REVIEW: NEUROBIOLOGY

# The Presenilins in Alzheimer's Disease—Proteolysis Holds the Key

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Alzheimer's disease (AD) research has shown that patients with an inherited form of the disease carry mutations in the presenilin proteins or the amyloid precursor protein (APP). These disease-linked mutations result in increased production of the longer form of amyloid-β (the primary component of the amyloid deposits found in AD brains). However, it is not clear how the presenilins contribute to this increase. New findings now show that the presenilins affect APP processing through their effects on γ-secretase, an enzyme that cleaves APP. Also, it is known that the presenilins are involved in the cleavage of the Notch receptor, hinting that they either directly regulate γ-secretase activity or themselves are protease enzymes. These findings suggest that the presenilins may prove to be valuable molecular targets for the development of drugs to combat AD.

Worldwide, about 20 million people suffer from age-related dementia caused by AD. A very small fraction of AD cases are caused by autosomal dominant mutations in the genes encoding presenilin (PS) proteins 1 and 2 and the APP. Study of familial AD cases has illuminated the pathological mechanisms involved in the major, sporadic form of the disease. Disease-linked mutations in *PS1*, *PS2* and *APP* result in an increase in the production of the 42-amino acid peptide form of amyloid-β ($A\beta_{42}$), which is a major component of the amyloid plaques deposited in the brains of AD patients. Three enzymes, α-, β- and γ-secretase, cleave the transmembrane APP into Aβ fragments of different sizes. Recent research demonstrates the involvement of presenilins in the formation of Aβ through their effects on γ-secretase, and connects them to the signaling pathway mediated by the Notch receptor. These findings define presenilins and γ-secretases as molecular targets for developing drugs to combat AD and hint at the potential side effects that could be associated with such drugs. Curiously, AD research links diverse fields such as developmental biology and neurobiology through genetic and biochemical studies in fish, flies, and worms.

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## Subcellular Localization of Presenilin Proteins

The presenilins are serpentine integral membrane proteins (Fig. 1). By using the indices of Kyte and Doolittle, ten hydrophobic regions, linked by short hydrophilic sequences, can be identified (*1*). The presenilins do not contain a signal peptide, and the hydrophilic amino-terminal domain protrudes into the cytoplasm. A second hydrophilic domain, between transmembrane domains (TM) 6 and 7, and the relatively hydrophobic carboxyl-terminal tail also protrude into the cytoplasm (*2, 3*). Although the proposed structure implies eight transmembrane domains (Fig. 1) (*3, 4*), alternative models for the insertion of presenilin into biological membranes are possible (*5*). Studies of endogenous presenilins are required to answer this important question more definitively because transient overexpression of presenilins may lead to abnormal membrane insertion.

Presenilin proteins have been localized to the endoplasmic reticulum (ER) and the Golgi subcellular compartments (*2, 6, 7*). The finding of overexpressed presenilin proteins within Golgi compartments should, however, be interpreted with caution, because recent evidence indicates that membrane proteins with many transmembrane domains (such as the presenilins and the cystic fibrosis transmembrane regulator) can accumulate in structures called aggresomes. These cytoplasmic structures merely reflect cell stress and overloading of the proteasome compartment (*8*). They accumulate at the microtubule organizing center, which is located in many cells near the nucleus and close to the Golgi apparatus. Endogenous presenilins, in contrast, have a relatively limited subcellular

