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A unifying model for functional difference and redundancy of presenilin-1 and -2 in cell apoptosis and differentiation

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Abstract

Mutations in genes encoding the highly homologous proteins presenilin-1 and -2 (PS1 and PS2) are linked to the early onset of Alzheimer's disease (AD). Here, we report that polyclonal antibodies against *Xenopus* PSβ (PS2), but not PSα (PS1), suppress the in vitro apoptotic activation of *Xenopus* egg extracts. To clarify the relationship between structural and functional differences in presenilins, we searched for presenilin homologues in various living sources, and found that presenilins were divided into three distinct groups, named α-, β- and γ-types, based on the size of the large hydrophilic loop (HL) regions as follows: HLα/HLβ/HLγ = 4:3:6. No such size conservations were found in the N-terminal (NT) hydrophilic regions. Phylogenetic studies revealed that the presenilin genes were duplicated independently in different lineages of phyla/divisions, suggesting that there were functional requirements for and constraints on the generation and conservation of these HL sizes. On the basis of these findings, we propose a model postulating that both PS1 and PS2 can be differentiative or apoptotic when they are proteolytically processed within the HL regions or not, respectively, and PS1 may be more sensitive than PS2 to auto-proteolytic cleavage due to the larger size of the HL region of the former. Furthermore, the model assumes that C-terminal fragments (CTF) stabilized by phosphorylation may inhibit both the activities due to the dominant-negative effect. The model explains not only the functional redundancy but also apparently conflicting observations reported so far for PS1 and PS2.

Keywords: APP; Alzheimer's disease; β-amyloid; Dominant negative; Notch; γ-Secretase; Processing

1. Introduction

Mutations in human genes encoding two highly homologous proteins, presenilin-1 and -2 (PS1 and PS2), have been found in a number of pedigrees of familial Alzheimer's disease (FAD) (Sherrington et al., 1995; Rogaev et al., 1995). It has been reported that presenilins are autocatalytic aspartyl proteases that hydrolyze β-amyloid precursor protein (βAPP) and Notch protein, which are responsible for AD genesis and Notch signaling, respectively (Wolfe et al., 1999). The proposed structure of presenilins possesses eight transmembrane domains (TM I to TM VIII) with a large hydrophilic loop (HL) region flanked by TM VI and TM VII (Li and Greenwald, 1998). The hydrophobic amino acid sequences in the TM domains are highly conserved between human PS1 and PS2, as well as among various presenilin homologues from different living sources. In particular, two aspartate residues, one in TM VI and the other in TM VII, are conserved without exception among all the presenilin

Abbreviations: AD, Alzheimer's disease; Aβ, amyloid β-peptide; CTF, C-terminal fragment; FAD, familial AD; FLM, full-length molecule; HL, hydrophilic loop; MBT, midblastula transition; NT, N-terminus; NTF, N-terminal fragment; PBS, phosphate buffered saline (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4 at pH 7.4); PS, presenilin.

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homologues so far identified. These residues are thought to be located at the active center of presenilins, and to be responsible for hydrolyzing their own molecules into N- and C-terminal fragments (NTF and CTF), and in addition for hydrolyzing βAPP and Notch to yield amyloid β-peptide (Aβ) and NICD peptides, which are responsible for apoptosis of neuronal cells and embryonic cell differentiation, respectively (Steiner et al., 1999). The high structural similarity between PS1 and PS2 suggests that their physiological functions may be redundant or overlapping at least in part (Li and Greenwald, 1997).

On the other hand, several lines of evidence suggest that there may be functional differences between PS1 and PS2. For instance, (1) there is a strong bias in the frequency of FAD mutations in PS1 and PS2, namely 24 vs. 1, (2) PS1 knock-out (KO-) mice are embryonic lethal or die just after birth (Wong et al., 1997; Shen et al., 1997), while PS2 KO-mice live without serious health problems (Herreman et al., 1999), and (3) PS1 gene expression is down-regulated prior to apoptosis and tumor suppression (Roperch et al., 1998), while over-expression of the PS2 gene induces apoptosis (Wolozin et al., 1996). These observations would support the idea that PS1 and PS2 may play different biological roles (Lee et al., 1996).

In fact, we have observed that during *Xenopus* embryogenesis, PSα (PS1) and PSβ (PS2) show different behaviors at the levels of both gene expression and post-translational modifications. Briefly, although both the PSα and PSβ mRNAs are present at high levels in oocytes, the former is mostly (>80%) degraded upon oocyte maturation while the latter remains present at a high level until the midblastula transition (MBT) (Tsujimura et al., 1997). Moreover, PSα protein exists as processed molecules in oocytes and early embryos, as in the brain, while PSβ is present mainly (90%) as a full-length molecule (FLM) in early embryos (Watanabe et al., 2003). Furthermore, phosphorylation occurs efficiently (80%) on PSα but not PSβ CTFs during early embryogenesis (Watanabe et al., 2003), at which time cells are potentially apoptotic (Newmeyer et al., 1994; Sible et al., 1997) and Notch signaling is suppressed (Coffman et al., 1993). Finally, at MBT, the major species of PSα CTF is switched to the unphosphorylated one and the processed form of PSβ becomes predominant over the unprocessed one (Watanabe et al., 2003). The differential switching of gene expression and post-translational modifications of PSα and PSβ suggest that PS1 and PS2 play different roles during embryogenesis.

