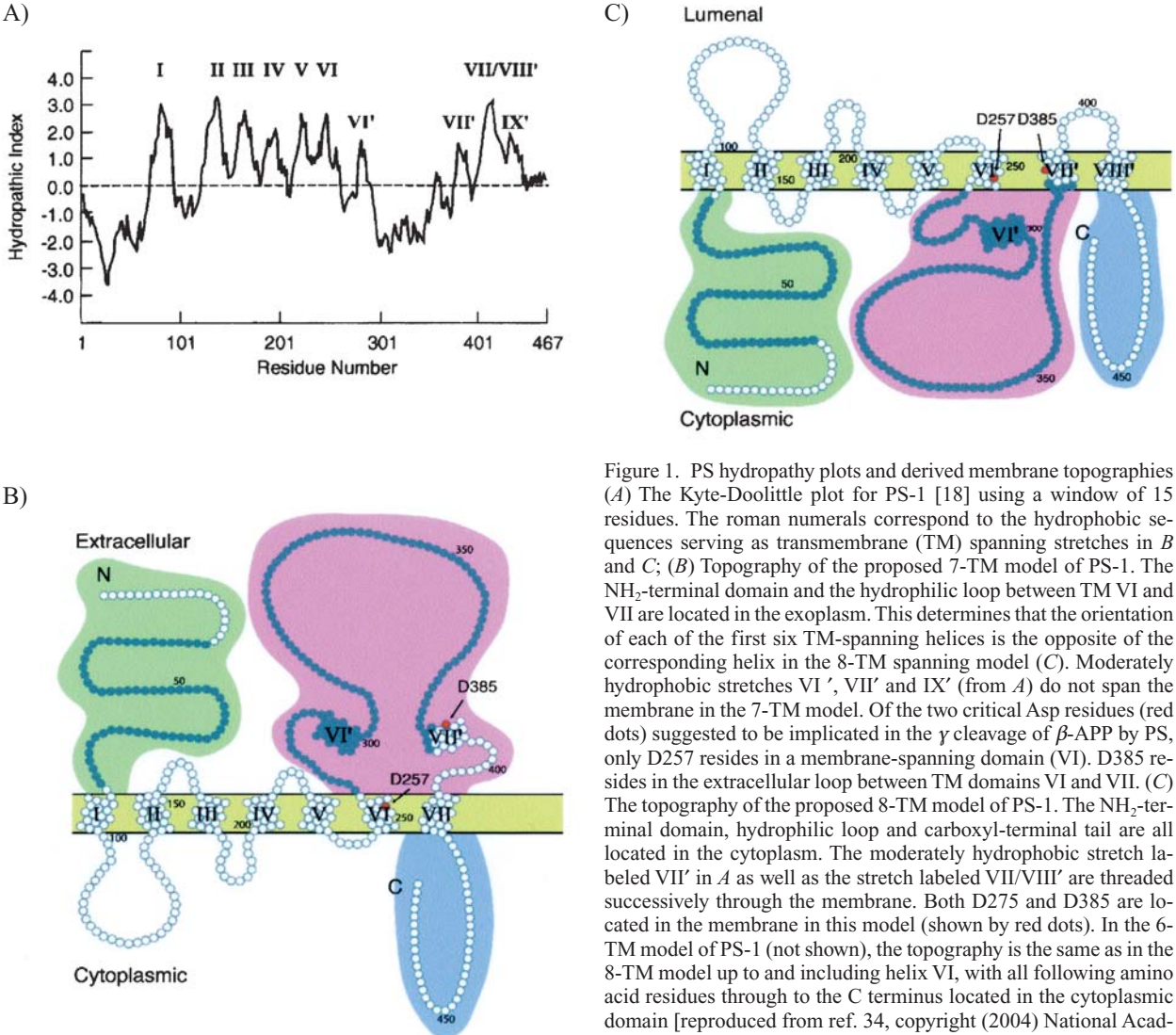

Figure 1. PS hydropathy plots and derived membrane topographies (*A*) The Kyte-Doolittle plot for PS-1 [18] using a window of 15 residues. The roman numerals correspond to the hydrophobic sequences serving as transmembrane (TM) spanning stretches in *B* and *C*; (*B*) Topography of the proposed 7-TM model of PS-1. The NH_2-terminal domain and the hydrophilic loop between TM VI and VII are located in the exoplasm. This determines that the orientation of each of the first six TM-spanning helices is the opposite of the corresponding helix in the 8-TM spanning model (*C*). Moderately hydrophobic stretches VI ′, VII′ and IX′ (from *A*) do not span the membrane in the 7-TM model. Of the two critical Asp residues (red dots) suggested to be implicated in the γ cleavage of β-APP by PS, only D257 resides in a membrane-spanning domain (VI). D385 resides in the extracellular loop between TM domains VI and VII. (*C*) The topography of the proposed 8-TM model of PS-1. The NH_2-terminal domain, hydrophilic loop and carboxyl-terminal tail are all located in the cytoplasm. The moderately hydrophobic stretch labeled VII′ in *A* as well as the stretch labeled VII/VIII′ are threaded successively through the membrane. Both D275 and D385 are located in the membrane in this model (shown by red dots). In the 6-TM model of PS-1 (not shown), the topography is the same as in the 8-TM model up to and including helix VI, with all following amino acid residues through to the C terminus located in the cytoplasmic domain [reproduced from ref. 34, copyright (2004) National Academy of Sciences, USA].

evidence for a schematic *one-dimensional* topography of the PS polypeptide chain in the direction perpendicular to the plane of the membrane.

Using the indices of Kyte and Doolittle [24], we observe that the hydropathy plots of both PS-1 and PS-2 suggest the presence of ten hydrophobic regions of varying length and hydrophobicity. On the basis of this result (fig. 1A), the discoverers of PS suggested that the proteins spanned the membrane seven times (7-TM) [1]. In this model, only the first six and the ninth hydrophobic region (VII/VIII′ in fig. 1A) are considered both long enough and hydrophobic enough to span the bilayer. The amino terminus and large acidic loop face the same side of the membrane, assumed to be the lumen of the ER whereas the carboxyl terminus faces the opposite side, assumed to be the cytoplasm. Our partial immunofluorescence results on the cell surface localization of the presenilins considered in the previous section are also consistent with this 7-TM topography of the cell surface PS molecules. Slunt et al. [25] used another algorithm to predict the secondary structure of PS-1. They proposed a 9-TM model in which only the eighth hydrophobic region does not cross the lipid bilayer. In this model, the carboxyl terminus of the protein and the large hydrophilic loop are positioned at the same side of the membrane while the amino terminus faces the opposite side.