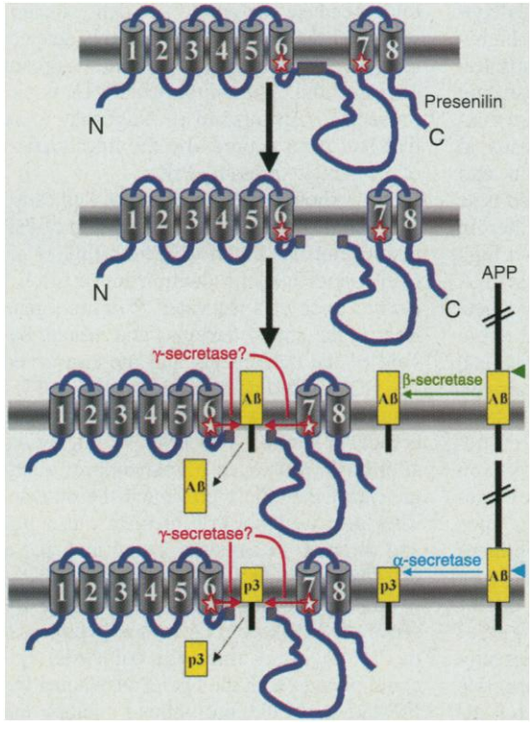


**Fig. 1.** Presenilins regulate the processing of APP. The APP is a transmembrane protein that is cleaved into several Aβ peptide fragments by α-, β- or γ-secretases. Presenilin mutations that result in clinical outcomes all cause an increased production of a slightly longer (42–amino acid) variant peptide ($A\beta_{42}$) that is highly amyloidogenic. It has been proposed that presenilins modulate the activity of γ-secretases or are themselves γ-secretases, although there is no direct proof that presenilins have proteolytic activity. Activity of the presenilins resides in their amino (N)- and carboxyl (C)-terminal fragments, which exist together with other proteins in a complex in the endoplasmic reticulum and Golgi apparatus.

distribution; they are found in the early compartments of the biosynthetic pathway (*9, 10*). Confocal and electron microscopy, combined with subcellular fractionation experiments, show that presenilins in neurons reside in the smooth and rough ER, the ER-Golgi intermediate compartment (ERGIC), and, to a limited extent, in the cis-Golgi, but not beyond (*9*). The localization of the presenilins in the nuclear membrane (*11*) is compatible with their localization in the ER, given the continuity of this structure with the ER. Subcellular fractionation experiments demonstrating the enrichment of presenilins in other vesicular structures (*12*) remain to be confirmed by immunolocalization studies. The cellular localization of presenilin proteins in ER and early Golgi overlaps to some degree with the intracellular site of generation of the highly amyloidogenic $A\beta_{42}$ (*13, 14*). Moreover, immature (uncleaved) APP, which is located in the ER and the early Golgi, has been demonstrated to coimmunoprecipitate with the presenilins (*15*). Although the interaction of presenilins with APP is still controversial (*16*), the results suggest that the presenilins and APP may interact directly in early compartments of the protein transport pathway.

### A Toxic Gain of Function

All presenilin *PS1* and *PS2* mutations in familial AD are missense mutations, which cause single amino acid substitutions in the primary structure of the presenilins (*17*). Even the pathological activity of the exceptional PS1Δexon9 splice mutation (*18*) now turns out to be a consequence of the amino acid substitution at the artificial splice site (*19*). Therefore, the clinical *PS* mutations are rather conservative, probably because more drastic mutations causing large truncations, deletions, or frameshifts of *PS* interfere with vital functions, as observed in *PS1*-deficient mice (*20–22*). Therefore, *PS* mutations apparently do not substantially affect the intrinsic physiological function of the presenilins, but cause an additional gain of (toxic) function. This gain of function appears to be very similar for all *PS* mutations. They all cause an increase in the production of $A\beta_{42}$ of about 1.2- to 3-fold (*23–25*). Because this peptide variant is also produced under normal conditions, but at lower levels, *PS* mutations cause a rather subtle change in γ-secretase cleavage of APP without affecting other vital functions. This conclusion is corroborated by the demonstration that transgenic mice expressing human presenilins engineered to contain mutations found in familial AD do not display obvious abnormalities, apart from an increase in $A\beta_{42}$ production (*24, 26*). Moreover, mutant *PS1* genes can functionally rescue the fatal phenotype in mice that have their *PS1* gene ablated (*27*). However, an apparent problem remains because, although wild-type human presenilin can functionally replace its homolog, *sel-12*, in the worm *Caenorhabditis elegans*, human presenilins containing mutations that have clinical outcomes cannot (*28*). Thus, it seems likely that *PS* mutations cause other subtle malfunctions, in addition to increased $A\beta_{42}$ production, that can be detected in *C. elegans* but remain elusive in mouse and human. The recent finding that presenilins with mutations do not restore Notch signaling in *PS1*-deficient cells should provide further insight into this paradox (*29*). Furthermore, the increased sensitivity of neurons expressing mutated presenilins to apoptotic or necrotic stimuli could reflect a subtle dysfunction in the mutated proteins (*30*), although the relevance of these findings to AD remains to be demonstrated in vivo.