In this work, we studied the influence of anti-PSα and anti-PSβ antibodies on the in vitro activation of potential apoptotic capability of *Xenopus* egg extracts, analyzed the phylogenetic trees of different types of presenilins, and localized the time of presenilin gene duplication on the universal evolutionary tree. We discuss the possible relationship between the functions and structural differences of presenilins and finally propose and mathematically analyze a model for the differential involvement of PS1 and PS2 in cell differentiation and apoptosis assuming a pivotal role of CTF phosphorylation. This model explains both the differences and redundancy of *ps1* and *ps2* genes in biological functions and FAD genesis.

2. Materials and methods

2.1. *In vitro apoptosis assay using* Xenopus *laevis egg extracts*

Frog egg extracts were prepared according to Newmeyer and Wilson (1991). Polyclonal antibodies against *Xenopus* PSα and PSβ, AbPSαCTF and AbPSβCTF, have been described (Watanabe et al., 2003). The cytoplasmic extracts supplemented with mitochondrial fractions were divided into four tubes containing phosphate buffered saline (PBS), rabbit IgG, AbPSαCTF or AbPSβCTF kept at 4 °C for 30 min, and incubated at 15 °C for 3 h for apoptotic activation. The in vitro apoptotic activity was measured before (time 0) and after (3 h) apoptotic activation as fluorescence generated by cleavage of DEVD-MCA (Peptide Institute, Osaka, Japan) by caspase-3 and other group II caspases according to the manufacturer's instructions.

3. Results and discussion

3.1. *Apoptosis assay using* Xenopus *egg extracts*

Since *Xenopus* embryos employ apoptosis to remove damaged cells before but not after MBT (Hensey and Gautier, 1997) and this capability can be detected in vitro as apoptotic activation of *Xenopus* egg extracts (Newmeyer et al., 1994), we examined the possible influence of antibodies against PSα and PSβ on the apoptotic activation of *Xenopus* egg extracts. We observed that AbPSβCTF nearly completely suppressed the in vitro apoptotic activation of *Xenopus* egg extracts, while AbPSαCTF did not (Fig. 1). This observation suggesting that PSβ may be required for the apoptotic activation during *Xenopus* early embryogenesis appears to be consistent with a previous report on the pro-apoptotic activity of mouse PS2 (Vito et al., 1996). On the other hand, it should be emphasized that AbPSαCTF did not inhibit apoptotic activity of *Xenopus* egg extracts, suggesting that PSα may be required for some function(s) other than apoptosis at least during early embryogenesis. Since the major components of PSα and PSβ in *Xenopus* early embryos are processed and unprocessed molecules, respectively (Watanabe et al., 2003), it is not clear whether these results reflect functional differences between PSα and PSβ themselves or conformational change of presenilins. If PS1 and PS2 per se are functionally redundant, it is most plausible that the functional differences may be due to conformational differences. In any case, this may be the first direct indication of functional differences between the two presenilins obtained by comparative studies.

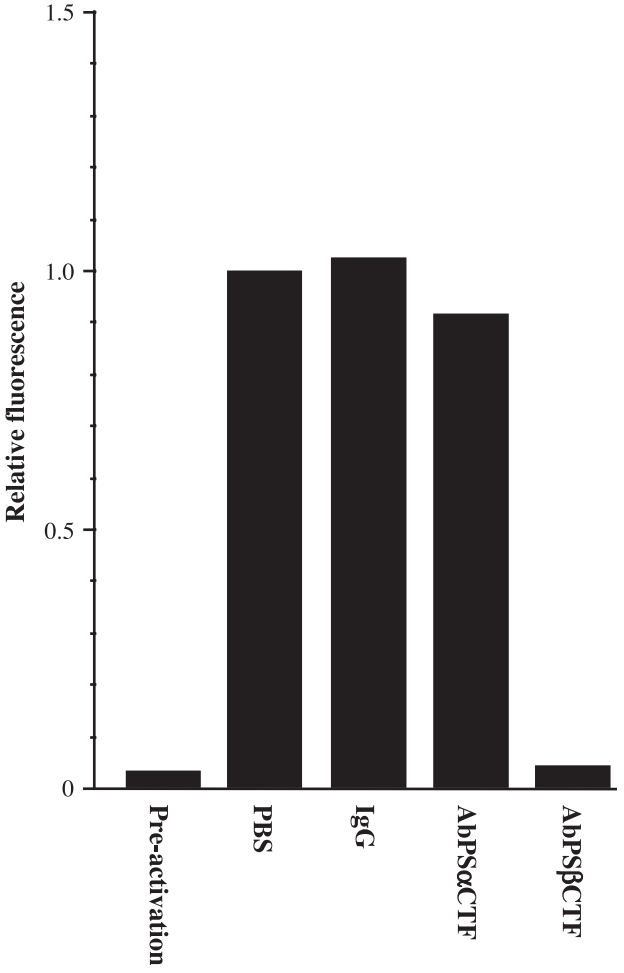


Fig. 1. Effect of anti-PSα and anti-PSβ antibodies on in vitro apoptotic activation of *Xenopus* egg extracts. *Xenopus* egg extracts were combined with mitochondrial fractions at 4 °C and divided into four tubes containing PBS, rabbit IgG, AbPSαCTF or AbPSβCTF. Fluorescence of free MCA released from DEVD-MCA via cleavage by caspases was measured (Ex380 nm and Em460 nm) as described in Section 2. The caspase activities of the respective samples were expressed as fluorescence relative to that of the sample with PBS. The relative fluorescence value designated "pre-activation" is for the sample with PBS (0.038) at time 0, and other pre-activation samples with IgG, AbPSαCTF and AbPSβCTF also gave values within a similar range, namely 0.02 to 0.04.