In 1997 we further investigated the membrane topography of PS [26] by immunofluorescence labeling of live as well as permeabilized cells with a battery of six independently prepared polyclonal anti-peptide antibodies, directed against specific extramembrane domains of the PS proteins that were predicted to protrude from one or other side of the membrane in different models (fig. 1B, C). We provided strong evidence that the PS molecule in the cell surface spanned the membrane seven times, with either the 6-TM or 8-TM topographies ruled out. Another subsequent study [27], showing that cell surface PS has its N-terminal domain extracellularly positioned, was also consistent with a 7-TM topography of cell surface PS.

In the same year, De Strooper et al. [28] addressed the issue of whether two major hydrophilic domains of PS-1, the N terminus and the hydrophilic loop, are oriented toward the cytoplasmic or luminal side of the ER. They engineered a Myc tag at these two regions and selectively permeabilized the plasma membrane only, or the plasma membrane and the ER, of transfected cells. Antibodies directed toward the amino-terminal domain and large loop displayed similar immunofluorescence staining patterns in digitonin (which selectively permeabilizes the plasma membrane but not the ER) and saponin (which permeabilizes both the plasma membrane and the ER) permeabilized cells. Based on control experiments using antibodies to the KDEL ER retention signal (a luminal epitope of the ER), which did not show immunofluorescence staining in digitonin-treated cells, the authors concluded that both the amino terminus and large loop faced the cytoplasm. Their data were compatible with a 7-TM model with, however, both the amino-terminal domain and the hydrophilic loop located in the cytoplasm. In their model, the C terminus would be located luminally. These investigators therefore examined PS orientation only in the ER membrane, and not at the cell surface. A weakness of their method was that a ten-amino-acid Myc tag (EQKLISEEDL) was inserted immediately after the initiator Met in the N-terminal domain of PS-1. In the native molecule, the sequences immediately following Met in the N-terminal domain are relatively more hydrophobic (MTELPAPLSYF) and this difference may well affect the orientation of the N-terminal domain of the Myc fusion construct.

