Presenilins: clarification of contradictory observations using molecular and developmental animal models

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Abstract: Presenilins (PS1 and PS2) were discovered first as human genes through linkage analyses of pedigrees with familial Alzheimer's disease. The PS proteins may form the catalytic core of a high molecular weight aspartyl protease complex that cleaves β-amyloid precursor protein (APP) within the transmembrane (TM) domain, yielding neurotoxic and apoptotic products such as Aβ peptide and APP C-terminal intracellular domain (AICD) fragments. Accumulating evidence indicates that PS proteins also cleave a number of type I TM proteins involved in various multicellular functions such as cell differentiation and cell-cell interaction/adhesion. Notch1 is one such protein, from which Nβ peptide and Notch intracellular domain (NICD) fragments are similarly generated. Thus, AICD and NICD fragments should conflict with each other by transducing contradictory signals to the nucleus. PS proteins undergo autocatalytic processing to form a heterodimeric complex from the full-length (FL) molecule. There appear to be two main hypotheses for the functional differences between the FL and heterodimeric form of PS proteins; 1) FL-PS is inactive and premature, and is activated by autolytic processing to form an active form of PS proteins as a heterodimeric complex (Wolfe's model), and 2) FL-PS and heterodimeric PS are responsible for cell apoptosis and differentiation, respectively, both of which are inhibited by stabilized C-terminal fragments (the Unifying Model). Recent reports suggest that PS-mediated proteolytic activities on APP and Notch are differentially regulated, and FL-PS is responsible for the apoptotic activation of cells. These observations seem to favor the Unifying Model rather than Wolfe's model, unless additional factors are presumed. We provide an overview of controversial observations obtained in human PS proteins and try to reconcile these data with genetic and developmental studies of *ps* genes in *Caenorhabditis elegans* and *Xenopus laevis*.

Key words: Alzheimer's disease, Apoptosis, Differentiation, Development, APP, Notch, Multicellular transition.

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Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder of the central nervous system, the common form of dementia characterized by β-amyloid (Aβ) plaques and neurofibrillary tangles in the brain. Presenilins 1 and 2 (PS1 and PS2) are highly homologous multi-spanning transmembrane (TM) proteins that were first discovered as genes co-segregating with familial Alzheimer's disease (FAD)[1-3]. The two *ps* genes are highly conserved throughout vertebrates. PS proteins may form the catalytic core of a high molecular weight γ-Secretase complex that hydrolyzes a number of type I TM proteins, among which β-amyloid precursor protein (APP) and Notch1 have been characterized most extensively. APP and Notch1 are cleaved similarly at multiple cleavage sites by PS-mediated, regulated intramembrane proteolysis (RIP) in the TM regions, generating Aβ/AICD and Nβ/NICD fragments, respectively (Fig. 1). The putative proteases responsible for these cleavages have been called historically by different names such as 'γ-secretase' for APP and 'S3-protease' for Notch1. Both are now often referred to simply as 'γ-Secretase', although whether or not γ-secretase and S3-protease are in fact the same remains questionable.

The γ-secretase is a promiscuous protease that cleaves APP at the multiple γ-sites in the TM domain, resulting in a variety of Aβ peptides consisting of 38 to 43 amino acid (aa) residues, $Aβ_{38}$ to $Aβ_{43}$, among which $Aβ_{40}$ is the major product with the wild-type PS proteins (Fig. 1C). The PS FAD mutants may increase relative levels of the more amyloidogenic Aβ variant $Aβ_{42}$ against $Aβ_{40}$[4]. A similar phenomenon has been reported for Notch1 proteolysis, $Nβ_{25}$ against $Nβ_{21}$, with PS FAD mutations[5]. Although APP and Notch1 are cleaved by PS-mediated endoproteolysis, these substrates are responsible for contradictory cellular functions such as cell apoptosis and differentiation, respectively. No sequence similarity is shared between their cleavage site regions in the TM domains, except the valine residues next to the ε- and S3-cleavage sites for AICD and NICD, respectively (Fig. 1C).

Thus, one could ask whether these two instances of PS-mediated endoproteolytic activity are indistinguishable or discrete. In this review, we summarize recent controversial observations from pharmacological and biochemical studies concerning PS action on APP and Notch1, as well as those obtained from molecular genetic and developmental studies using non-mammal animal models, by focusing on two models proposed for the regulation of PS-mediated proteolytic activity, Wolfe's model[6] and the Unifying Model[7].