3.2. Different types of presenilins

The major structural differences between PS1 and PS2 reside in the two large hydrophilic regions, namely the N-terminal (NT) and hydrophilic loop (HL) regions. If PS1 and PS2 play different roles during early embryogenesis, it is likely that their functional differences may be ascribed to either the NT or HL region. Therefore, we compared these regions among various presenilin homologues. Although the amino acid sequences of these hydrophilic regions are conserved only very poorly, the HL sizes relative to that of human PS1 can be grouped into three distinct types, named α, β and γ, with the values of 0.94–1.08, 0.75–0.80 and 1.37–1.63, irrespective of the living sources, while the NT sizes vary between 0.15 to 1.46 relative to that of NT of human PS1 (Table 1). In other words, the HL sizes can be given approximately by integer ratios of HLα/HLβ/HLγ = 4:3:6, whereas no such regularity exists in NT sizes. These results may suggest that the HL rather than NT sizes are biologically significant or functionally critical. Then, we constructed phylogenetic trees to evaluate the functional constraints during evolution leading to conservation of the HL sizes, and estimated the approximate time of gene duplication in presenilin genes during evolutionary history.

Table 1
Typing of presenilin homologues from various organisms[a]

Source[b]	Presenilin	Total	Size of				gi/ci numbers
			NT	reNT	HL	reHL	
α type							
Human	PS1	467	81	1.00	116	1.00	1709856
Frog	PSα	433	47	0.58	115	0.99	6093849
Fish	PS1	456	69	0.85	116	1.00	4583564
Threadworm	SEL12	461	49	0.60	126	1.08	5759133
Sponge	PSα	476	83	1.02	121	1.04	our data
Thale cress	PSα	397	17	0.21	108	0.93	4567215
β type							
Human	PS2	448	87	1.07	102	0.78	1709858
Frog	PSβ	448	90	1.11	99	0.77	6093851
Fish	PS2	449	85	1.05	97	0.74	6900019
Threadworm	HOP1	358	13	0.16	95	0.72	7496294
γ type							
Lancelet	BfPS	525	93	1.15	174	1.37	17529693
Sea squirt	PSγ	504	63	0.78	171	1.47	0100144063
Snail	PSγ	582	118	1.46	207	1.63	11066248
Fly	DmPS	541	103	1.27	181	1.43	2194201
Threadworm	SPE4	465	16	0.20	193	1.52	3881734
Thale cress	PSγ	453	12	0.15	183	1.44	9454484
Rice	PSγ	478	31	0.38	179	1.41	6539565
Not defined							
Slime mold	PS?	>284	?	?	?	?	19215242
Choanoflagellate	PS?	>103	?	?	?	?	Our data
Trypanosome	PS?	>187	?	?	?	?	6771022
Entamoeba	PS?	>272	?	?	?	?	11823207

[a] Living organisms belonging to different classes or phyla/divisions are listed. Sizes are given as the number of amino acid residues or relative values. "NT" and "HL" means the N-terminal hydrophilic region and the hydrophilic loop region, respectively. "reNT" and "reHL" are relative sizes to NT and HL of human PS1, respectively. The ci (sea squirt) and gi (others) numbers are for the JGI (http://genome.jgi-psf.org/ciona4/ciona4.home.html) and NCBI (http://www.ncbi.nlm.nih.gov/) DNA databases, respectively.

[b] Human, *Homo sapiens*; Frog, *Xenopus laevis*; Fish, *Danio rerio*; Threadworm, *Caenorhabditis elegans*; Sponge, *Ephydatia fluviatilis*; Thale cress, *Arabidobsis thaliana*; Lancelet, *Branchiostoma floridae*; Sea squirt, *Ciona intestinalis*; Snail, *Helix lucorum*; Fly, *Drosophila melanogaster*; Rice, *Oryza sativa*; Slime mold, *Dictyostelium discoideum*; choanoflagellate, *Monosiga ovata*; trypanosome, *Trypanosoma brucei*; entamoeba, *Entamoeba histolytica*. In the last four species, the HL types could not be defined due to the lack of information.