Doan et al. [29] followed the same strategy of selective permeabilization of the plasma membrane and ER of transiently transfected CHO cells that were stably expressing β-APP 695, using DNA constructs coding for proteins in which selected PS domains were fused to β-APP. This group also examined only orientation in the ER and Golgi membranes. For both the wild type and PS-1Δ9 variant that lacks exon 9 (amino acids 290–319), their results led them to conclude that the PS proteins had an 8-TM topography with the N-terminal domain, large hydrophilic loop and the C-terminal domain all in the cytoplasm (fig. 1C).

Li and Greenwald [30, 31] proposed the same 8-TM model on the basis of gene fusion studies with various truncated portions of SEL-12, (a *Caenorhabditis elegans* PS-1 homolog), and human PS-1 itself, fused to the reporter protein β-galactosidase after each hydrophobic domain of SEL-12 or PS-1. In this approach, fusions that expressed β-galactosidase activity in intact cells would have a cytoplasmic disposition of β-galactosidase, since this enzyme is active only within the cytoplasm, but not in an extra-cytosolic compartment. In these studies, the activity of β-galactosidase was detected when the enzyme was fused following either hydrophobic domains 2, 4, 6, 7, 9 or 10 (II, IV, VI, VI′, VII/VIII′ and IX′ in fig. 1A), leading the authors to locate these hydrophobic domains as aligned in the native SEL-12 and PS-1 molecules in the 8-TM model (fig. 1C). The authors concluded that the SEL-12 and PS proteins were absent from the cell surface, and confined to intracellular membranes.

In a similar study of fused truncated PS-1 molecules, however, Lehmann et al. [32] came to a different conclusion: PS spanned the ER membranes six times, leading to the 6-TM model (fig. 1C). In these studies, the authors employed as the reporter protein a portion of prolactin containing glycosylation sites, and used it to make fusion constructs after each hydrophobic domain of PS-1. They then monitored the glycosylation pattern of the expressed fusion proteins. Glycosylation of a particular fusion pro-

tein was taken to indicate the translocation of the reporter group to the lumen of the ER. Glycosylated proteins were formed in chimeras with fusion junctions after hydrophobic domains 1, 3 and 5 of PS-1 (I, III and V in fig. 1A). Based on these results, the authors concluded that PS-1 traverses the membrane six times (6-TM), with both the N and C termini located in the cytoplasm.

In another similar approach using glycosylation, Nakai et al. [33] fused truncated PS fragments to the cytoplasmic portion of the *Escherichia coli* leader peptidase (LP). The cytoplasmic region of LP possesses N-glycosylation acceptor sites; thus the researchers examined the expressed fusion peptides for glycosylation. They provided evidence for a 7-TM model for PS-1 which, however, was quite different from the one that we proposed [26, 34], locating the amino-terminal domain in the cytoplasm and the C terminus in the lumen. Thus, they found that fusion constructs containing either hydrophobic domains 1, 3 and 5 or 10 (I, III, V and IX′ in fig. 1A) of PS-1 formed glycoproteins; however, those with regions 7, 8 and 9 (VI′, VII′ and VII/VIII′ in fig. 1A) did not. Thus, in their 7-TM model, domain 9 does not span the bilayer but 10 does and the C-terminal tail is luminal.

The 7-TM model that we proposed based on our immunofluorescence studies is significantly different from any of the other models that have been put forward [25, 28–30, 32, 33], with the PS molecule nearly completely inverted in the membrane (fig. 1B) as compared with the 6-TM or 8-TM models (fig. 1C). In addition, in this 7-TM model, most of the individual transmembrane helices are oriented in opposite directions compared to the 6-TM or 8-TM models, with most of the predicted extramembranous domains protruding from opposite sides of the membrane. Furthermore, certain functionally critical amino acid residues of PS are located differently in the three different models either in the aqueous phase or in the membrane interior.

