Protein family review

The presenilins

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Summary

The presenilins are evolutionarily conserved transmembrane proteins that regulate cleavage of certain other proteins in their transmembrane domains. The clinical significance of this regulation is shown by the contribution of presenilin mutations to 20-50% of early-onset cases of inherited Alzheimer's disease. Although the precise molecular mechanism underlying presenilin function or dysfunction remains elusive, presenilins are thought to be part of a complex of proteins that has 'γ-secretase cleavage' activity, which is clearly central in the pathogenesis of Alzheimer's disease. Mutations in presenilins increase the production of the longer isoforms of amyloid β peptide, which are neurotoxic and prone to self-aggregation. Biochemical studies indicate that the presenilins do not act alone but operate within large heteromeric protein complexes, whose components and enzymatic core are the subject of much study and controversy; one essential component is nicastrin. The presenilin primary sequence is remarkably well conserved in eukaryotes, suggesting some functional conservation; indeed, defects caused by mutations in the nemotode presenilin homolog can be rescued by human presenilin.

Gene organization and evolution history

The *presenilin 1* (*PS1*) gene on human chromosome 14 (14q24.3) was initially discovered by genetic analysis of a subset of pedigrees in which the Alzheimer's disease is transmitted as a pure autosomal dominant trait [1]. The closely related *PS2* gene on chromosome 1 (1q42.2) was identified subsequently by sequence homology [2,3]. Both *PS1* and *PS2* genes are organized into ten translated exons that display tissue-specific alternative splicing [2,4-7]. The functions and biological importance of differentially spliced presenilin variants are poorly understood; differential expression of isoforms may lead to differential regulation of the proteolytic processing of the β-amyloid precursor protein (βAPP; see later). For example, aberrant *PS2* transcripts lacking exon 5 increase the rate of production of amyloid β peptide (Aβ, the neurotoxic peptide implicated in Alzheimer's disease) [8], whereas naturally occurring isoforms without exons 3 and 4 and/or without exon 8 do not affect production of Aβ [6,9].

GenBank database searches using the full length *PS1* sequence suggest that presenilin-like proteins are phylogenetically ancient and well-conserved across diverse eukaryote species, including plants, molluscs, insects, fish, birds, and mammals [10-16]. Functional conservation of presenilins in most non-human species is undetermined, except in the nematode *Caenorhabditis elegans*, in which a deficiency in *Sel-12*, the *PS1* homolog, induces an egg-laying defect that can be rescued by expression of human *PS1* [17,18]. Additional presenilin homologs were recently identified in disparate eukaryotes by their homology to the PS1 transmembrane domains, suggesting that the presenilin family may be more common than previously contemplated [19,20].

Characteristic structural features

Mammalian PS1 and PS2 are synthesized as 50 kDa polypeptides, each predicted to traverse the membrane 6-10 times;

the amino and carboxyl termini are both oriented towards the cytoplasm [21]. The current model, with eight transmembrane domains, is shown in Figure 1. More than 100 different missense mutations and two splicing-defect mutations in the *PS1* gene have been reported (Table 1) [22,23]. These are dispersed throughout the *PS1* sequence, with the majority of mutations clustered near membrane interfaces in the highly conserved transmembrane domains or in hydrophobic residues in either the amino-terminal domain or the putative loop domain between transmembrane domains 6 and 7.

Following synthesis, the PS1 and PS2 holoproteins undergo tightly regulated, but imprecise, endoproteolysis in their third cytoplasmic loop domain to generate an approximately 35 kDa amino-terminal fragment and an 18-20 kDa carboxy-terminal fragment, which remain associated with each other [24]. It is clear that cleavage of presenilins following export from the endoplasmic reticulum is governed by additional rate-limiting factors, such as nicastrin (see below), because overexpressed presenilins readily saturate the processing machinery and accumulate as holoproteins [25]. An additional proteolytic pathway is known to involve members of the caspase 3 family of proteases and may be involved in apoptosis [26].