3.3. Presenilins in various living organisms

To date (May 9, 2003), we have identified, by searching in the DNA databases as well our own data, 32 presenilin homologues (E value $\leq 1.4 \times 10^{-9}$), including partial ones, in 21 species from 11 different phyla/divisions including protozoa, plants and animals (Fig. 2). Although we have also identified genes encoding presenilin-like proteins, hereinafter termed pseudo-presenilins, in some archaebacterial species (for instance, gi:18160164 in *Pyrobaculum aerophilum*), they were excluded from our studies even though the two conserved aspartate residues seemed to be present. This is because the homology with human presenilins is not sufficient (E value $= 1.2 \times 10^{-2}$) to expect common biological functions, and in addition the number of TM domains is fewer than eight in all cases (data not shown). Thus, we have confirmed the absence of presenilin genes in at least 63 bacterial species, including archaebacteria and eubacteria, for which the entire genome sequences are available. Moreover, it was found that fungi possess no presenilin genes, irrespective of whether they are budding, fission or bisexual yeast such as *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe* and *Neurospora crassa*. This was rather unexpected since, in general, genes possessed by both animals and plants are also possessed by fungi, because the Kingdom Fungi branched from the Kingdom Animalia after the Kingdom Plantae did (Fig. 2). This suggests that there are both presenilin-possessing and -lacking protozoa, which are related to the ancestors of plants, animals and fungi. This is in fact the case, since presenilin genes were found in protozoa close to animals and plants (*Monosiga ovata* and *Trypanosoma brucei*, respectively) but not in those close to fungi (*Encephalitozoon cuniculi*). Furthermore, it was surprising that presenilin gene-possessing protozoa included not only multicellular protozoan species such as cellular slime molds (*Dictyostelium discoideum*) but also unicellular species such as entamoeba (*Entamoeba histolytica*), choanoflagellates (*M. ovata*) and trypanosomes (*T. brucei*). On the other hand, the absence of presenilin homologues was confirmed in other unicellular protozoa such as malaria parasites (*Plasmodium falciparum*). Consequently, it is plausible that the first presenilin gene may have been generated by the recruitment of some gene similar to pseudo-presenilins very early in protozoan history (around 1.0 to 1.2 billion years ago), and later transmitted to ancestral protozoans that evolved into animals and plants, but not to those that evolved into fungi (Fig. 2). This is consistent with the idea that *T. brucei*, *M. ovata* and *E. cuniculi* may be ancestral species of plants, animals and fungi, respectively (data not shown).

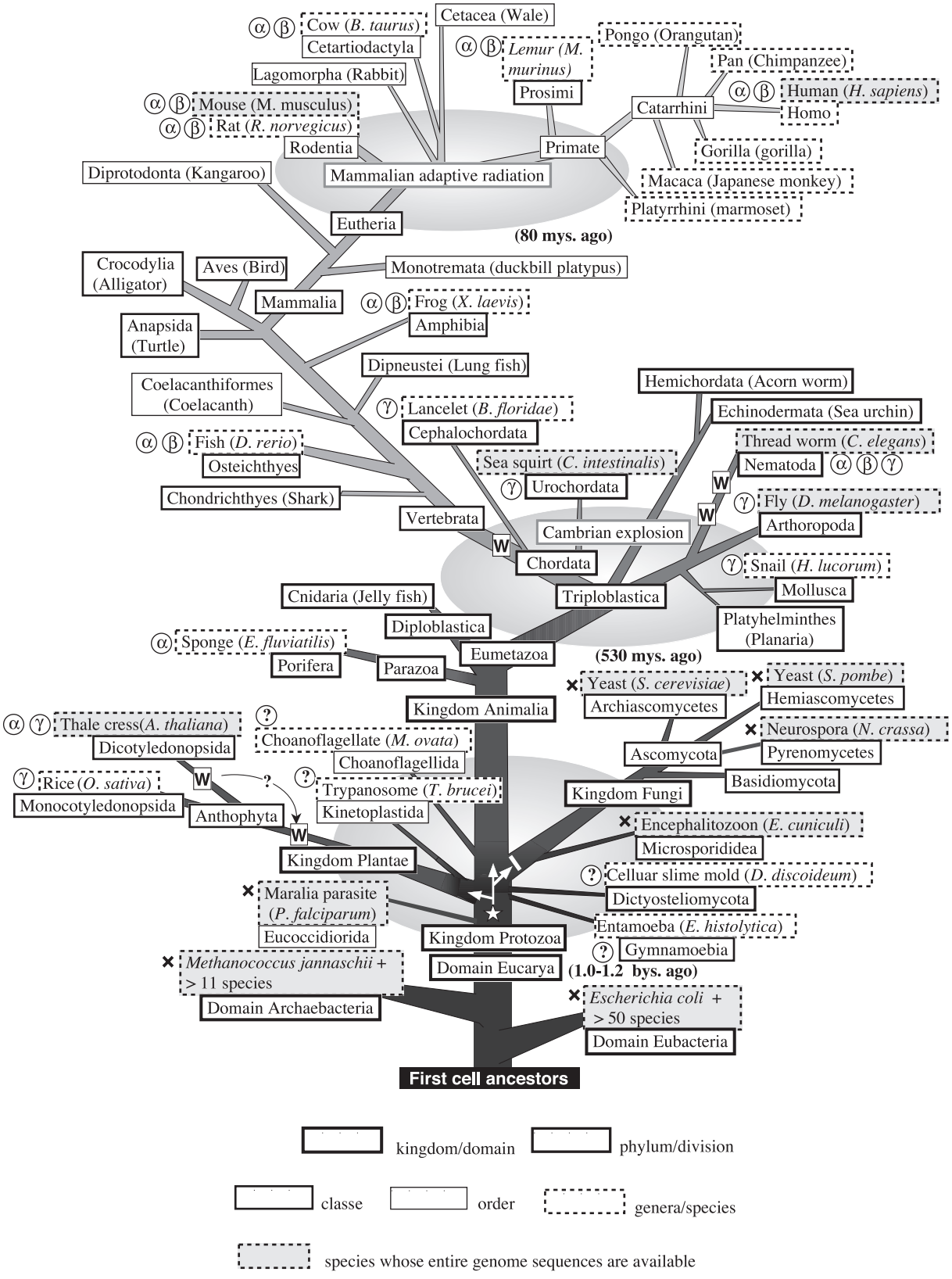
3.4. Phylogenetic examination of presenilins

