Presenilins in their infancy

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Familial forms of Alzheimer's disease are caused by mutations in the genes encoding the presenilins, which are integral membrane proteins. Presenilins have been shown to interact with β-amyloid precursor proteins and Notch receptors. Several recent studies have examined the role of presenilins in Notch processing.

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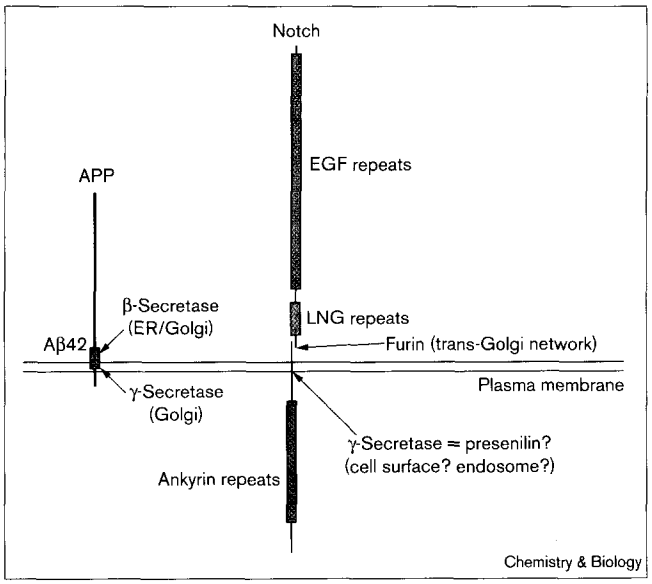
Alzheimer's disease is a degenerative disorder that causes progressive memory and cognition decline during late adult life, and is accompanied by the gradual formation of extracellular neuritic plaques in the brain. An important constituent of these plaques is Aβ42, a highly amyloidogenic peptide that is proteolytically derived from a much larger β-amyloid precursor protein (APP). APP is a single-pass transmembrane protein that is subject to several alternative proteolytic cleavages. To generate the toxic Aβ42 form, APP is cleaved by two enzymatic activities: β-secretase, which cleaves in the extracellular domain, causing the release of soluble derivatives of the protein, and γ-secretase, which cleaves within the transmembrane domain (Figure 1). The enzymes have not yet been identified, however.

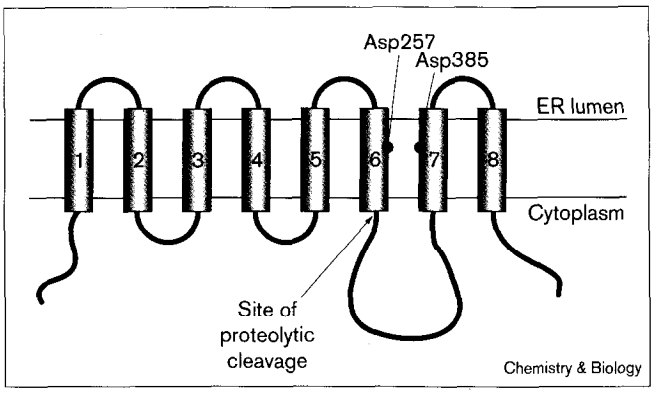
Three genes that, when mutated, lead to the relatively early onset of familial Alzheimer's disease (FAD) have been identified. The first locus encodes APP itself. The *APP* FAD missense mutations increase the amount of secreted Aβ42. The two other loci encode novel integral membrane proteins, called presenilin-1 and presenilin-2 (PS1 and PS2; see [1] for a review). PS1 and PS2 are highly homologous; they have 67% amino acid sequence identity. Analysis of the membrane topology of PS1 revealed that PS1 consists of eight transmembrane (TM) domains, with both the carboxyl and amino termini on the cytosolic side (Figure 2; [2]). As the vast majority of FAD cases are caused by missense mutations in the *PS1* gene, most studies have focused on the function of PS1. FAD *PS1* mutations (more than 50 have been described so far) occur at positions conserved between PS1 and PS2. These structurally and/or functionally important residues are located within all TM domains as well as within the large intracellular loop, so their identification has not revealed any specific domains essential for the function of PS1. Both PS1 and PS2 are ubiquitously expressed, and they are found predominantly associated with intracellular membranes—in the endoplasmic reticulum (ER) and Golgi apparatus [3]. A small fraction of PS1 is cleaved in the loop domain separating TM6 and TM7 (Figure 2). The resultant amino- and carboxy-terminal fragments form stable 1:1 heterodimers that may correspond to the biologically active form of PS1. Co-immunoprecipitation experiments indicate that PS1 and PS2 directly interact with the immature form of APP in the ER [4,5], where Aβ42 is probably generated [6]. Moreover, transfection studies showed that FAD mutations in presenilins altered APP processing, leading to an increased accumulation of Aβ42 isoforms [7–9]. Finally, the level of γ-secretase activity was found to be reduced in mice homozygous for a *PS1* deletion [10]. Although these data indicate that presenilins directly regulate APP processing, both the non-pathological activity of presenilins and the dominant effect of FAD mutant presenilin remain to be defined.