Localization and function

Human *PS1* and *PS2* have distinct patterns of expression in human tissues. Whereas *PS1* is transcribed uniformly throughout the brain and in peripheral tissues, the *PS2* transcript is expressed at relatively low levels in the brain, except in the corpus collosum, where it is high; it is highly expressed in some peripheral tissues, such as pancreas, heart, and skeletal muscle [27]. The low PS2 levels in brain and the compensatory activity provided by PS1 may explain why *PS2* mutations are infrequent and incompletely penetrant compared with *PS1* mutations, which are fully penetrant [28,29].

The βAPP protein is cleaved by three different activities, called α-, β- and γ-secretases, to generate Aβ and other fragments. Members of the Notch family, which are involved in developmental signaling in many animals, undergo cleavage

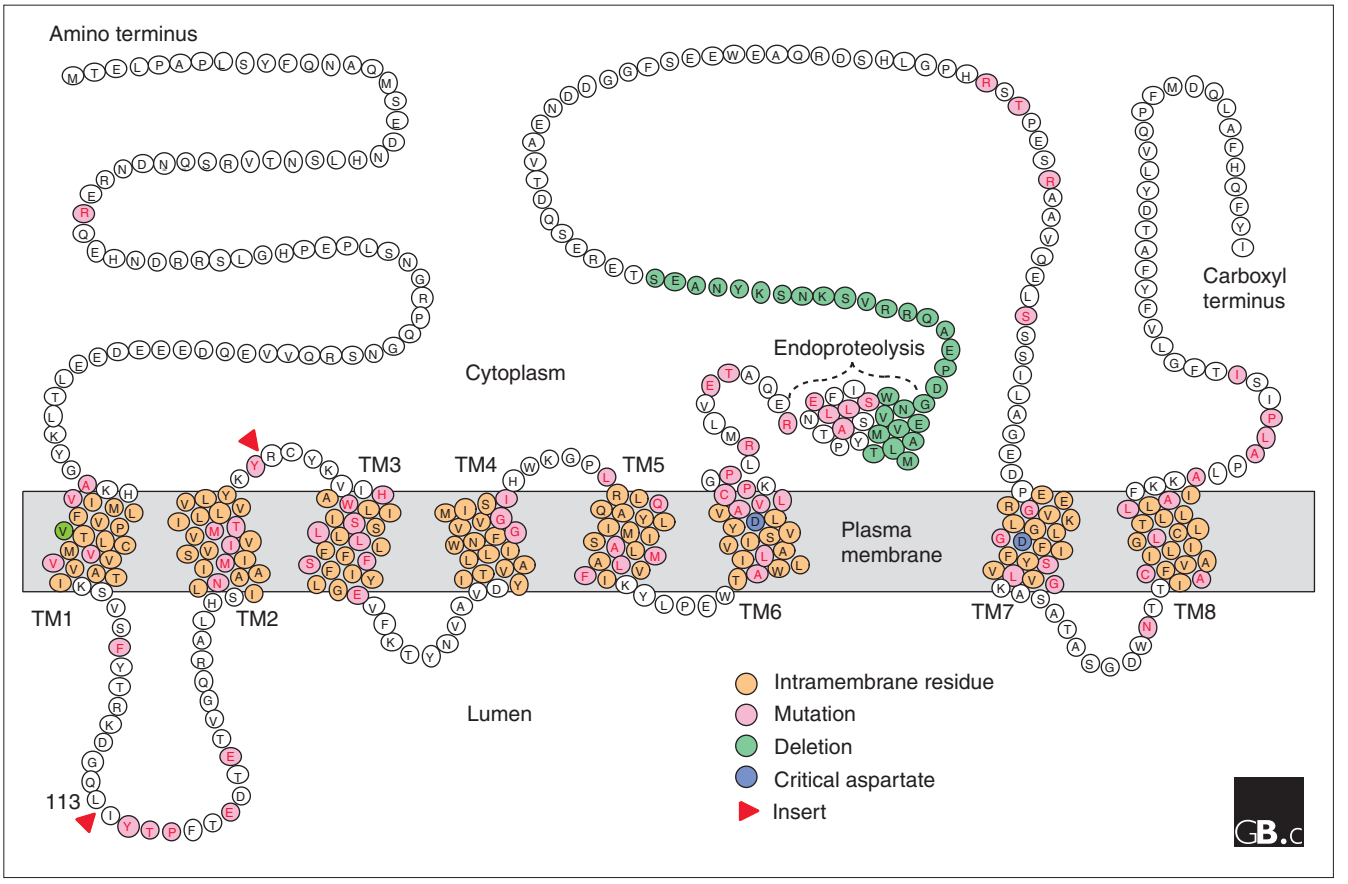

Figure 1
A molecular model of Presenilin-1. The protein is thought to have eight transmembrane domains. Residues associated with mutations found in familial Alzheimer's disease are colored as indicated in the key. 'Endoproteolysis' indicates the approximate site of the imprecise cleavage of the molecule.

Table 1

Mutations in the presenilin genes

PS1

Codon	Location	Mutation	Phenotype
35	Amino-terminal domain	Arg->Gln	FAD
79	Amino-terminal domain	Ala->Val	FAD, onset 64 years
82	TM1	Val→Leu	FAD, onset 55 years
94	TM1	Val→Met	See [71]
96	TM1	Val→Phe	FAD, onset 53 years
105	TM1/TM2 loop	Phe→Leu	FAD, onset 52 years
113-114 (insert)	TM1/TM2 loop	Insert Thr	FAD, onset 35 years
115	TM1/TM2 loop	Tyr→His	FAD, onset 37 years
115	TM1/TM2 loop	Tyr→Cys	FAD, onset 42 years
116	TM1/TM2 loop	Thr→Asn	FAD, onset 37 years
117	TM1/TM2 loop	Pro→Leu	AD, onset 28 years
120	TM1/TM2 loop	Glu→Asp	FAD, onset 48 years
120	TM1/TM2 loop	Glu→Lys	FAD, onset 37 years
123	TM1/TM2 loop	Glu→Lys	FAD, onset 56-62 years
135	TM2	Asn→Asp	FAD, onset 36 years
139	TM2	Met→Thr	FAD, onset 49 years
139	TM2	Met→Val	FAD, onset 40 years
139	TM2	Met→Ile	AD
139	TM2	Met→Lys	FAD, onset 37 years
143	TM2	Ile→Thr	FAD, onset 35 years
143	TM2	Ile→Phe	FAD, onset 55 years
146	TM2	Met→Leu	FAD, onset 45 years
146	TM2	Met→Val	FAD, onset 38 years
146	TM2	Met→Ile	FAD, onset 40 years
147	TM2	Thr→Ile	FAD, onset 42 years
156 + insert	TM3 interface	Tyr→ (Phe,Ile,Tyr)	FAD
163	TM3 interface	His→Arg	FAD, onset 50 years