To estimate the approximate time of the gene duplication of presenilins on the universal evolutionary tree, the phylogenetic trees of presenilins were constructed by using two methods (Fig. 3). The tree constructed using the NJ method (Saitou and Nei, 1987) suggests that presenilin genes were duplicated independently in vertebrates and plants (Fig. 3A). On the other hand, the tree constructed using the ML method (Yang, 1994; Katoh et al., 2001) suggested that another duplication event occurred twice independently in nematodes (Fig. 3B). In any case, both the methods suggested that the α- and β-types of presenilins were generated independently in different phyla/divisions. These results strongly suggest that there were functional requirements for and constraints on the generation and conservation of certain sizes of HL regions in presenilins.

3.5. Alternative proteolytic processing model for presenilins

To explain the results obtained from our studies as well as results previously reported by others, we constructed a simple model, named the alternative proteolytic processing model, which involves both different and redundant functions of PS1 and PS2. We postulate that presenilins with larger HL sizes would be more sensitive to autocatalytic proteolysis in this region (Shirotani et al., 2000). Thus, PS1 and PS2 may exist in general mainly as processed and unprocessed molecules, respectively, although the level of these molecules in either presenilin depends on the tissue and developmental stage. NTF + CTF and FLM are further postulated to promote differentiation and apoptosis, respectively. When CTF is phosphorylated, it is stabilized but its counterpart, NTF, is degraded so as to maintain the level of NTF at a ratio of 1:1 to that of unphosphorylated CTF (Lau et al., 2002). Consequently, an excess of phosphorylated CTF may act as a dominant-negative inhibitor against both NTF + CTF and FLM (Vito et al., 1996). The model can then be summarized as in Fig. 4.

Fig. 2. Presence/absence, and generation and duplication times of presenilin genes. The evolutionary tree was constructed according to Miyata et al. (data not shown). Symbols; ☆ and Ⓦ on the tree indicate approximate times of generation and duplication of presenilin genes, respectively. Open arrows above the open star indicate the directions of presenilin gene transmissions at the branching points of Kingdoms from Protozoa to Plantae, Animalia and Fungi. Note that presenilin genes were not transmitted to the ancestral species of fungi. Species in which presenilin genes were found are marked by "α", "β", "γ" and/or "?" in circles, which indicate the presence of α-, β-, γ- or undefined type of presenilins, respectively. Species in which the absence of presenilins was confirmed are indicated by " × ". The appearance of Domain Eucarya or Kingdom Protozoa (1.0–1.2 billion years ago), Cambrian explosion (530 million years ago) and mammalian adaptive radiation (80 million years ago) are shown by gray ovals. Species whose entire genome sequences are available are in gray boxes. The branching point of *P. falciparum* is a tentative proposal.


Cetacea (Wale)
α β Cow (B. taurus)
Cetartiodactyla
α β Lemur (M. murinus)
Pongo (Orangutan)
Pan (Chimpanzee)
Lagomorpha (Rabbit)
Prosimi
α β Human (H. sapiens)
Catarrhini
Homo
α β Mouse (M. musculus)
α β Rat (R. norvegicus)
Rodentia
Primate
Gorilla (gorilla)
Diprotodonta (Kangaroo)
Mammalian adaptive radiation
Macaca (Japanese monkey)
Platyrrhini (marmoset)
Eutheria
(80 mys. ago)
Crocodylia (Alligator)
Aves (Bird)
Monotremata (duckbill platypus)
Mammalia
α β Frog (X. laevis)
Anapsida (Turtle)
Amphibia
Dipneustei (Lung fish)
Hemichordata (Acorn worm)
Coelacanthiformes (Coelacanth)
γ Lancelet (B. floridae)
Echinodermata (Sea urchin)
Cephalochordata
α β Fish (D. rerio)
Thread worm (C. elegans)
Sea squirt (C. intestinalis)
Nematoda
α β γ
Osteichthyes
W
γ Urochordata
γ Fly (D. melanogaster)
Chondrichthyes (Shark)
W
Vertebrata
Arthoropoda
Cambrian explosion
W
Chordata
γ Snail (H. lucorum)
Cnidaria (Jelly fish)
Triploblastica
Mollusca
Diploblastica
Platyhelminthes (Planaria)
α Sponge (E. fluviatilis)
Eumetazoa
(530 mys. ago)
Porifera
Parazoa
Yeast (S. cerevisiae)
Yeast (S. pombe)
Hemiascomycetes
Archiascomycetes
Kingdom Animalia
α γ Thale cress(A. thaliana)
?
Choanoflagellate (M. ovata)
Neurospora (N. crassa)
Dicotyledonopsida
Choanoflagellida
Ascomycota
Pyrenomycetes
γ Rice (O. sativa)
W
?
?
Trypanosome (T. brucei)
Basidiomycota
Monocotyledonopsida
Anthophyta
Kinetoplastida
Kingdom Fungi
W
Encephalitozoon (E. cuniculi)
Microsporididea
Kingdom Plantae
? Celluar slime mold (D. discoideum)
Maralia parasite (P. falciparum)
Dictyosteliomycota
Eucoccidiorida
Entamoeba (E. histolytica)
Kingdom Protozoa
? Gymnamoebia
Methanococcus jannaschii + > 11 species
Domain Eucarya
(1.0-1.2 bys. ago)
Escherichia coli + > 50 species
Domain Archaebacteria
Domain Eubacteria
First cell ancestors
kingdom/domain
phylum/division
classe
order
genera/species
species whose entire genome sequences are available