Topology and structure of PS proteins

Hydropathy profiles predict that PS proteins have 10 hydrophobic regions (HRs 1 to 10 from the N- to C-termini). There is no consensus, however, concerning the number of membrane-spanning TM domains; the estimation range is from six to nine[8-16]. The discrepancy could possibly be attributable to technical differences such as antibodies or tagging, but it may also be possible that PS proteins are subjected to conformational changes depending on activities and/or cellular conditions. Although other models remain feasible, the eight-TM domain model proposed by Li and Greenwald[9] seems to be most frequently cited. In this model, HRs 1 to 6, and 8 and 9 are membrane-crossing domains, denoted TMs I to VIII, respectively. A large hydrophilic loop (HL) region between K264 and K380 (hereafter, aa positions refer to human PS1 unless otherwise stated), flanked by TMs VI and VII, would thus reside

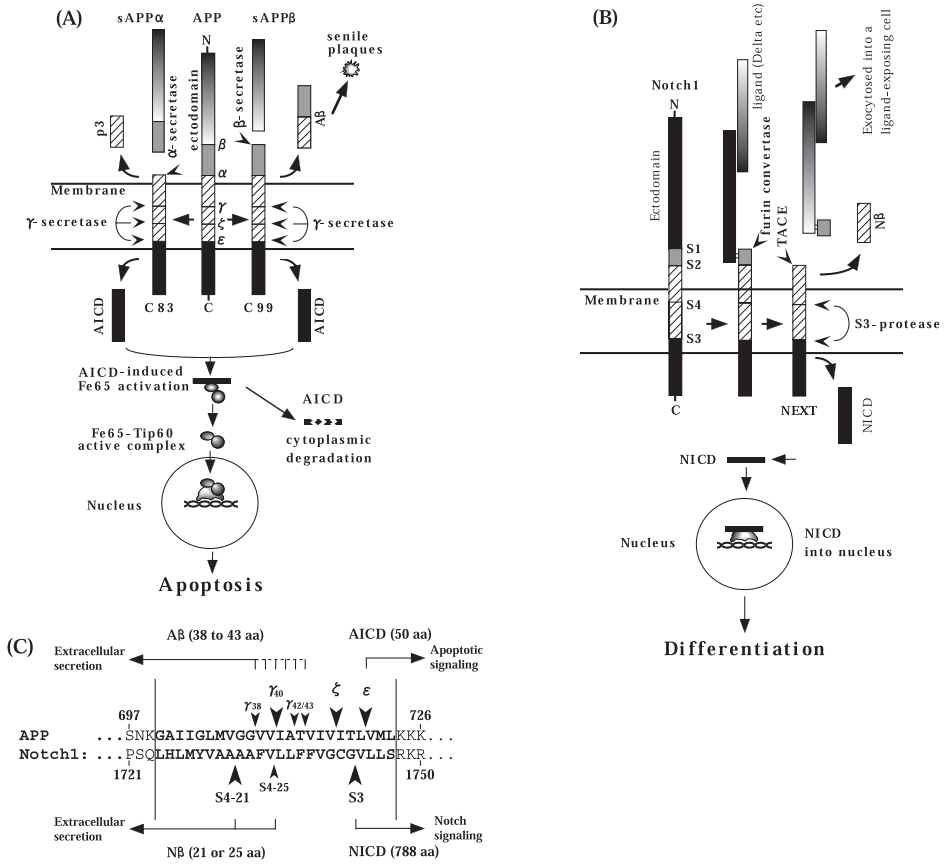

Fig. 1. PS-mediated RIP of APP and Notch1

APP and Notch1 are initially synthesized as 82–87 kDa proteins, depending on splicing variants, and as ~300 kDa precursors, respectively. The final product, AICD or NICD, generated from APP or Notch1, is released into cytoplasm and transduces a signal to the nucleus for promoting transcriptional activation toward cell apoptosis or differentiation, respectively. (A) The first cleavage of APP in the ectodomain by α- or β-secretase generates the C83 or C99 fragment, consisting of the C-terminal 83 or 99 aa residues embedded in the membrane, respectively. Subsequently, C83 or C99 is cleaved sequentially at the ε-/ζ-/γ-cleavage sites by the PS-mediated γ-secretase in the TM domain, resulting in generation of the N-terminal half p3 or Aβ peptide, and the C-terminal half AICD fragment[88-90]. Aβ is secreted extracellularly, accumulating as senile plaques in the brain; AICD released in the cytoplasm activates the Fe65-Tip60 complex to translocate into the nucleus to promote gene transcription for apoptosis, while AICD itself is degraded in the cytoplasm[35)91)]. "sAPPα" and "sAPPβ" are α- and β-secretase releasing APP-derived N-terminal fragments, respectively. The arrowheads indicate respective cleavage positions by α-, β- and γ-secretases. (B) Notch1 is constitutively cleaved by furin convertase at the S1-cleavage site in the trans-Golgi; the generated extracellular ectodomain fragment remains noncovalently linked, forming a heterodimeric receptor. Then, a cell-surface ligand, such as Delta or Jagged, on a neighboring cell attaches to the ectodomain. Subsequently, the second cleavage is mediated by TNFα-converting enzyme (TACE) at the S2-cleavage site located 13 aa residues outside of the membrane. Subsequently, the Notch1 ectodomain is removed and endocytosed into a Notch-ligand-exposing cell. The remaining Notch1 extracellular truncation (NEXT) fragment is cleaved by the PS-mediated S3-protease at the S3-/S4-cleavage sites in the TM domain[5)92)]. The final products, namely Nβ and NICD, are released; the latter translocates into the nucleus and interacts directly with transcription factors, leading to the induction of genes promoting cell differentiation. The arrowheads indicate cleavage positions by furin convertase (S1), TACE (S2), and PS-mediated S3-proteases (S3 and S4). (C) Sequence comparison of the cleavage site regions by PS-mediated RIP in TM domains between human APP (770 aa) and mouse Notch1 (2531 aa). The cleavage sites are indicated by arrowheads, denoting ε-/ζ-/γ- and S3-/S4-cleavage sites for APP and Notch1, respectively. Note that PS-mediated RIP exhibits promiscuous cleavages in APP between γ_{38}- and γ_{43}-sites as indicated (dashed line). The γ_{40} or S4-21 site is the major position cleaved in APP or Notch1, respectively, with the wild-type PS. The aa residues shown by bold letters are those located within the TM domains as flanked by two vertical lines. The numbers at both ends of the aa sequences indicate aa positions from the N-termini. Arrows indicate positions of Aβ/AICD and Nβ/NICD fragments and their destinations. In these regions, no sequence differences are found between human and mouse APPs, while S1722 and L1726 are replaced by A1733 and F1737 in human Notch1.

