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SPECIAL ISSUE

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The physiological role of presenilins in cellular differentiation: lessons from model organisms

Abstract Mutations in the human presenilin genes cause the most frequent and aggressive forms of Alzheimer's Disease. They results in an increase of the 42 amino acid variant of amyloid β peptide that rapidly aggregates into neurotoxic plaques. In addition, lack of presenilin activity prevents the proteolytic cleavage of the Notch receptor of intercellular signaling. The biological role of presenilins is evolutionary conserved in animals. This review summarizes recent results obtained from animal models to understand presenilin activity and malfunction.

Key words Alzheimer's Disease · Presenilin · Caenorhabditis elegans · Notch receptor

Introduction

The discovery of the role of presenilin proteins in the early onset of familial Alzheimer's Disease raised many issues concerning the biological function of these proteins. Some clues about their function were obtained within the past four years from work done in mouse, and, more recently, in Drosophila. The most data, however, were collected from experiments in a small invertebrate animal, the nematode *C. elegans*. For someone who is not a developmental biologist, reports about this work are notoriously difficult to read. In this article, I try to summarize the most recent data. I will mostly focus on results obtained from *C. elegans* and would like to discuss how work in this animal, in combination with other model systems, may help to uncover the details of presenilin function.

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C. elegans may be ideally suited to analyze the molecular genetics of neural development and function

Since December 1998, *C. elegans* is the only multicellular animal whose genome (100 megabases) has been fully sequenced (The *C. elegans* Sequencing Consortium, 1998). In comparison, only 14% of the genome of the fruit fly Drosophila melanogaster has been determined so far. About 60% of the currently known human genes have a homologue in *C. elegans* (Sonnhammer and Durbin 1997). *C. elegans* is ideally suited for many analyses, especially of genes involved in nervous system development and function. The position, lineage, and ancestry of its 959 cells, as well as the function of many of these cells, are known in detail. The detailed anatomy and synaptic connectivity of each neuron in its nervous system has been analyzed on the level of electron microscopy (White et al. 1986). Although the *C. elegans* nervous system is undoubtedly built extremely simply, it is highly specialized. Its 302 neurons belong to 118 functional classes, and share many developmental and functional similarities with the human nervous system. *C. elegans* uses the same neurotransmitters as vertebrates and the pharmacology of their receptors is strikingly similar. *C. elegans* has a short generation time (3 days) and can easily be cultivated on agar plates or in microtiter wells. There are mutants for more than 3000 genes readily available which can be stored in frozen stocks and recovered easily. *C. elegans* is ideally suited for large-scale functional analyses and drug discoveries (Rand and Johnson 1995).

In many instances, the analysis of gene functions is therefore faster, less expensive, and more efficient in *C. elegans* than in the more complicated model organisms like the mouse, while still providing significant insights into the function of mammalian genes and pathways. Therefore, *C. elegans* has been used in a fast growing number of cases, including the projects in our laboratory, as a model to understand the function and regulatory properties of human genes. Already from these studies we

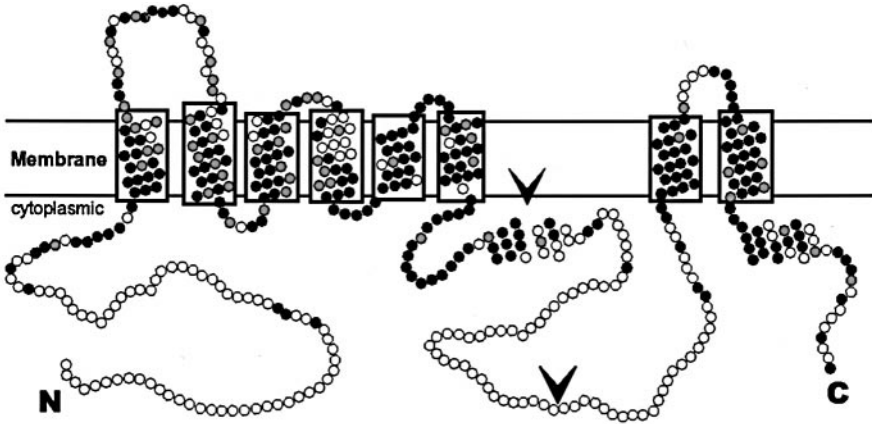

Fig. 1 Structural model of the SEL-12 presenilin. Transmembrane domains are indicated by boxes, amino acids are represented by spheres. Identical residues of human and *C. elegans* presenilins are shown in black, while similar side chains are indicated in grey. Proteolytic cleavage sites are shown by arrows

can conclude that homologous genes perform similar or identical tasks in different organisms, even though the visible phenotype caused by eliminating or reducing gene activity may be different.