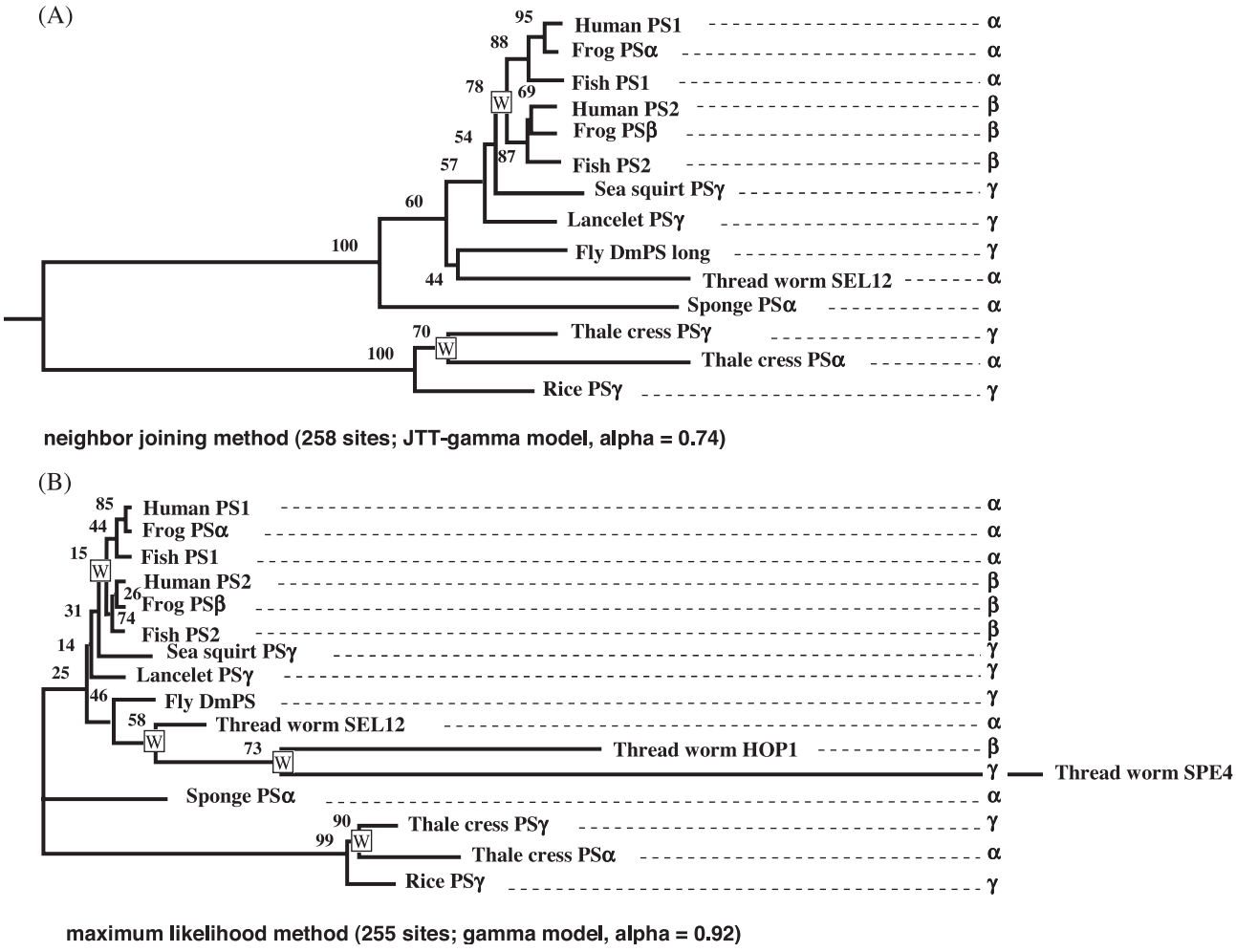


Fig. 3. Molecular phylogenetic trees inferred by the neighbor joining method (Saitou and Nei, 1987) under the JTT-model (A) and by the genetic algorithm-based maximum likelihood method (Katoh et al., 2001) (B). (A) The evolutionary rate heterogeneity among sites was taken into account, assuming Yang's discrete gamma model (Yang, 1994) and optimized shape parameter alpha of 0.74; highly homologous sites without gap sites were selected for the comparison. Two presenilins of the threadworm, namely HOP1 and SPE4, were omitted because their high evolutionary rates might have caused a long-branch attraction (LBA) artifact. (B) The evolutionary rate variation among sites was corrected by assuming the gamma distribution with optimized shape parameter alpha of 0.92; gap sites were excluded from the comparison. Using either method, it was revealed that presenilin genes could have been duplicated at least twice (A) or four times (B), independently in different phyla/divisions as indicated by W. The numbers at each branching node represent the bootstrap probability. "α", "β" and "γ" are as in Table 1.