in the cytoplasm together with both the N- and C-termini[8)11)12)].

PS proteins undergo an autocatalytic cleavage in the HL region to generate the N- and C-terminal fragments (NTF and CTF, respectively)[17)]. PS1 usually exists as a heterodimeric form of the NTF+CTF complex localized primarily to the Golgi compartment. When PS proteins are exogenously over-expressed, unprocessed full-length (FL) molecules accumulate without altering the level of NTF+CTF complexes in the cell. Overproduction of FL-PS molecules in transfected cells results in an increased susceptibility to apoptotic stimuli or can directly cause apoptosis[18)19)]. The active form of the γ-Secretase complex contains at least four proteins, including Nct, Pen2, Aph1, and either one of PS proteins[20)]. Pen2 appears to play a central role in the regulation of the autolytic processing of FL-PS molecules[21-24)].

Molecular genetics and evolution of *ps* genes

To date, *ps* homologous genes have been identified in many phyla/divisions, including animals, plants, and protozoans. The absence of *ps* genes has been confirmed in a number of species of fungi and prokaryotes[7)].

It is generally believed that *ps* FAD mutations are gain-of-function mutations because they are dominantly expressed with 100% penetration in FAD pedigrees. However, the first evidence indicating that *ps1* FAD mutations are loss-of-function has been produced in a study of Notch over-expression phenotypes in the threadworm, *Caenorhabditis elegans*. This study screened for suppressor genes of a gain-of-function mutation of *lin12*, the *notch* homologue in *C. elegans*. A gene named *sel12* (*suppressor/enhancer of lin12*) was then identified as a loss-of-function mutant that rescued the Lin-12 over-expression phenotype of defective vulva formation. The *sel12* mutation alone resulted in an egg-laying defect phenotype. Sel12 is 50% identical to human PS proteins[25)]. Introduction of the wild-type human *ps1* into a *C. elegans sel12* mutant rescued the egg-laying defect, while the *ps1* FAD mutant did not, implying that *ps1* FAD mutations are loss-of-function in Notch signaling[26)]. This may be consistent with the observation that replacement of L286 with charged aa residues in human PS1 —where a naturally occurring FAD mutation has been identified—(1) showed the FAD phenotype by increases in $A\beta_{42/43}$ versus $A\beta_{40}$, (2) inhibited Notch S3-protease activity, and (3) abolished NICD transport to the nucleus[27)]. Human PS proteins also demonstrated a limited identity of 20% to the protein encoded by *spe4* (*spermatogenesis*) in *C. elegans*, which is involved in the formation and stabilization of the fibrous body membrane organelle during worm spermatogenesis[28)]. In a search of the *C. elegans* DNA database, a gene named *hop1* (*homologue of presenilin*) was identified[29)]. Hop1 is 30% identical to human PS1 and PS2. The ratio of HL sizes in Sel12, Hop1, and Spe4 can be given approximately as 4 : 3 : 6, which corresponds to that of PS1 and PS2 in vertebrates and PSγ in chordates, respectively. The HL regions of various PS proteins from animals and plants are classifiable into either one of these three types, named the α-, β- and γ-types[7)], even though aa sequences are highly divergent in the overall HL regions among different species (Table 1). Such size regularity is not found in the N-terminal (NT) hydrophilic regions, implying that the PS HL size, rather than the overall HL aa sequences, may have been under evolutionary constraints. In this context, it may be worth noting that expression of anti-sense *ps1* results in a failure of the clones to differentiate concomitant with increased apoptosis, while cells transfected with anti-sense *ps2* differentiate normally, indicating some functional difference between the α- and β- type PS proteins[30)]. Interestingly, some unicellular