163	TM3 interface	His→Tyr	FAD, onset 47 years
165	TM3	Trp→Cys	FAD, onset 42 years
169	TM3	Ser→Leu	FAD, onset 31 years
169	TM3	Ser→Pro	FAD, onset 35 years
171	TM3	Leu→Pro	FAD, onset 40 years
173	TM3	Leu→Trp	FAD, onset 27 years
177	TM3	Phe→Ser	FAD
178	TM3	Ser→Pro	FAD
184	TM3	Glu→Asp	FAD
206	TM4	Gly→Ser	FAD
209	TM4	Gly→Val	FAD, onset 30-48 years
209	TM4	Gly→Arg	FAD, onset 49 years
213	TM4 interface	Ile→Thr	FAD, onset 42-48 years
213	TM4 interface	Ile→Leu	FAD
219	TM4 interface	Leu→Pro	FAD
219	TM4 interface	Leu→Phe	See [71]
222	TM5	Gln→Arg	FAD
231	TM5	Ala→Thr	FAD, onset 52 years
231	TM5	Ala→Val	FAD
233	TM5	Met→Thr	FAD, onset 35 years
233	TM5	Met→Leu	FAD, onset 46 years
235	TM5	Leu→Pro	FAD, onset 32 years
237	TM5	Phe→Ile	AD with spastic paraparesis, 31 years
246	TM6	Ala→Glu	FAD, onset 55 years
250	TM6	Leu→Ser	FAD, onset 53 years
260	TM6	Ala→Val	FAD, onset 40 years
261	TM6	Val→Phe	FAD
262	TM6	Leu→Phe	FAD, onset 50 years
263	TM6/TM7 loop	Cys→Arg	FAD, onset 47 years
264	TM6/TM7 loop	Pro→Leu	FAD, onset 45 years
267	TM6/TM7 loop	Pro→Ser	FAD, onset 35 years
269	TM6/TM7 loop	Arg→Gly	FAD, onset 47 years
269	TM6/TM7 loop	Arg→His	FAD, onset 47 years
273	TM6/TM7 loop	Glu→Ala	FAD, onset 63 years
274	TM6/TM7 loop	Thr→Arg	FAD
278	TM6/TM7 loop	Arg→Thr	FAD, onset 37 years
280	TM6/TM7 loop	Glu→Ala	FAD, onset 47 years
280	TM6/TM7 loop	Glu→Gly	FAD, onset 42 years
282	TM6/TM7 loop	Leu→Arg	FAD, onset 43 years
285	TM6/TM7 loop	Ala→Val	FAD, onset 50 years
286	TM6/TM7 loop	Leu→Val	FAD, onset 50 years
290	TM6/TM7 loop	Ser>Cys	FAD, onset 39-50 years
291-319 deletion	TM6/TM7 loop	Shortened loop	FAD
352 (insert)	TM6/TM7 loop	Insert Arg	FAD
354	TM6/TM7 loop	Thr→Ile	FAD
358	TM6/TM7 loop	Arg→Gln	FAD
365	TM6/TM7 loop	Ser→Tyr	FAD
378	TM7	Gly→Glu	FAD, onset 35 years
384	TM7	Gly→Ala	FAD, onset 35 years
390	TM7	Ser→Ile	FAD, onset 39 years
392	TM7	Leu→Val	FAD, onset 25-40 years
394	TM7	Gly→Val	FAD
405	TM7/TM8 loop	Asn→Ser	FAD, onset 48 years
409	TM8	Ala→Thr	FAD, onset 58 years
410	TM8	Cys→Tyr	FAD, onset 48 years
418	TM8	Leu→Phe	FAD
424	TM8	Leu→Arg	FAD, onset 33 years
426	TM8	Ala→Pro	FAD, onset 48-60 years
431	Carboxy-terminal domain	Ala→Glu	FAD
434	Carboxy-terminal domain	Ala→Cys	FAD
435	Carboxy-terminal domain	Leu→Phe	FAD
436	Carboxy-terminal domain	Pro→Ser	FAD, onset 48-60 years
436	Carboxy-terminal domain	Pro→Gln	FAD, onset 48-60 years
439	Carboxy-terminal domain	Ile→Val	FAD