Figure 1



Processing of APP and Notch. APP is subject to alternative proteolytic cleavages. Maturation of APP by β-secretase in the endoplasmic reticulum (ER) and/or Golgi, and by γ-secretase in the Golgi leads to the extracellular release of the amyloidogenic Aβ42 peptide. Notch is first processed by furin in the trans-Golgi network to form functional heterodimeric receptors at the cell surface. The binding of Delta to the epidermal growth factor (EGF) repeats of Notch leads to the intracellular processing of Notch at a site adjacent to the transmembrane domain, that is otherwise inhibited by the Lin-12/Notch/Glp-1 (LNG) repeats of Notch. This proteolytic step may be catalyzed by a γ-secretase, either at the cell surface or following endocytosis of the activated receptor.

Figure 2



Schematic representation of PS1. Membrane topology and position of the two aspartate residues, Asp257 in TM6 and Asp385 in TM7, required for γ-secretase activity and presenilin endoproteolysis at a site adjacent to the large loop region residing on the cytoplasmic side of the ER membrane.

One important clue came from the genetic identification of a presenilin homologue, SEL-12, in *Caenorhabditis elegans* as a *lin-12* suppressor [11]. The *lin-12* gene encodes a transmembrane receptor involved in regulating many cell-fate decisions during development. Loss-of-function mutations in the *sel-12* gene reduce signalling by the LIN-12 receptor in various developmental contexts in *C. elegans*, therefore raising the possibility that presenilins were involved in cell–cell interactions during normal development. Homologues of *lin-12* exist in *Drosophila* and vertebrate species, and are known as Notch receptors. The structure and function of the LIN-12/Notch receptors have been evolutionarily conserved from nematode to man [12]. Likewise, the SEL-12 protein shows about 50% amino acid sequence identity to PS1 and PS2, and has the same membrane topology as PS1 and PS2 [13,14]. SEL-12 localizes in ER and Golgi membranes [15]. Furthermore, both PS1 and PS2 can substitute for SEL-12 *in vivo*. In this assay, however, FAD mutant PS1 had a reduced level of activity, and had no detectable dominant effect [16]. Finally, mouse embryos homozygous for a *PS1* deletion show developmental defects associated with decreased Notch signalling [17], strongly supporting the idea that presenilins participate in Notch signalling.

Signalling by the LIN-12/Notch receptors appears to involve several proteolytic processing steps. Western blot analysis suggests the existence of many processing events, of which only two have been characterized. The extracellular domain of Notch is first processed by furin in the trans-Golgi network [18,19]. The two processed subunits that result from this cleavage remain tightly associated and together form a heterodimeric functional Notch receptor at the cell surface. Upon ligand binding, a second cleavage occurs at a conserved intracellular valine residue located very close to the transmembrane domain. This cleavage releases the large intracellular domain of the receptor, called the Notch intracellular domain (NICD). This domain, which contains a functional nuclear localisation signal, translocates into the nucleus and acts as a transcriptional co-activator to trigger the expression of Notch target genes [20–23]. The similarities observed between the proteolytic processing of APP and Notch raise the possibility that presenilins regulate the processing of these two proteins in a similar manner.

Recent biochemical and genetic studies show that presenilins are needed to generate the processed forms of Notch required for signalling, thereby emphasizing the connection between the processing of both APP and Notch. First, consistent with earlier findings in worms and mice, loss-of-function mutations in *presenilin* in *Drosophila* lead to cell-fate transformations associated with lack of Notch signalling activity. Although Notch appears unable to signal, Notch biosynthesis, cleavage by furin and accumulation at the apical plasma membrane appear to be normal [14,24,25]. Likewise, mutant *PS1* mouse cells produce normal amounts of furin-processed Notch [26]. Second, the *presenilin* loss-of-function phenotype is rescued by an activated form of the receptor that is similar to NICD [15,25], indicating that presenilin activity is not required for the nuclear function of NICD. Together, these findings indicate that presenilins act downstream of the furin-mediated maturation of Notch and upstream of the nuclear function of NICD. One obvious possibility is that presenilins regulate the ligand-induced intracellular cleavage of Notch. Presenilins may therefore either act as γ-secretases or stimulate γ-secretase activity for both APP and Notch.