Table 1. Types of PS homologues from various organisms

Sources	Presenilins	aa sizes of			Relative sizes of	
		Total	NT	HL	NT	HL
α *type*						
Human	PS1	467	81	116	1.00	1.00
Frog	PSα	433	47	116	0.58	1.00
Fish	PS1	456	69	116	0.85	1.00
Threadworm	SEL12	444	49	126	0.60	1.09
Sponge	PSα	476	83	121	1.02	1.04
Thale cress	PSα	397	17	108	0.21	0.93
β *type*						
Human	PS2	448	87	91	1.07	0.78
Frog	PSβ	449	90	89	1.11	0.77
Fish	PS2	441	85	86	1.05	0.74
Threadworm	HOP1	358	12	84	0.15	0.72
γ *type*						
Lancelet	BfPS	525	93	164	1.15	1.41
Sea squirt	PSγ	504	63	171	0.78	1.47
Snail	PSγ	582	118	196	1.46	1.69
Fly	DmPS	541	103	170	1.27	1.47
Threadworm	SPE4	465	23	182	0.28	1.57
Thale cress	PSγ	453	12	172	0.15	1.48
Rice	PSγ	478	31	168	0.38	1.45

NT and HL stand for N-terminal and hydrophilic loop regions. See Section 3.

organisms such as choanoflagellates (*Monosiga ovata*), which are thought to be the prototype of animals and that exhibit multicellular functions such as cell-cell interactions and communication, may also have a *ps* gene[7].

Type I transmembrane proteins as substrates

Accumulating evidence implicates PS proteins as the catalytic core of a multi-protein complex that executes the unusual cleavage of its substrates during RIP[31)32)]. To date, in addition to APP and Notch1, a number of Type I TM proteins have been identified as substrates for PS-mediated RIP. This list includes not only synonymous proteins, such as APP homologues like APLP1 and 2 and Notch1 homologues like Notch2 through 4, but also other proteins as listed in Table 2. Although both APP and Notch1 are processed by PS-mediated endoproteolysis, their products, AICD and NICD, exert contradictory effects on cell fate, cell apoptosis and differentiation, respectively. In this context, it may be worth noting that AICD inhibits the NICD signaling cascade[36)]. Although no data are available as

Table 2. Substrates for PS-mediated endoproteolytic processing

Substrates	ICD functions[a]	Refs.
APP	Apoptotic activation	Cao and Südhof, 2001[33]
	by AICD binding	Kinoshita et al., 2002[34]
	to Fe65-Tip60	Cao and Südhof, 2004[35]
	Anti-Notch signaling	Roncarati et al., 2002[36]
Notch1	Cell differentiation	De Strooper et al., 1999[37]
		Fortine, 2001[31]
CD43	Cell interaction	Andersson et al., 2005[38]
CD44	Cell adhesion	Lammich et al., 2002[39]
	Anti-apoptosis	Lakshman et al., 2005[40]
E-cadherin	Cell adhesion	Marambaud et al., 2002[41]
ErbB-4	Cell proliferation	Ni et al., 2001[42]
	and differentiation	
LRP	Blocking of Fe65-Tip60	May et al., 2002[43]
	activated by AICD	Kinoshita et al., 2003[44]
Nectin-1	Cell adhesion	Kim et al., 2002[45]
NRADD	Cell apoptosis	Gowrishankar et al., 2004[46]
Delta	Notch ligands	Ikeuchi and Sisodia, 2003[47]
Jagged		

[a] ICD stands for intracellular domain. See Section 4.

yet demonstrating an inhibitory effect by NICD on AICD, at least some ICDs produced similarly can transduce anti-apoptotic signaling or even directly oppose AICD signaling[40)44)].