Our results supporting the 7-TM topography in figure 1B [26], despite the specificity controls that we employed, were initially dismissed by the field as artifactual due to antibody heterogeneity, and the 8-TM model has been nearly universally adopted as correct since 1996 among AD investigators.

As a result of the discordance of the conclusions of independent investigations, we recently reinvestigated and reported [34] the membrane topography of the human PS proteins in cell surface membranes using newer and better reagents. We carried out further immunofluorescence experiments, using this time as the primary antibodies monoclonal antibodies (mAb) directed either to the amino-terminal domain of human PS-1 (rat mAb 1563, Chemicon), or the large extramembranous loop region of human PS-1 following TM helix VI (mouse mAb 5232; Chemicon). The cells employed in these new studies were embryonic stem (ES)-derived cells, from PS-1$^{-/-}$ and PS-2$^{-/-}$ (double-null) mice, kindly provided by Drs D. Donoviel and A. Bernstein [35]. The ES cells were examined either untransfected, or after transfection with PS-1. We also employed the polyclonal rabbit antipeptide antiserum (AbC1), which we had used in our earlier work [26], directed to the carboxyl-terminal domain of PS-1. Double-immunofluorescence labeling was performed with one primary Ab directed to a PS-1 determinant, and a second primary Ab directed either to tubulin or actin, the latter two in order to test whether there was access of these antibodies to the cytoplasm of fixed but not permeabilized cells, or to cells fixed and permeabilized with Triton X-100. The rat mAb 1563 directed to the N-terminal domain of PS-1 immunolabeled the surfaces of PS-1-transfected ES double-null cells that were fixed but not permeabilized. (Their impermeability was shown by their inability to be labeled for tubulin.) Untransfected double-null ES cells similarly treated were not labeled by rat mAb 1563. Furthermore, the surface labeling of the PS-1 N-terminal domain by rat mAb 1563 was inhibited in the presence of an excess of the *specific* fusion protein of the N-terminal domain of PS-1 with FLAG, but was not inhibited by an excess of the *non-specific* fusion protein of the N-terminal domain of PS-2 with FLAG. Our results therefore unambiguously demonstrated that the N-terminal domain of PS-1 is exposed on the extracellular surface of PS-1-transfected ES cells.

The mouse mAb 5232 directed to the large-loop region of PS-1 likewise labeled the surfaces of fixed, but not permeabilized, PS-1-transfected ES cells, which could not be labeled either for actin or for polyclonal rabbit AbC-1. The C-terminal domain of PS-1 is therefore located, as found in our previous studies [26], on the side of the surface membrane *opposite* to the loop region of PS-1, thus placing the loop region and the N-terminal domain on the *same* side of the membrane. Correspondingly, cytoplasmic labeling of fixed and permeabilized PS-1-transfected ES cells was observed for actin and for AbC1. Untransfected ES cells were not labeled with the mAb 5232 to the loop region, whether permeabilized or not.

We also examined the immunolabeling of *endogenous* PS-1. Because our monoclonal primary Abs to PS-1 were directed to domains of the human protein, the endogenous PS-1 was examined with human DAMI cells in similar immunofluorescence experiments with both the N-terminal-specific and loop-specific monoclonal antibodies to PS-1. The results were entirely consonant with the 7-TM topography of endogenous PS-1 in the cell surface membrane, and, together with the results from the PS-1-transfected double-null ES cells, completely contradicted the predictions of the 8-TM or 6-TM topographies. Thus the new data [34] confirmed the 7-TM topography of cell surface PS (fig. 1B) derived in our earlier study [26].

How can the entirely different conclusions regarding an 8-TM (or other) topography rather than a 7-TM one for

PS in ER membranes be explained? Several possibilities may be entertained. One is that the PS molecule exhibits *both* the 7-TM and (perhaps to a larger extent) the 8-TM topography in the ER membrane, but *only* the 7-TM form undergoes intracellular transport to the plasma membrane. Reports of a few such cases of integral proteins with two topographies have appeared [36–38]. It is important to point out that so far we have only studied the topography of cell-surface PS-1, whereas all other studies have only analyzed PS-1 in the ER and Golgi membranes. Further studies to determine whether the PS proteins simultaneously exhibit both the 7-TM and 8-TM topographies in the ER membrane could examine this possibility, for example, by double electron microscopic immunolabeling.