### Presenilins Stimulate Notch Signaling

Several lines of evidence have recently converged, shedding further light on how presenilin proteins modulate cellular differentiation and embryonic development. Studies in mouse, the fruit fly *Drosophila melanogaster*, and *C. elegans* imply that presenilins control the Notch signaling pathway, which is important for cell fate decisions during embryogenesis, hematopoiesis, and neuronal stem cell differentiation. Notch has pathological roles in leukemia, cervical and colon carcinoma, and the multisystem Alagille syndrome. Missense mutations in the human Notch3 gene cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) (*31*). It is therefore tempting to speculate that certain aspects of the clinical picture observed in families with presenilin mutations could be due to disturbed Notch signaling (*29*), although this remains to be demonstrated. In any event, shortly after the discovery of the *PS* genes, Greenwald and colleagues provided functional evidence for the involvement of presenilins in Notch signaling. They found that a suppressor of a gain-of-function mutation in the *lin-12* gene (a homolog of Notch) was the *C. elegans* homolog of human presenilins (*32*). They called the new gene *sel-12* (suppressor-enhancer of *lin-12*). Human *PS1* and *PS2* genes can functionally replace *sel-12*, which indicates that human presenilins are involved in Notch signaling (*28*). A similar link between presenilin function and Notch signaling was established in *D. melanogaster*. Loss-of-function mutations of the single presenilin gene in flies resulted in a classic Notch-like phenotype (*33, 34*). The phenotype of mice homozygotic for *PS1* null mutations ($PS1^{-/-}$) is more complex. The mice display severe growth retardation, most pronounced in the caudal region (*20–22*), and die shortly before or at birth. In their brains, underdevelopment of the subventricular zone and severe vascular lesions and hemorrhages are observed (*20, 35*). The cortical dysplasia, featuring leptomeningeal fibrosis and overmigration of cortical plate neurons into the marginal zone, resembles human lissencephaly type 2, linking *PS* deficiency to neuronal migration disorders (*35*). The cerebral problems, as well as the disturbed development of somites, can be partially explained by deficient Notch signaling in the *PS1*-deficient mice. Mice with inactivated Notch1 genes display abnormal somite formation and severe growth retardation (*36*). They do not, however, survive beyond embryonic day 11, in contrast to the $PS1^{-/-}$ mice (*20, 21*). Mice that are deficient in both *PS1* and *PS2*, however, display a full Notch-deficient phenotype, indicating that *PS2* compensates partially for the loss of function in *PS1*-deficient mice (*37, 38*).

Notch is a large, type I integral membrane protein. It is proteolytically processed in its extracellular domain by furin (*39*) or the metalloproteinase kuzbanian (*40*) or both (Fig. 2). The signal transduction cascade is initiated by binding of the Notch receptor to a member of the DSL (Delta, Serrate, Lag-2) ligand family. Upon binding, the cytoplasmic domain of Notch is cleaved, released, and then translocated to the nucleus (*41–44*). This Notch intracellular domain by itself has no DNA binding properties but rather acts as a coactivator, binding to a member of the CSL [CBF1, Su (H), Lag-1] DNA binding protein family (*45*). Presenilin could act at any point in the Notch signaling pathway. However, a reasonable guess based on a comparison between Notch cleavage and APP processing suggests that the presenilins may control the cleavage of Notch. APP, like Notch, is first cleaved by α- or β-secretases in its ectodomain. The resulting carboxyl-terminal fragment then becomes the substrate for γ-secretase, which cleaves the fragment