If this model is adapted to *Xenopus* early embryos (Watanabe et al., 2003), one can analyze the model mathematically as follows. During early embryogenesis, both cell differentiation and apoptosis should be suppressed. Then, we obtain

$$(\alpha_{\mathrm{PCTF}} + \beta_{\mathrm{PCTF}}) > (\alpha_{\mathrm{N+C}} + \beta_{\mathrm{N+C}}) + (\alpha_{\mathrm{FLM}} + \beta_{\mathrm{FLM}}),$$

in which α_{PCTF} and β_{PCTF} are the amounts of PSα and PSβ CTFs phosphorylated in a cell, $\alpha_{\mathrm{N+C}}$ and $\beta_{\mathrm{N+C}}$ are the amounts of NTF + CTF complex, and α_{FLM} and β_{FLM} are the amounts of FLM, respectively. Providing that the initial amounts of presenilin molecules are given by α and β, presenilins are processed with the efficiencies of k_α and k_β and CTFs are phosphorylated with the efficiencies of p_α and p_β, for PSα and PSβ, respectively, we obtain

$$\alpha_{\mathrm{PCTF}} = p_\alpha k_\alpha \alpha$$

$$\beta_{\mathrm{PCTF}} = p_\beta k_\beta \beta$$

$$\alpha_{\mathrm{FLM}} = (1 - k_\alpha)\alpha$$

$$\beta_{\mathrm{FLM}} = (1 - k_\beta)\beta$$

$$\alpha_{\mathrm{N+C}} = k_\alpha \alpha (1 - p_\alpha)$$

$$\beta_{\mathrm{N+C}} = k_\beta \beta (1 - p_\beta)$$

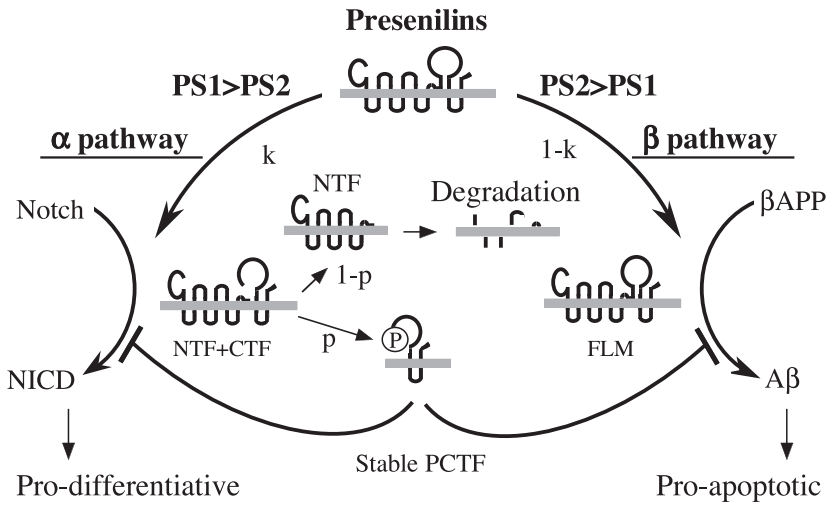


Fig. 4. Alternative proteolytic processing model for different and redundant involvement of PS1 and PS2. After autocatalytic processing in the HL region, presenilins are converted to NTF + CTF complexes with the efficiency of k (α pathway), and those not processed remain as FLM with the efficiency of $1 - k$ (β pathway). PS1 and PS2 enter both the pathways but preferentially the α and β pathways, respectively, as indicated by PS1>PS2 and PS2>PS1 due to the HL size differences (see Section 3.5). Then, the CTF is phosphorylated with the efficiency of p, yielding a stable form of CTF (PCTF), without forming a complex with its counterpart NTF, the latter of which is then degraded. NTF + CTF and FLM are postulated to be pro-differentiative and pro-apoptotic, respectively. Therefore, it is likely that Notch and βAPP may be preferentially cleaved by NTF + CTF and FLM, respectively. PCTF blocks the overall activities of NTF + CTF and FLM with equal probabilities as a dominant negative inhibitor.