Another possibility is that the evidence for the 8-TM and the other topographies discussed above is faulty. The topographical results obtained for PS-1 from the membrane topography of fusion proteins containing truncated fragments of PS-1 fused to different reporter groups discussed above, which have been the main evidence for the 8-TM and other topographies of PS-1 in the ER membrane, demonstrate in fact that such experiments give different and inconsistent results, depending on the reporter group used. The method has therefore been shown to be unreliable. This is also the inference to be drawn from other studies that have shown that the membrane topography of a native multi-TM-spanning integral protein can depend on the sequences of remote as well as contiguous residues within the protein [39–44]; these residues may inadvertently be altered when constructing fusions between truncated fragments and various reporter groups. In studies of the polytopic eukaryotic protein, cig 30 [45], C-terminally truncated constructs of the protein were analyzed and the first TM segment was found to be sufficient for translocation of the luminally oriented N-terminal domain; however, membrane targeting is inefficient unless TM1 is followed by a sufficiently long stretch of the polypeptide of the wild-type protein. In contrast, in a mutant with additional positively charged residues, efficient translocation is observed only when two

Table 1. Positive-in rule for predicting orientation of the first TM helix

Protein	N side	C side	Orientation	Reference
PS-1	–4.5	+2	predict N out/C in	1
PS-2	–0.5	+2	predict N out/C in	2
Sel-12	–3	+1.5	predict N out/C in	73
SPP (human)	0	+1	known N out/C in	74
SPP (*C. elegans*)	–2	+4	known N out/C in	75
PSH 3 (human)	0	+1	known N out/C in	76

Table 2. Some proteins that interact with the PS-1 or PS-2 hydrophilic loop

Protein	PS	Method	Function	Reference
δ-Catenin	PS-1	yeast two-hybrid	signaling	49
β-Catenin	PS-1	yeast two-hybrid, Co/IP	signaling	77
GSK-3β	PS-1	Co-IP	Signaling	78
Tau	PS-1	Co-IP	actin binding	79
Filamin	PS-1, PS-2	yeast two-hybrid, direct binding	cytoskeleton	79
NRAP	PS-1	yeast two-hybrid, Co/IP	not known	80
Calmyrin	PS-2	yeast two-hybrid, Co-IP	cell death	81
HC5/ZETA	PS-1	yeast two-hybrid, Co-IP	protein degradation	82
MOCA/ PBP	PS-1, PS-2	yeast two-hybrid, Co-IP	APP processing	52
Rab 11	PS-1, PS-2	yeast two-hybrid, Co-IP	vesicular transport	83
Restin	PS-1	yeast two-hybrid	cytoskeleton	84
Syntaxin 1A	PS-1	yeast two-hybrid, direct binding	Ca^{2+} signaling	85
Sorcin	PS-2	Co-IP	Ca^{2+} signaling	86
DRAL	PS-2	yeast two-hybrid	LIM family protein	87
Methyl transferase	PS-1, PS-2	yeast two-hybrid, Co-IP	methyl transferase	88

GSK-3β, glycogen synthase kinase 3β; NPRAP, neural plankophilin-related armadillo protein; HC5/ZETA, 20S catalytic particle of the 26S proteome; MOCA/PBP, modifier of cellular adhesion/PS-binding protein; DRAL, member of LIM protein family; Co-IP, co-immunoprecipitation.

TM segments are present, and only if they are followed by a sufficiently long polar domain. Details of the structural features that determine the orientation and succession of intercalation of domains of polytopic integral proteins are not yet fully understood [46, 47] especially for 7-TM proteins [48]. We [26, 34] examined only the topography of *intact* and unmodified cell surface PS-1, both endogenous and transfected. Therefore, in our experiments, it is the native integral membrane state of PS (including its associated proteins) that has been examined.

Furthermore, in favor of the exterior orientation of the N-terminal domain of the PS proteins [as in the 7-TM model (fig. 1B), but not the 8-TM (fig. 1C)] is the compliance with the 'positive-in' rule [47] which states that the difference in the net charge of the first 15 residues of the two aqueous domains flanking the first transmembrane domain generally determines the membrane orientation of the transmembrane domain, with the more positive of the two aqueous domains facing the cytosol. Table 1 shows that the net charge differences spanning helix I of the PS proteins and some of their homologs predict an orientation that is in keeping with the 7-TM topography.