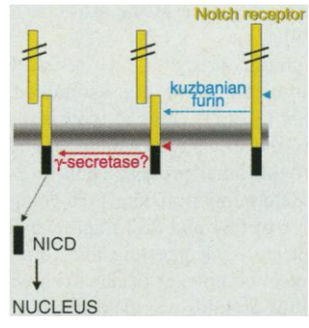


**Fig. 2.** Endoproteolytic processing of the Notch receptor. The carboxyl-terminal cleavage of Notch occurs close to its transmembrane domain resulting in the formation of an intracellular domain (NICD) that moves to the nucleus and acts as a coactivator of transcription factors. It has been postulated that cleavage of Notch resembles the γ-secretase-cleavage of APP and that Notch cleavage may be facilitated by presenilins.

within its transmembrane domain, liberating Aβ (Fig. 1).

Deficiency in *PS1* results in a relatively selective inhibition of γ-secretase-mediated processing of APP (*46*). In an analogous fashion, Notch1 is no longer proteolytically cleaved in neurons and fibroblasts derived from $PS1^{-/-}$ mice (*47*). In a complementary approach using *PS*-deficient flies, Struhl and Greenwald were able to demonstrate that presenilin is indeed required for the ligand-induced release of the Notch intracellular domain (*34*). The analogy between Notch and APP processing has been pushed even further with the demonstration that a series of γ-secretase inhibitors interfere with proteolytic processing of both APP and Notch (*47*). Moreover, presenilin function in Notch signaling and the proteolytic processing of Notch can be inhibited by dominant negative mutations in *PS1* (*48*) and *PS2* (*37*). Therefore, presenilins control the proteolysis of the integral membrane domains of APP (*46*), the mammalian APP homolog APLP-1 (*49*), and Notch (*29, 34, 37, 47*), and probably other substrates still to be identified.

## Proteolytic Maturation of the Presenilins

Presenilins not only control proteolysis of other proteins, but also are themselves proteolytically processed (*50*). In vivo, only very small amounts of the holoprotein (the uncleaved, full-length PS) can be detected, primarily in the nuclear envelope (*9*), whereas relatively high levels of the ~30-kD amino-terminal fragment and the ~20-kD carboxyl-terminal fragment of presenilins are observed in all tissues and cell lines analyzed so far (*50, 51*) (Fig. 1). Proteolytic processing in general is not affected by the clinical PS mutations (*52*), with the exception of the PS1Δexon9 mutation (*18*). However, the pathological activity of this mutation is independent of the deletion and is due to the point mutation generated at the artificial cleavage site (*19*).

Coimmunoprecipitations reveal that the presenilin fragments are assembled into a complex (*16, 53*) (Fig. 1), consisting of the amino- and carboxyl-terminal fragments together with other proteins such as β-catenin (*54*). Heterodimeric complexes composed of mixtures of PS1 and PS2 fragments have not been observed, suggesting that formation of the protein complexes occurs even before the presenilin proteins are cleaved (*55*). Formation of the complex is highly regulated. Presenilin molecules that are not incorporated into the complex are rapidly degraded by several proteases, including the proteasome caspases and calpain-like enzymes (*56, 57*). The fragments in the complex escape proteolytic degradation and have a half-life of more than 24 hours (*51, 55, 57*). On the basis of these findings, it appears that the complex is the biologically active form of the presenilins. This is supported by recent results showing that a recombinant amino-terminal fragment (artificially expressed by the insertion of a stop codon at the site for endoproteolytic cleavage) containing a familial AD-associated mutation does not stimulate $A\beta_{42}$ production even upon inhibition of its proteolytic degradation (*57*). Additional coexpression of the carboxyl-terminal fragment of PS1 from a separate cDNA construct does not reconstitute $A\beta_{42}$ generation (*58*). This supports our view that endoproteolysis and incorporation of presenilin fragments into complexes occurs soon after their biosynthesis and is required for normal presenilin function probably because it confers stability on the fragments. However, when mutations are engineered into the cleavage sites of the presenilins so that they cannot be cleaved, the production of Aβ is maintained (*59, 60*). Thus, it appears that endoproteolysis of the presenilins is not needed for activation of their putative enzyme activities but may be required to convert unstable presenilins into stable complexes (*57*).