Thus, PS-mediated RIP may be a general mechanism activating signaling pathways downstream of Type I TM receptors. The ICD fragments released by PS-mediated cleavage may transduce signals that fall into one of the conflicting functional categories, possessing pro- or anti-apoptotic function, the latter of which may be accompanied by induction of various multicellular functions such as cell differentiation and cell-cell interaction/adhesion.

Post-translational modifications and two models for PS activation

1. Autolytic processing and phosphorylation

Two post-translational modifications of PS proteins take place in the HL region, endoproteolytic processing and phosphorylation. The heterogeneous endoproteolytic cleavages occur between T291 and A299, resulting in generation of the NTF+CTF complex[48)]. The phosphorylation at T354 functions to stabilize selectively CTF, resulting in elevation of relative amounts of CTF to NTF to maintain a 1:1 ratio between NTF and unphosphorylated CTF[49)]. The putative protease that endoproteolytically processes FL-PS proteins has been referred to as "presenilinase" but is likely presenilin itself[17)]. Auto-

lytic processing may occur during protein trafficking from the ER to the Golgi compartments because FL-PS molecules can be found in the ER, while the NTF and CTF are detected primarily in the Golgi[50-52]. One of the PS1 FAD mutants, the ΔE9 variant—which lacks a segment of T291 to S319 and has a S290C substitution because of an Exon 9 deletion that cannot be cleaved—is found in both the ER and Golgi fractions as an FL molecule[53-55]. The blocking of autolytic processing does not seem to prevent FL-PS molecules from being trafficked normally[56].

2. Models for the regulation of PS-mediated endoproteolysis

Wolfe et al[58] reported that either mutation of the conserved aspartate residue at D257 in TM VI or D385 in TM VII resulted in a large accumulation of human FL-PS1 molecules in one of the three transfected CHO cell lines. They observed in these cell lines that either the FL-PS mutant protein did not exhibit γ-secretase activity. It has been also reported that a PS1 D257A or D385A substituion abolishes Notch S3 cleavage[59]. Based on these observations, a model was proposed (Fig. 2A) in which FL-PS is activated by autolytic processing to form a heterodimeric NTF+CTF complex capable of cleaving both APP- and Notch-based substrates such as C83/C99 and NEXT, respectively[6]. In support of this model, Xia et al[60] reported that a transgenic mouse line expressing PS1 D257A or D385A mutant exhibits neither autolytic processing of PS1 nor Aβ production.

Hashimoto-Gotoh et al[7] have proposed the Unifying Model (Fig. 2B) to explain the distinct behaviors of PS1 and PS2 during oogenesis and embryogenesis in *Xenopus laevis*. Although both *psα* and *psβ* (corresponding to human *ps1* and *ps2*) mRNAs are prominent during oogenesis, *psα* mRNA is degraded (~80%) upon oocyte maturation, while *psβ* mRNA remains prominent in the early embryos until the midblastula transition (MBT) around stage 8, then is degraded quickly to levels below those of *psα*[61] (Fig. 3A, left panel). PSα protein is entirely processed, while PSβ is mostly unprocessed in the early embryos. PSα CTF is fully phosphorylated on T320 (corresponding to T354 in human PS1) in the early embryos, but after MBT, the major component of PSα CTF is unphosphorylated, switching slowly from phosphorylated (Fig. 3A, right panel). Then, the majority of PSβ is processed only in the tailbud embryos at stage 28[62]. Moreover, anti-PSβ antibodies suppress apoptotic activation of *Xenopus* egg extracts nearly completely at similar levels of Bcl-2, while anti-PSα antibodies show no effect[7] (Hashimoto-Gotoh et al., unpublished data). Considering the fact that *Xenopus* early embryos are potentially apoptotic and suppressive of Notch signaling until MBT[63-65], these data were interpreted to indicate that FL and heterodimeric PS molecules may be responsible for cell apoptosis and differentiation, respectively, and stabilized PSα CTF may inhibit both activities through a dominant-negative effect[7]. Then, the model predicts that expression levels of *psα* relative to those of *psβ* and the phosphorylation efficiency of PSα CTF may be critical for determining cell fates (Fig. 3B).