PS2

Codon	Location	Mutation	Phenotype
62	N-term	Arg→His	AD, onset 62 years
122	TM1/TM2 loop	Thr→Pro	FAD, onset 46 years
141	TM2	Asn→Ile	FAD, onset 50-65 years
148	TM2	Val→Ile	AD, Onset 71 years
239	TM5	Met→Val	FAD, onset variable 45-84 yrs
239	TM5	Met→Ile	FAD, onset 58 years

Compiled from [2,70,71]. Abbreviations: AD, Alzheimer's disease; FAD, familial Alzheimer's disease; TM, transmembrane segment; TM1/TM2 loop, the loop between transmembrane segments 1 and 2. The age of onset of disease is given if it is known.

at a site (S3) within the transmembrane domain to release an intracellular domain (NICD). It is well established that presenilins are required for the γ-secretase cleavage of βAPP and for the S3 cleavage of Notch-family receptors [30]. For βAPP processing, γ-secretase cleavage is the final step of two distinct proteolytic pathways involving either an α-secretase - which precludes Aβ peptide formation - or a β-secretase, which releases the Aβ peptide, comprising the 40 or 42 carboxy-terminal residues of βAPP. It is uncertain whether the γ-secretase cleavage event occurs at the plasma membrane or during trafficking of βAPP. The usual downstream effect of presenilin mutations in individuals with presenilin-linked familial Alzheimer's disease is the accumulation of Aβ in the brain [31,32] and a shift in the site of the γ-secretase cleavage of βAPP to produce the longer Aβ peptide, spanning residues 1-42 (Aβ42). These main features can be recapitulated in cell culture or in animal models expressing mutant forms of PS1 [33-35]. Conversely, PS1-deficient mice are impaired in γ-secretase activity, have reduced Aβ secretion, and accumulate γ-secretase substrates (the carboxy-terminal βAPP fragments derived from α- and β-secretase processing; see Figure 2) [36].