The molecular basis for the critical involvement of both presenilin fragments within the complex could be the localization of the two functionally important aspartate residues in domains TM6 and TM7 (Fig. 1), which are part of the amino- and carboxyl-terminal fragments, respectively (*61*). Both residues are required for presenilins to be functionally active.

## To Cleave, or Not to Cleave: That Is the Question

When either one of the aspartates in the TM6 or TM7 domains of PS1 were replaced with another amino acid, the resulting PS1 variant no longer underwent endoproteolytic processing (*61*) (as is the case with the cleavage site mutations) (*59, 60*), and the accumulating full-length protein replaced the endogenous presenilins (*61, 62*). However, in contrast to the cleavage site mutations, the aspartate mutations resulted in a substantial inhibition of the production of Aβ and p3 (the APP fragment generated by α- and γ-secretase cleavage) (*61*). This was accompanied by the accumulation of the carboxyl-terminal fragments of APP (produced by α- or β-secretase cleavage), which are the immediate substrates of γ-secretase. Because similar effects are observed when the *PS1* gene is inactivated (*46*), it has been presumed that the *PS1* variant bearing aspartate mutations acts as a dominant-negative mutant for wild-type *PS1* (*61*). This observation, together with the fact that Aβ generation is also blocked by aspartyl protease inhibitors (*63*), recently led Wolfe and colleagues (*61*) to propose that *PS1* itself is an unusual aspartyl protease. Owing to its involvement in the cleavage of APP, the presenilins may also turn out to be the long-sought-after γ-secretase (Fig. 1). Aspartyl proteases, like the presenilins, require two aligned aspartate residues in their catalytic domain. The proteolytic maturation of the presenilins may be reminiscent of the maturation of other proteases, which are first expressed in the cell as inactive proenzymes and then become activated after cleavage (Fig. 1). However, this hypothesis may not be compatible with the above-mentioned data demonstrating that uncleavable presenilin derivatives maintain their capacity to promote Aβ production from APP (*59, 60*).

Additional support for the hypothesis that PS1 may be an aspartyl protease comes from in vitro experiments. Amyloid-β production is inhibited in a cell-free, in vitro translation system that uses microsomes (an artificially generated vesicular cell fraction) obtained from cells that express PS1 containing aspartate mutations. It is interesting that Aβ generation in this system is dependent on an acidic pH, which is in agreement with the hypothesis that aspartyl proteases are involved (*61*). The critical aspartates are conserved in evolution and play a functionally similar role in human PS2 (*37*), zebrafish PS1 (*62*), and the *C. elegans* presenilin (*48*). The results thus establish that the aspartates in TM6 and TM7, which are buried in the membrane, are required for the γ-secretase cleavage of APP and the generation of the Notch intracellular domain. It should be emphasized, however, that direct evidence for the conclusion that PS1 and PS2 represent the γ-secretase activity itself is still lacking because so far no direct evidence for proteolytic activity has been observed for either presenilin. It therefore remains possible that presenilin proteins influence γ-secretase activity indirectly. Presenilins may control membrane insertion of γ-secretase or may behave as cofactors stimulating their catalytic activity. An analogy can be drawn with the regulation of the site 1 cleavage of sterol regulatory element binding proteins, which is controlled by a multitransmembrane cleavage-activating protein located in the ER (*64*).