The 7-TM model, however, is not consistent with the in vitro interactions reported between certain cytoplasmic proteins and the loop region of the PS proteins (which is luminal in the 7-TM model), listed in table 2. Examples include the cytosolic armadillo family proteins such as β-catenin, δ-catenin and p0071 [49–51] and MOCA [52], a member of the DOCK family of proteins that contains a Src homology 3 domain at the N terminus and several Crk-binding motifs near the C terminus. As table 2 shows, however, the majority of the evidence for such PS-interacting proteins has generally been obtained using the in vitro yeast two-hybrid system, a screening technique that likely does not permit the PS proteins to adopt their native membrane state. Thus the use as bait of protein fragments whose folding and binding properties may be distinct from those of the intact membrane-interrelated proteins raises many questions about the significance of the observed interactions in the absence of direct in vivo validation.

In view of all these considerations, we believe that the particular 7-TM topography of the PS molecules and their close homologs shown in fig. 1B should be accepted as most likely correct, pending more definitive structural studies of PS by high-resolution X-ray crystallographic investigation.

Some functional considerations:

The 7-TM topography of PS leads to a number of functional possibilities regarding the proteins. One concerns a proposal that PS is a heterotrimeric G protein-coupled receptor (GPCR). Although PS does not exhibit any substantial amino acid homologies with any of the approximately 1000 GPCRs so far examined, the fact that all of these GPCRs are 7-TM integral proteins, with many showing no sequence homologies with any others, allows for the possibility that PS molecules are also GPCRs. Furthermore, evidence from in vitro experiments [53] with fragments of PS-1 has suggested that within the 39-amino-acid residue carboxyl-terminal domain of PS-1 (located in the cytoplasm in almost all topographic models of PS-1 in the membrane; see table 3 for a list of cytoplasmic proteins that interact with the carboxyl terminal region of PS) there exists a specific binding and regulating domain for the brain G_o protein. The domain of PS-1 that binds G_o in vitro also shows some local amino acid sequence homologies with the G-binding domains of two other GPCR proteins, the D2 dopaminergic [54] and the 5HT-1B receptors [55], as well as the G-protein-activating oligopeptide, mastoparan [56] (fig. 2). The possibility that PS-1 may be a GPCR has not, however, been further reported upon since the original publication [53], and requires more adequate exploration in in vivo experiments.

Interactions of PS with β-APP

In 1996, shortly after the discovery of the PS proteins we proposed [57] a model based on certain precedents in other systems [58, 59] that the formation of the neurotoxic AD polypeptide, Aβ, from β-APP requires a specific cell-cell interaction in the brain that is mediated by the direct and specific interaction of β-APP on one cell surface with either PS-1 or PS-2 on the other, as the first step in the eventual production of Aβ. This model was based on its striking analogy to precedents in *Drosophila* eye

Table 3. Some proteins that interact with the PS-1 or PS-2 C-terminal domain

Protein	PS	Method	Function	Reference
Go	PS-1	Co-Ip in PS+ cells	G protein signaling	53
Bcl-X_L	PS-1/PS-2	yeast-two-hybrid/Co-IP	cell death	89
Calsenilin	PS-2	yeast-two-hybrid/Co-Ip	cleavage	90
PSAP	PS-1	yeast-two-hybrid	adaptor	91

PSAP, PS-1-associated protein; Co-IP, co-immunoprecipitation.