Differential regulation of PS-mediated proteolysis

1. Mutagenesis of conserved aspartyl residues

In contrast to the report by Wolfe et al[58], multiple independent groups have demonstrated that the PS1 D257 mutation does not suppress PS1-mediated APP γ-cleavage[66-69]. Furthermore, mouse PS2 mutants accumulating FL molecules exhibit enhanced Aβ generation[70], and the PS1 ΔE9 mutant expressed exogenously in PS-null cells accumulates as an FL molecule that is active as γ-secretase, as assessed by AICD generation[57]. By analyzing PS1 mutants at Y288, Laudon et al[56] have shown that the

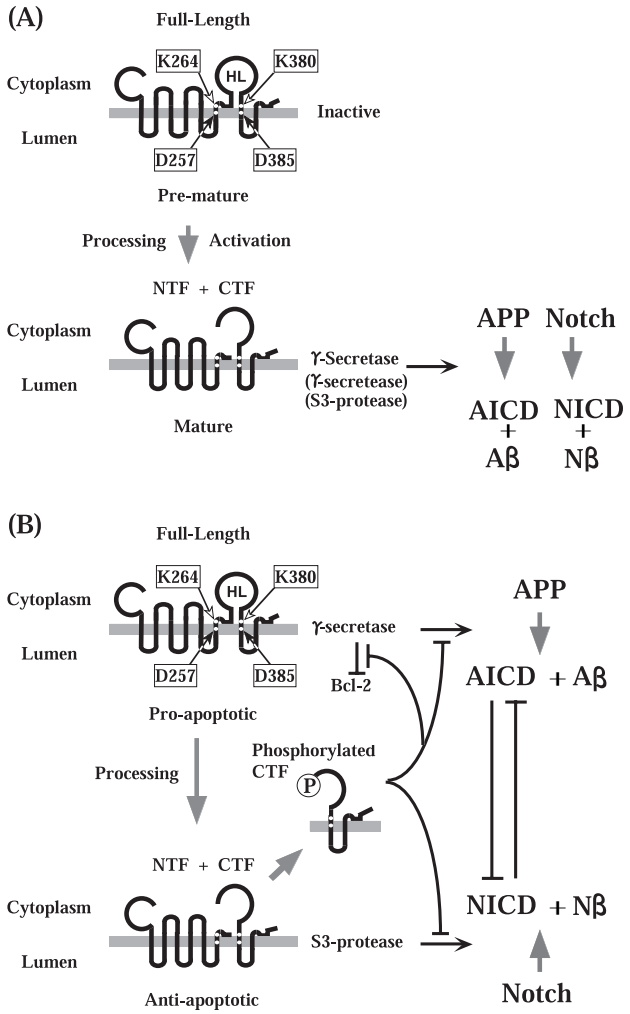

Fig. 2. Two models for regulation of PS-mediated proteolytic activities.

The membrane topology of human PS proteins presented here is based tentatively on the eight-TM model[9]. The aa positions are indicated according to the human PS1 structure. Autolytic cleavage of FL-PS1 occurs heterogeneously at or near V292 to A299 in the HL region. The aa positions of the N- and C-terminal ends of the HL region, K264 and K380, respectively, are shown by open arrows on the FL molecules. The two critical aspartate residues, D257 and D385, are indicated by closed arrows, located within TMs VI and VII, respectively. (A) Wolfe's model assumes that FL-PS molecules are the premature, inactive form of PS proteins, which should be processed by autolysis to form the heterodimeric NTF+CTF complex as an active γ-Secretase responsible for both APPγ- and Notch S3-cleavages. (B) The Unifying Model postulates that FL-PS and the NTF+CTF complex of PS cleave preferentially, rather than selectively, pro- and anti-apoptotic substrates such as APP and Notch, respectively. S3-protease and γ-secretase activities are distinguishable and mainly exerted by PS1 and PS2 molecules, respectively. PS1 is assumed to be more sensitive to PS-autolysis because of its larger HL size compared to PS2. FL-PS may directly antagonize the anti-apoptotic function of Bcl-2[86]. Phosphorylated PS1 CTF with a prolonged stability[49] may block both FL- and heterodimeric (NTF+CTF) PS activities in a dominant-negative manner. AICD inhibits the NICD signaling cascade[36], and NICD may also inhibit AICD signaling, as LICD from LRP (low-density lipoprotein receptor-related protein) does (Table 2).