The major problem with the hypothesis that presenilins are proteases is their subcellular localization. Presenilin proteins have been localized to early transport compartments (*2, 6, 7, 9, 10*), whereas abundant γ-secretase activity is restricted to late transport compartments and the endosomal pathway (*14, 65*). The same holds true for the release of the Notch intracellular domain, which occurs after ligand binding by Notch at the cell surface (*41, 44*). Moreover, proteolytic generation of the Notch intracellular domain is completely inhibited on retention of Notch in the ER by Brefeldin A (*41*), although presenilins are located in the ER and

the early Golgi. One possible interpretation is that minute amounts of presenilin are present at the cell surface where Notch cleavage occurs (*66*). However, the experimental evidence for this possibility remains controversial, because these findings are inconsistent with our current understanding of presenilin topology (*2–5*). There is indirect evidence for a role for PS1 in protein maturation and trafficking, suggesting an alternative interpretation of the experimental data. For instance, glycosyl maturation and phosphorylation of the tyrosine kinase receptor TrkB is reduced in cells derived from *PS1* knockout mice, leading Sisodia and co-workers (*49*) to propose that presenilins are a sorting platform for selected cell surface proteins. Reduced proteolytic cleavage of APP in these mice is then considered to be a consequence of the APP being unable to reach an as yet undefined "processing" compartment in the cell.

## Are We Getting Close to Therapy?

It is obvious that AD research is ultimately oriented toward one major goal: a therapy for this tragic disease. Is all the recent excitement about presenilins and their possible γ-secretase activity warranted in this regard? Certainly, seeking drugs that are capable of blocking γ-secretase activity and hence the production of Aβ is a major goal. Currently, however, there are no guarantees that lowering the amyloid burden in the brain using such drugs will indeed reduce neuronal degeneration. But a recent study showed that immunizing young transgenic mice (which develop neuropathological features of AD as they age) with $A\beta_{42}$ blocked amyloid plaque deposition (*68*). In another encouraging study, Vassar and colleagues have identified and characterized an APP-specific β-secretase (called BACE for beta-site APP-cleaving enzyme), which may provide another valuable molecular target in the development of AD drugs that inhibit secretase activity (*69*).

It should be remembered that γ-secretases and other secretases evolved to fulfill several important physiological functions in addition to cleavage of APP. Thus, it is probable that γ-secretase inhibitors would, for example, interfere to a certain degree with Notch signaling and could thus result in defective hematopoiesis (*47*). Finding the correct therapeutic window for new drugs will therefore turn out to be essential for the treatment of AD. The problem of side effects with γ-secretase inhibitors due to their ability to block Notch signaling could probably be solved. It is well established that only very small amounts of the Notch intracellular domain are required for normal signal transduction in the Notch pathway (*43, 44*). Indeed, it has been debated for years whether such a Notch derivative might even exist. Therefore, partial inhibition of presenilin function may be sufficient to reduce Aβ generation to therapeutically relevant levels, while production of the Notch intracellular domain can be maintained at sufficient levels to cause only minimal side effects. In this regard, it should be noted that mice with only one functional *PS1* allele ($PS1^{+/-}PS2^{-/-}$) remain healthy (*38*), which suggests that a therapeutic window for the partial inhibition of presenilin function does exist. A second indication that side effects of γ-secretase inhibitors could be overcome lies in the fundamental mechanism of proteolytic processing of APP and Notch. Notch cleavage is sensitive to subtle mutations at the cleavage site (*44*), whereas APP processing is not (*67*). This suggests that the proteases involved in Notch and APP processing are related, but probably not identical. This obviously challenges the idea that presenilins are identical with the γ-secretase, but the exploitation of these small differences may lead to a more specific drug to treat AD.

Although we are still a long way from treating AD patients effectively, it is likely that presenilins and the β- and γ-secretases will be valuable molecular targets for the development of drugs to lower the production of Aβ. Inhibiting or modulating presenilin activity might indeed turn out to be a possible treatment for the many patients suffering from this devastating disease.

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