Table 4. Some proteins that interact with full-length PS-1- or PS-2

Protein	PS	Method	Function	Reference
β-APP	PS-1, PS-2	cell-cell adhesion Co-IP Co-IP	Aβ production	60 62 63
Bcl-2	PS-1	yeast two-hybrid	signaling	92
Jif-1	PS-1	yeast two-hybrid	signaling	93
Notch	PS-1	Co-IP	signaling	94
Nicastrin	PS-1, PS-2	Co-IP, direct binding	PS complex	95
Cadherin	PS-1	Co-IP	adhesion	96

Co-IP, co-immunoprecipitation.

development [58, 59]. That same year we presented the first evidence for a specific interaction in cell culture between cell surface β-APP and either PS-1 or PS-2 [60] on a second cell. We demonstrated that when β-APP-transfected human DAMI cells (these cells were chosen for β-APP transfection because they normally express only negligible amounts of endogenous β-APP at the cell surface [61]) were mixed under appropriate conditions with either PS-1- or PS-2-transfected DAMI cells [60], cell-cell aggregation was observed under light microscopy that did not occur if vector-only transfected DAMI cells were used instead of either transfected cell. As a further control for the molecular specificity of the cell-cell aggregation, the experiment was carried out in the presence of an excess of soluble β-APP. Aggregation was inhibited in the presence of soluble β-APP, demonstrating directly that the binding of β-APP to the PS-expressing cells was involved in the cell-cell aggregation. Double-immunofluorescence experiments on specific cell aggregates with monoclonal antibodies to β-APP and polyclonal anti-peptide antibodies to PS-1 or PS-2 showed that each small aggregate consisted of mixtures of adjacent β-APP-expressing and PS-1- or PS-2-expressing cells. Two other groups [62, 63] have since confirmed, using another method (see table 4), that β-APP interacts with PS-1 and PS-2. Pradier et al. [64] mapped the β-APP/PS-binding domains and reported that the hydrophilic N-terminal domain of PS-2 was sufficient for interaction with APP, and that this fragment could displace the β-APP:PS-1 interaction. This is consistent with our observations (see below).

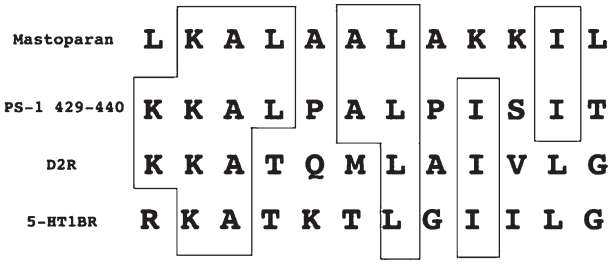

Figure 2. Sequence conservation among the carboxyl-terminal tails of PS-1 and PS-2, mastoparan and some GPCRs.The G_o-binding domain of PS-1 identified by Smine et al. [53] possesses sequence similarity to mastoparan and the GPCRs D2 dopaminergic receptor (D2R) and the 5HT-1B receptor [Reproduced from ref. 53 with permission from *Journal of Biological Chemistry*].

There is now substantial, but not uncontested [65, 66], evidence to support the claim that PS is itself the protein with γ-secretase activity, when it is part of a complex containing nicastrin, Pen-2 and Aph-1. The complex is involved in the intramembranous cleavage of several type I transmembrane proteins, including β-APP, Notch and ErbB4 [65]. In PS-1/PS-2 double knock-out cells, Aβ secretion into the medium is completely inhibited. Biochemical studies show that the production of the Notch intracellular domain is also entirely eliminated with deletions of both PS-1 and PS-2 genes. But the possibility exists that in this case, one of the other components of the PS complex that may be the true γ-secretase is synthesized but is not transported to the cell surface without PS. Nicastrin is not likely to be a protease [65].

Several other observations strongly support the idea that the PS may be the γ-secretase enzyme. The first involves two highly conserved Asp residues at positions 257 and 385 of PS-1. Substituting these with Ala leads to defects in Aβ secretion and the accumulation of C-terminal fragments of β-APP [67]. This has led to the suggestion that Asp 257 and Asp 385 could be within the active site of a new di-aspartyl protease, PS. Furthermore a short stretch of conserved residues that surround Asp 385 in PS-1 are similar to the putative aspartyl catalytic domain of the bacterial polytopic membrane protease called prepilin peptidase [68], prompting the suggestion that the motif might be important for proteolytic cleavage within TM domains. However, single replacement of Asp 257 or formation of a deletion construct of PS-1 that lacks Asp 257 [65], allows the PS complex to retain activity, casting

doubt on PS being a classical aspartyl protease. In addition, the expression of PS-1 with substitution of Asp 257, or both Asp 257 and Asp 385, has differing effects on the cleavage of β-APP and Notch. The second observation in support of PS having γ-secretase activity is that PS-1 and γ-secretase activity co-elute and/or co-precipitate in biochemical fractionation studies [69]. The third observation is that several potent aspartyl protease transition state analog inhibitors have been developed that inhibit Aβ production, and that can be selectively cross-linked to PS-1 or PS-2 in photoaffinity labeling experiments [70].