Mutation of two highly conserved aspartate residues in the transmembrane domains of PS1 (Asp257 and Asp385, shown in blue in Figure 1) inactivates γ-secretase activity and reduces Aβ secretion [37]. The sequence motif around Asp385 is somewhat similar to a sequence within prepilins, a family of bacterial peptidases [38]; this has promoted speculation that presenilins are themselves aspartyl proteases responsible for γ-secretase activity and that the critical Asp257 and Asp385 residues form that catalytic center of the γ-secretase. Additional support for the idea that presenilins are the proteases that have γ-secretase activity comes from studies in which photoactivated inhibitors of γ-secretase activity were found to bind to PS1 and PS2 [39,40].

It should be noted that forms of PS1 with the D257A or D385A mutations integrate poorly into the heteromeric complexes that are considered necessary for γ-secretase function, raising the possibility that these transmembrane-domain mutations disable PS1 structurally [41]. Moreover, several lines of evidence show that the regulation of βAPP and Notch cleavage differs, however, and such evidence is difficult to reconcile with a direct enzymatic role for PS1 in γ-secretase cleavage. First, a naturally occurring splice variant of PS1 lacking the region (encoded by exon 8) that contains the critical Asp257 allows Aβ production but not cleavage of Notch [42]. Second, different presenilin mutations differentially affect Aβ production and Notch cleavage [43-45]. Third, some recently discovered γ-secretase inhibitors preferentially affect processing βAPP over that of Notch [46]. Together, these findings suggest the presenilins regulate proteolysis indirectly, perhaps by an effect on trafficking of βAPP or Notch or by activation of the γ-secretase.

The biological purpose of presenilin-dependent γ-secretase cleavage of βAPP is still unknown. By analogy with the signaling pathway downstream of cleaved Notch and NICD, recent studies have raised the intriguing possibility that the short-lived carboxyl-terminal stub of βAPP, called βAPP intracellular domain (AICD), is released into the cytoplasm following γ-secretase cleavage and translocates to the nucleus (Figure 2), where it may regulate expression of components involved in mobilizing intracellular calcium stores [47-49]. Another proposal implicates βAPP as a regulator of the axonal transport of a subset of vesicles ferrying cargo to nerve terminals. This view is derived from the observations that βAPP interacts directly with the light chain of the transport protein kinesin [50], that the transport of a vesicular compartment containing PS1 and β-secretase depends on βAPP [51], and that deletion of the *Drosophila* βAPP-like gene (dAPPL) or overexpression of either dAPPL or human βAPP in *Drosophila* disrupts axonal transport [52,53]. In this scheme, γ-secretase cleavage of the βAPP by presenilin-containing complexes releases the carboxy-terminal portion of βAPP that connects the transport vesicle to the transport machinery through interaction with kinesin, thereby disengaging the vesicle from microtubules upon arrival at its destination. Thus, presenilins may influence diverse cellular processes, such as intracellular signaling and axonal traffic.

In vitro studies of detergent-solubilized membranes show that γ-secretase activity resides within large multisubunit complexes that also contain presenilins. If presenilin molecules are excluded from these complexes, they are rapidly targeted for proteosome-mediated degradation [54]. On density gradients, presenilin holoproteins and the amino- and carboxy-terminal fragments of presenilins co-elute with high-molecular-weight markers (180 kDa for the holoproteins and 250-1000 kDa for the fragments [25,55]), presumably because they are part of larger complexes, and antibodies to PS1 coimmunoprecipitate heteromeric protein complexes that contain γ-secretase activity [56]. Conversely, affinity isolation with γ-secretase inhibitors co-purifies protein complexes containing PS1 [39,40]. Members of the Armadillo protein family (β- and δ-catenin, neural plakophilin-related armadillo protein (NPRAP), and p0071) [55,57,58] interact with presenilins but are not required for γ-secretase activity *in vitro* [40]. Other interactions whose role in γ-secretase activity is unknown have been reviewed previously [22].