Several observations are consistent with this possible role for presenilins. The ligand-dependent intracellular processing of Notch, as revealed using an indirect nuclear activity assay, requires presenilin activity in *Drosophila*. Furthermore, the ligand-independent processing of a membrane-bound form of constitutively activated Notch also requires presenilin activity [24]. Similarly, the constitutive intracellular processing of a membrane-bound form of activated Notch is greatly reduced — but not abolished — in mutant *PS1* mouse neurones [26]. In addition, the γ-secretase activity that generates Aβ42 from APP shares pharmacological properties with the activity that produces NICD from Notch. Both activities are inhibited by MDL 28170, a peptide aldehyde calpain inhibitor; MG132, another peptide aldehyde protease inhibitor with relatively low specificity; and MW167, a difluoro ketone peptide analogue designed on the basis of the site of cleavage by γ-secretase in APP [26]. Finally, site-directed mutagenesis experiments show that two conserved aspartate residues located in TM6 and TM7 of PS1 are required for γ-secretase activity [27] (Figure 2). These

residues are also essential for the cleavage of PS1 in the loop separating TM6 and TM7 (whereas γ-secretase activity does not strictly depend on PS1 processing). As these effects were seen both in transfected cells and in cell-free microsomes prepared from transfected cells, they were interpreted to suggest that PS1 was either an essential cofactor for γ-secretase or γ-secretase itself, postulated to then be an autoactivated aspartyl protease [27].

A number of observations do not yet fit with the proposal that presenilins are directly involved in the processing of both Notch and APP, however. First, presenilins appear to localize predominantly in the ER and Golgi membranes. If the ligand-induced intracellular processing of Notch requires a direct interaction with presenilins, then activated Notch receptors would therefore probably have to be transported from the plasma membrane back to these subcellular compartments. In contrast with this prediction, PS1 co-immunoprecipitated with precursor forms of the Notch receptor in transfected human cells, indicating that PS1 interacts with Notch prior to the cleavage of Notch in the trans-Golgi network [28]. In addition, although signalling by a membrane-bound form of activated Notch in imaginal discs from *Drosophila presenilin* mutant larvae is greatly reduced, it is not abolished [25]. This result appears to contradict those obtained by Struhl and Greenwald [24] who used a nuclear-activity assay to detect indirectly the intracellular processing of Notch in the embryo. This difference might be interpreted in several ways. Some interpretations are consistent with presenilins directly regulating the intracellular processing of Notch. For instance, presenilin activity might be redundant in discs but not in embryos; also, the signalling assay used in discs may more sensitive than the nuclear-activity assay used in embryos. Alternatively, presenilin may act upstream of the intracellular processing step. In support of this hypothesis, western blot analysis of Notch carboxy-terminal fragments indicates that processing of Notch is specifically altered in *presenilin* mutant larvae. In both wild-type and *presenilin* mutant larvae, four processed Notch carboxy-terminal species (115–120 kDa) can be detected. The smaller forms predominate in wild-type larvae, however, whereas the largest of these four species accumulate in *presenilin* mutant larvae. This suggests that loss of presenilin activity blocks an as yet uncharacterized extracellular processing event of furin-processed Notch [25]. In addition, expression of a dominant-negative form of the Kuzbanian extracellular protease, which was shown to process the Notch ligand Delta [29], leads to an increase in the accumulation of the smallest carboxy-terminal 115–120 kDa species. Interestingly, the effect of Kuzbanian appears to depend on presenilin activity [25]. Because Kuzbanian is thought to act upstream of Notch activation at the cell surface, this result may be interpreted to suggest that *Drosophila* presenilin activity is required prior to the activation of Notch by Delta. Together, these results favour the view that presenilins regulate the trafficking and processing of Notch. Such a model would be consistent with the role of the *C. elegans* presenilin-related SPE-4 protein that is involved in vesicular trafficking during spermatogenesis [30].

What to make of these reports? First, whether presenilins are indeed γ-secretase or whether they regulate the trafficking of both Notch and APP remains an open and exciting issue. Second, it now seems clear that alteration in Notch signalling is not directly linked to Alzheimer's disease. Finally, the studies suggest that the processing of Notch may be more complex than initially anticipated. Clearly, it will be important to determine the structures of the various 115–120 kDa carboxy-terminal Notch fragments identified by Ye *et al.* [25] and to establish their putative role in Notch signalling. In view of this complexity, it might be attractive to speculate that Notch, like APP, is subject to several alternative proteolytic cleavages, and that presenilin may play a role in regulating these cleavage events as Notch is transported towards the plasma membrane.

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