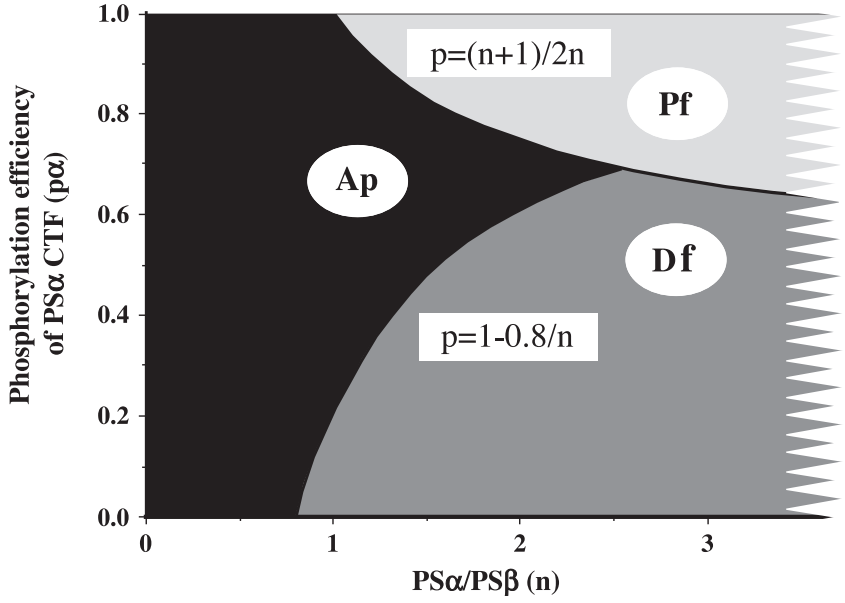


Fig. 5. Phosphorylation efficiency (p_α) of PSα CTF as a function of the ratio of PSα to PSβ protein synthesis (n) for regulating the choice among differentiation (Df), apoptosis (Ap) and proliferation (Pf) states in *Xenopus* early embryos. Above the curve given by $p_\alpha=(n+1)/2n$, where $1<n$, embryonic cells will be proliferative, as shown in the light gray area (Pf). Below the curves given by $p_\alpha=1-0.8/n$, where $0.8<n<2.6$, and $p_\alpha=(n+1)/2n$ ($n>2.6$), cells will be differentiative as shown in the dark gray area (Df). In-between, cells are potentially apoptotic as shown in the closed area (Ap). The relative rate of PS_α to PS_β protein synthesis should be between 1 and 2.6 for switching *Xenopus* embryonic states among Df, Ap and Pf by PSα CTF phosphorylation.

By introducing these, we obtain

$$(p_\alpha k_\alpha \alpha + p_\beta k_\beta \beta) > (\alpha + \beta)/2.$$

If one introduces $k_\alpha = 1$, $k_\beta = 0.1$ and $p_\beta = 0$ as observed in early embryonic stages 2 to 6 in *Xenopus laevis* (Fig. 2 in Watanabe et al., 2003), we obtain

$$p_\alpha > (n+1)/2n,$$

where n is given by α/β.

On the other hand, if the balance between cell differentiation and apoptosis is determined by the ratio between NTF + CTF and FLM activities, the differentiation and apoptosis states will be represented by

$$(\alpha_{N+C} + \beta_{N+C}) > (\alpha_{FLM} + \beta_{FLM})$$

and

$$(\alpha_{N+C} + \beta_{N+C}) < (\alpha_{FLM} + \beta_{FLM}),$$

respectively. Then, the border between apoptosis and differentiation will be defined by

$$(k_\alpha \alpha + k_\beta \beta) - (p_\alpha k_\alpha \alpha + p_\beta k_\beta \beta) = (1 - k_\alpha)\alpha + (1 - k_\beta)\beta,$$

that is,

$$p_\alpha = 1 - 0.8/n,$$

where the activities of wild-type NTF + CTF and FLM are assumed to be equivalent per molecule for opposite directions, though this may well be altered by mutations such as found in FAD pedigrees. The results are diagrammed in Fig. 5.

The model developed above may explain the bias of mutation frequencies of FAD pedigrees, in terms of loss- and gain-of-function mutations in *ps1* and *ps2* genes, respectively, since the effective n values will be reduced in either case by a decrease of PS1 or an increase of PS2 activity. Similarly, this would also explain why PS1 KO-mice are lethal while PS2 KO-mice can survive. Furthermore, this model predicts that the PS2 expression level must be lower than that of PS1 in order to regulate the distribution of cells among the differentiative, apoptotic and proliferative/static states by the degree of CTF phosphorylation. In the case of *Xenopus* early embryos (Watanabe et al., 2003), p_α was 0.89 and n values should be between 1.3 and 2.6. Although it is not clear at the moment how NTF + CTF and FLM induce differentiation and apoptosis, respectively, the model appears to be compatible with the idea that they may preferentially cleave Notch and βAPP, respectively, although the model does not exclude the possibilities that other substrates may be involved in intramembranous cleavage by presenilins (Lammich et al., 2002) or that presenilins may require other components in order to exhibit their proteolytic activities (Herreman et al., 2003). In this context, it is of interest to point out that *Arabidobsis thaliana*, which shows no apoptotic phenotype, possesses no β-type presenilins. Concerning the γ-type presenilins, it has been reported that alternative splicing sites result in shorter HL variants in DmPS (Boulianne et al., 1997) and BfPS (Martinez-Mir et al., 2001). According to our model, it is crucial that PS1 synthesis should be more efficient than that of PS2 and that CTF phosphorylation should be highly efficient in order to avoid apoptosis. It is of interest to see what would fully enhance phosphorylation of presenilin CTFs in nerve cells, as progesterone does in *Xenopus* oocytes and whether some other steroid hormones such as estrogen would do so.

4. Note added in proof

After submission of this paper, in the newest disclosure of Rice Genbank (http://www.tigr.org/tdb/e2k1/osa1/), we found another possible presenilin (8360.m03641), which showed significant homology to the α-type presenilin of *A. thaliana*.

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