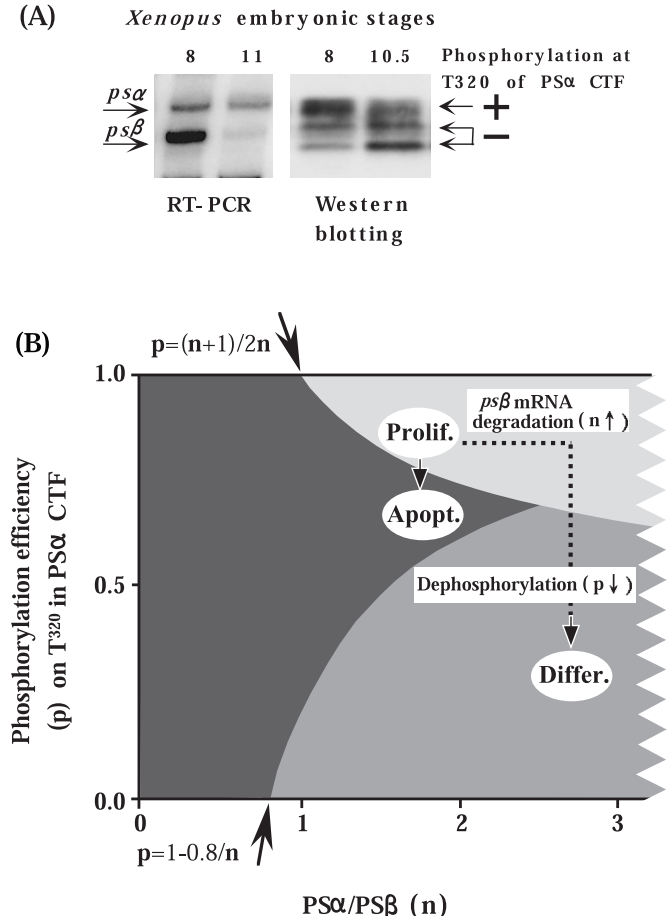

Fig. 3. Coordination between *psβ* mRNA degradation and dephosphorylation of PSα CTF after MBT (A), and the mathematical formulation of cellular conditions during *Xenopus* embryogenesis according to the Unifying Model (B).

(A) After MBT at stage 8, *Xenopus psβ* mRNA was specifically and quickly degraded as detected by RT-PCR[60]; coincidentally, the bulk of PSα CTF is dephosphorylated slowly as determined by Western blotting[62]. (B) The **p** and **n** values are those for phosphorylation efficiency of PSα CTF and for the ratio of the protein synthesis of PSα to that of PSβ. The equations were obtained by using experimental values for autolytic efficiencies of PSα and PSβ and the phosphorylation efficiency of PSα CTF in the early embryos of *X. laevis*[7]. The cellular phase of early embryos will move on from proliferation to apoptosis by reducing **p** before increasing **n**, but to differentiation after increasing **n**. **Apopt.**, **Prolif.** and **Differ.**, indicate apoptosis, proliferation and differentiation, respectively.

capacity for endoproteolysis of PS and γ-secretase activity can be separated. In summary, the aa substitution of D385A in PS1 is likely to result in suppression of all activities of PS autolysis and APP γ- and Notch S3-cleavages, while D257A may suppress PS autolytic processing and Notch S3-protease activities. Whether or not D257 is indispensable for APP γ-secretase activity, however, remains

controversial. To explain the apparently conflicting results, it is intriguing to speculate that the two conserved Asp residues may play somewhat different roles in the proteolytic reaction; for example, D257 for binding affinity and D385 for catalytic activity[71].

2. Competitive inhibitions

If γ- and S3-cleavages are conducted by a single catalytic activity, APP and Notch1 may act as competitive inhibitors against each other. The observation of competitive inhibition between APP- and Notch1-based substrates for PS-mediated endoproteolysis would, therefore, favor Wolfe's model. The reports seem controversial. While some groups demonstrated that these substrates did not compete[72)73)], others have observed competition[74)75)]. According to Schroeter et al[76], a competitive interaction between APP- and Notch1-based substrates in cells is only seen if the combined substrate concentration is in excess; merely altering the ratio of substrates does not result in competitive inhibition. This conclusion could be interpreted as that APP and Notch1 TM domains are cleaved in a similar manner but not recognized quite equally by the single activity. It has been reported that the synthetic peptide with 30 aa residues as encoded by human PS1 Exon 9 competitively inhibited γ-secretase activity[71]. It is of interest to see how this peptide interacts with PS autolytic processing and S3-protease activities.