More recently, PS1 and PS2 were found to interact with nicastrin, a novel single-pass transmembrane protein that is essential for processing of βAPP and Notch [59-61]. Nicastrin is clearly an important regulator of γ-secretase activity: nicastrin antibodies immunoprecipitate both presenilin and the active γ-secretase complex [40], and missense or deletion mutations within a conserved lumenal domain of nicastrin up- or down-regulate Aβ production in a manner that corresponds with PS1 binding, suggesting that γ-secretase

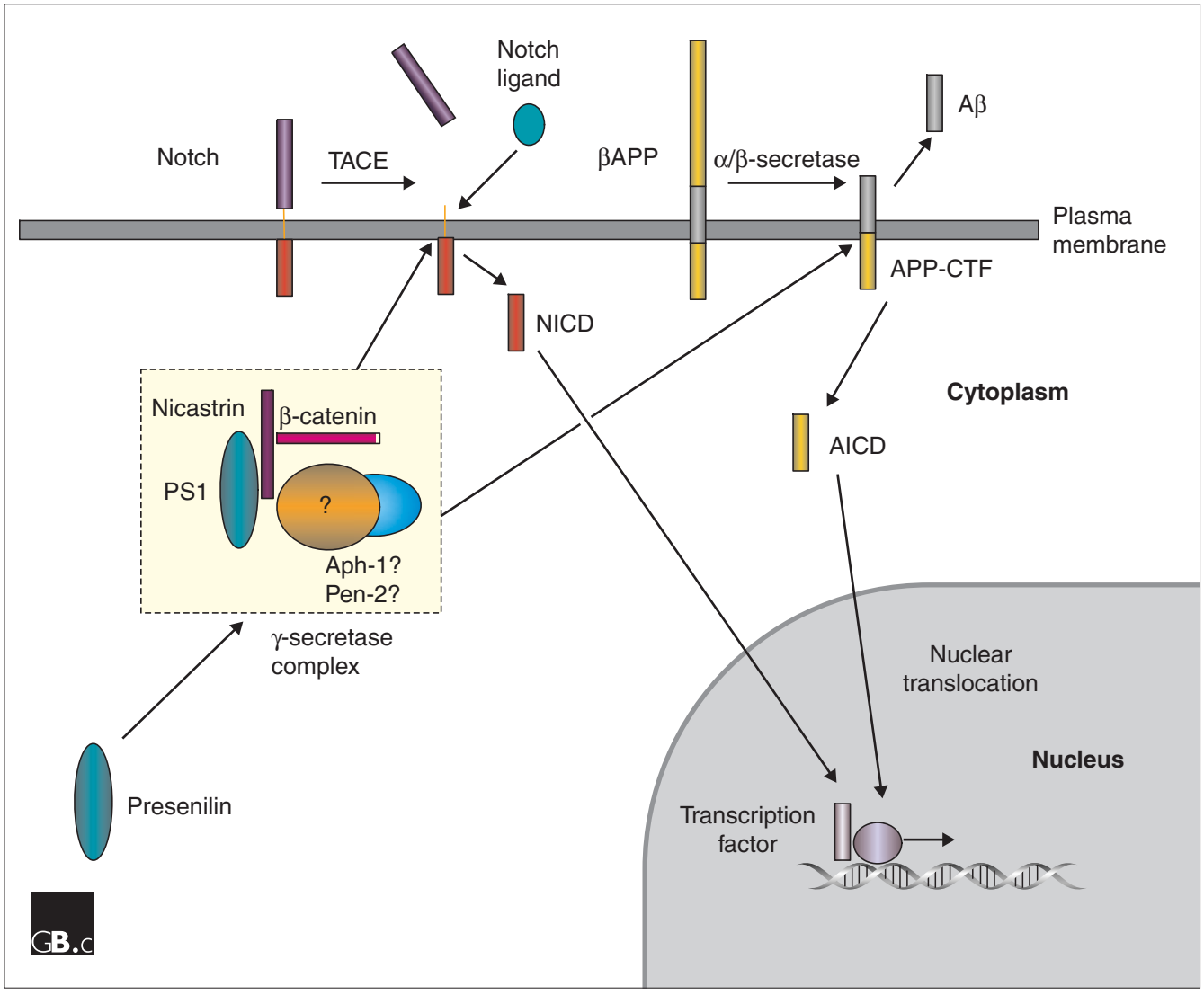

Figure 2
The role of presenilins in the γ-secretase cleavage of Notch and βAPP. Notch is cleaved by tumor necrosis factor α converting enzyme (TACE), and its ligand binds to the part of Notch that remains attached to the membrane. βAPP is cleaved by either the α-secretase pathway or the β-secretase pathway to give a membrane-bound carboxy-terminal fragment (APP-CTF). Subsequent γ-secretase cleavage (in the transmembrane domain) of Notch or APP-CTF produces carboxy-terminal intracellular domains, NICD and AICD, respectively, which enter the nucleus and are thought to regulate gene expression. The γ-secretase cleavage of βAPP also produces the neurotoxic Aβ peptide, but only if βAPP has been first cleaved by β-secretase (not α-secretase). The γ-secretase complex includes, in addition to PS1, the presenilin-binding protein nicastrin; members of the Armadillo protein family, such as β-catenin, have also been detected in presenilin complexes, although their role is not understood. Aph-1 and Pen-2 may also participate in the γ-secretase complex.

activity is generated only after an obligatory interaction between nicastrin and PS1 [59]. Notch cleavage is affected similarly by nicastrin mutations, albeit to a lesser extent [60]. Moreover, nicastrin is essential for the normal processing of both βAPP and Notch homologs in *Drosophila* and *C. elegans*, and human nicastrin can partially rescue mutants of the *C. elegans* nicastrin homolog Aph-2 [59,61-64], suggesting that nicastrin function and its interactions with presenilins are conserved widely in non-mammalian