If PS is indeed the γ-secretase enzyme, an apparently attractive feature for the two-aspartyl-active site proposal has been that in the schematic 8-TM topological representation of PS (fig. 1C), D257 within helix VI and D385 within the proposed helix VII′ of the 8-TM model could be located close to one another where they could co-operate to form a transition state complex with the susceptible peptide bond of APP that is assumed to be conveniently located between them. However, in the schematic 7-TM topology, D-385 is located in the exoplasm *outside* the membrane in the large-loop region following after helix VI, and may be far removed from D-257 (fig. 1B), so the attraction of the hypothesized transition state complex in the 8-TM topographic model disappears in the 7-TM model. Furthermore, members of a family of proteins that have been termed the presenilin homologs (PSHs) have been discovered [71]. Analysis has revealed that these are 7-TM proteins with a glycosylated amino-terminal domain located in the lumen and the carboxyl-terminal domain in the cytosol. PS and PSHs have two conserved Asp residues in the motifs YD and LGLGD within adjacently placed TM domains in topological models. A third stretch of homology comprises the PALP residues in the carboxyl terminus of the proteins. Apart from these three small conserved stretches, there is very little homology between the PSHs and PS. However, since having both Asps in similar domains in these two proteins seems more than simply coincidental, it has led to support for the 8-TM topography for PS and a partially homologous 7-TM structure for PSH.

At this stage, it should be emphasized again that diagrams such as those in figure 1B and C are only one-dimensional representations of the membrane topography of a protein such as PS, but no more than that. More detailed structural information, such as which of the TM helices are in close proximity, and hence which residues are apposed in the protein interior, can only be determined by information about the second and third dimensions of the molecule. This obvious point is made crystal clear by examination of the three-dimensional structure obtained by X-ray crystallography of the 7-TM protein rhodopsin [72] (see fig. 3). In this structure, the TM domains correspond closely to those found by Kyte-Doolittle plots, but the overall shape of the molecule in the plane of the membrane is roughly an oval instead of a linear one, and the arrangement of the 7-TMs with respect to one another shows no correspondence to their sequences in the proteins. Furthermore, the helices are inclined at various angles perpendicular to the membrane and several of them exhibit kinks along their lengths. Therefore, matters such as the true dispositions of D-257 and D-385 in the PS-1 structure, and the extent of structural homology between PS and PSH, will have to await high-resolution X-ray crystallographic analysis. Pending such analysis, the conclusion of this review is that the 7-TM topography of the native PS molecule in the plasma membranes of cells (fig. 1B) is most likely to be the correct one.

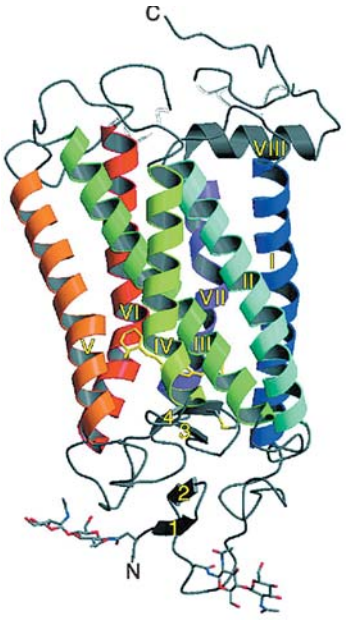

Figure 3. Schematic view of the X-ray structure of bovine rhodopsin parallel to the membrane. The seven hydrophobic transmembrane helices are labeled I through VII according to their order in the amino acid sequence. The outer surfaces of the helices coincide, more or less, with the outer surface of the surrounding membrane bilayer. *N* and *C* represent the N and C termini of the rhodopsin molecule on its exoplasmic and cytoplasmic sides, respectively. Several of the bound pigment molecules are labeled 1–4 [from ref. 72, copyright 2000, with permission from *Science*].

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