3. Pharmacological studies with γ-secretase inhibitors

Several transition-state analogue inhibitors of γ-secretase targeted the PS di-aspartyl active site[77)78)]. These inhibitors, labeling only the PS NTF+CTF complex but not FL-PS, blocked PS-mediated endoproteolytic activities against both APP and Notch1. The results have been argued as supporting Wolfe's model[79)80)]. In contrast, some of the transition-state analogues inhibited de novo Aβ generation without blocking de novo generation of NTF and CTF, while some others such as CM35 and MW167 affected both reactions, implying that 'presenilinase' and γ-secretase appear to be pharmacologically distinct[81]. Subsequently, Campbell et al[17] have reported that time-dependent de novo Aβ and AICD generation was not affected by inhibiting 'presenilinase' activity with MW167. There are also some inhibitors that are less effective against PS2 than against PS1 γ-secretase activities, suggesting that the PS2 γ-secretase may be a more potent enzyme with APP than PS1 is[77)82)83)].

Non-peptidic inhibitors, such as JLK2 and JLK6, markedly reduce Aβ generation and increase the levels of C83/C99 without affecting endoproteolysis of FL-PS and Notch S3-cleavage[84]. Petit et al[85] have shown that these inhibitors directly block Aβ formation in C99-expressing cells without affecting β-secretase-like activity. These results suggest that PS-mediated APP and Notch1 cleavage activities may be differentially regulated. Together, data from these inhibitor studies suggest that PS autolytic processing may be required for PS-mediated cleavage of Notch1 but not necessarily for APP. These data may be consistent with others, which indicate that proteolysis of APP and Notch1 are differentially regulated in the PS1 L286 mutant[27], or in splicing variant PS1 ΔE8, lacking the segment L258 to S289 and having a D257A substitution because of an Exon 8 deletion[66].

In this context, it should be noted that two recent observations address the possibility that FL-PS acts as a pro-apoptotic factor. The relative levels of FL-PS against NTF+CTF complex are increased upon suppressing *pen2* gene expression by RNA interference, and this results in caspase activation for apoptosis[86]. The second observation is that FL-PS promotes the degradation of FKBP38 and Bcl-2 and sequesters these proteins in the ER and Golgi compartments. This effect inhibits FKBP38-mediated mitochondrial targeting of Bcl-2, thus acting as a pro-apoptotic factor in a manner independent

of γ-secretase enzymatic activity[87].

Concluding remarks

In the last decade since the discovery of two human *ps* genes responsible for FAD, studies have greatly advanced our understanding of AD-related biology, especially the molecular mechanisms involved in PS-mediated regulation of APP and Notch signalings for cell apoptosis and differentiation, respectively. One of the most striking observations may be that the primary function of PS proteins may not be relevant to neural functions but instead is responsible for functions involved in biological reproduction and development. In fact, *ps* genes are found in the most primitive true animals such as sponges (*Ephydatia fluviatilis*) without any neural systems and also even in plants (Table 1).

A number of substrates, including Notch1, for PS-mediated RIP are involved in anti-apoptotic, and/or multicellular functions such as cell differentiation and cell-cell interaction/adhesion, while a few, such as APP, are pro-apoptotic (Table 2). Reports now suggest that the final steps of APP and Notch1 cleavages are differentially regulated, despite the fact that both are mediated by PS proteins. Because these discrete activities can be observed even for APP- and Notch1-based substrates such as C83/C99 and NEXT, respectively, the fact that they may be discrete should not be ascribed to ligands or proteases for the reactions occurring before PS-mediated cleavage. How PS can differentiate APP and Notch1 upon recognizing substrates and thus transduce contradictory signals to the nucleus in a manner dependent on the spatiotemporal requirement of cells remains to be elucidated.

One possibility may be that the levels of substrate expression determine the predominant cleavage pattern, for example APP or Notch1. In this case, Wolfe's model would be feasible, and both APP γ- and Notch S3-cleavages would occur in the Golgi compartment or at the plasma membrane. Another possibility may be that the point may be the difference of intracellular localization of APP and Notch1 to determine the preferential cleavage pattern. In this case, the Unifying Model would be feasible, and APP γ-cleavage would occur mainly in the ER, while Notch S3-cleavage would occur at the plasma membrane. Quantitative application of this model provides a mathematical formulation that correlates well with the patterns of gene expression and protein modifications observed in *Xenopus* embryonic development (Fig. 3A and B).

Studies of AD-related basic biology should provide a greater understanding of the molecular mechanisms concerning the most fundamental cell fate decisions among proliferation, differentiation, and apoptosis, which could be regulated, according to the Unifying Model, by rather simple mechanisms through the quantitative metabolic program of presenilins. In any case, the Presenilins may be key proteins in the transition from unicellular to multicellular organisms, evolutionarily and developmentally.

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