species. Only mature glycosylated nicastrin that has passed through the Golgi compartment interacts with PS1 and is included in γ-secretase complexes [65]; overexpressed nicastrin fails to mature normally and accumulates within the endoplasmic reticulum. Moreover, entry of each of nicastrin and PS1 into γ-secretase complexes appears to be regulated by the other protein: the loss of one partner destabilizes the other [61,63,66,67] .

Two potential new members of the PS-nicastrin complexes are homologs of Aph-1 and Pen-2, components of the

C. elegans Glp-1/Notch signaling cascade that interact genetically with Sel-12/presenilin and Aph-2/nicastrin [68,69]. Primary sequence analysis suggests that Aph-1 and Pen-2 have seven and two membrane spanning domains, respectively, that are conserved in their respective *Drosophila* and human homologs. Human Aph-1 and Pen-2 can rescue *C. elegans* mutants lacking their homologs only when both transgenes are present together, implying that they act in concert. Moreover, reduction of Aph-1 and Pen-2 expression in *Drosophila* cells by RNA inhibition reduces γ-secretase activity [69]. Reduced expression of nematode Aph-1 causes mislocalization of Aph-2/nicastrin [68], and both Aph-1 and Pen-2 are required to maintain presenilin levels [69], suggesting that they regulate, or are components of, the presenilin-nicastrin γ-secretase complexes.

Frontiers

The identification of the additional γ-secretase components within the presenilin complexes is clearly an important task that lies ahead. The complexes purified to date are quite large, partly because of membrane impurities that remain associated following treatment with gentle detergents and partly because of interacting proteins that are not related to γ-secretase activity but are necessary for trafficking and maturation of the complex. The genetic cause of at least half of all of cases early onset familial Alzheimer's disease remain unexplained, and some of the unknown genes may have products that may modulate presenilin activity within γ-secretase complexes.

Acknowledgements

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