Presenilins are structurally conserved

Soon after the human presenilin genes PS1 and PS2 were cloned, sequence database comparisons and Western blot analyses (C. Haass and R. Baumeister, unpublished results) revealed that similar genes and proteins exist in the nematode *C. elegans*. The gene products of *spe-4* and *sel-12* share 25% and 50 to 52% amino acid identity with PS1 and PS2, respectively. A third *C. elegans* presenilin, *hop-1* (initially termed *cps-3*) (Baumeister and Haass 1998), was discovered subsequently through the efforts of the *C. elegans* sequencing consortium. It is 31% and 32% identical to PS1 and PS2, respectively. Further studies using reporter gene fusions suggested that the topology of *sel-12* is similar to that of human PS1 and PS2 (Li and Greenwald 1996) (Fig. 1). In addition, most presenilin mutations identified from patients suffering from familial Alzheimer's Disease are located at positions that are conserved between human and *C. elegans* presenilins (Hardy 1997). From these data we and others inferred that *C. elegans* presenilins may be functionally conserved.

Prior to the identification of the presenilin genes in humans, mutations had been isolated in both *spe-4* and *sel-12*, which helped to indicate the function of the respective genes. Mutations in *spe-4* resulted in a lack of functional sperm, which prevents the selffertilization of the *spe-4* hermaphrodites (L'Hernault and Arduengo 1992). However, *spe-4* animals can produce progeny when mated with wild type male animals. The mutants in the *sel-12* gene were identified because defects in this gene ameliorated the phenotypic defect caused by a hyperactive mutant of the lin-12/Notch gene (the consequences of this mutation will be discussed later in this article). However, in a wild type background, mutations in *sel-12* cause a pronounced egg-laying defect (Levitan and Greenwald 1995) (Fig. 2). The recently isolated *hop-1* mutants do not reveal any significant phenotype (Westlund et al. 1999). Superficially, these phenotypes do not allow any functional correlation that helps to explain the onset of Alzheimer's Disease. As shown above, the structure and topology of presenilins in both *C. elegans* and humans is similar. The question that had to be resolved was whether these genes are still functionally conserved, even though both organisms are evolutionary separated by more than 500 million years.

Human and *C. elegans* presenilins are functionally conserved

In order to prove a functional conservation of human and *C. elegans* presenilins, we and others used related strategies. We reasoned that a functional conservation could be demonstrated if we succeeded in rescuing the egg-laying defect of the *C. elegans sel-12* mutant by expressing a functional copy of the human PS1 or PS2 gene in the animals. For this purpose, we isolated the *sel-12* promoter and cloned it in front of PS1 cDNA. This hybrid gene was transformed into *C. elegans sel-12* deficient animals by microinjection. For this procedure, a few nanoliters of the *sel-12*:PS1 gene were injected into the gonads of the animals, together with a reporter gene to identify the transgenic progeny of the treated animals. We then isolated the transgenic progeny of these animals and scored their egg-laying behavior (Baumeister et al. 1997). Strikingly, more than 90% of the animals laid eggs in a manner indistinguishable from wild type animals and displayed no retention of eggs in utero as is characteristic for the *sel-12* mutants (Fig. 2). Therefore, the expression of the human wild type PS1 gene from the *C. elegans sel-12* promoter was obviously sufficient to substitute for the defect in the endogenous *sel-12* gene. Taken together, PS1 functions indistinguishably from the endogenous *sel-12*, although both proteins share only 50% of their amino acid residues (Baumeister et al. 1997). The same result was also obtained when the human PS2 was expressed from the *sel-12* promoters (Brockhaus et al. 1998). In contrast, expression of FAD mutants from the same promoter did not rescue the egg-laying defect. Thus, *C. elegans* may serve as a test tube

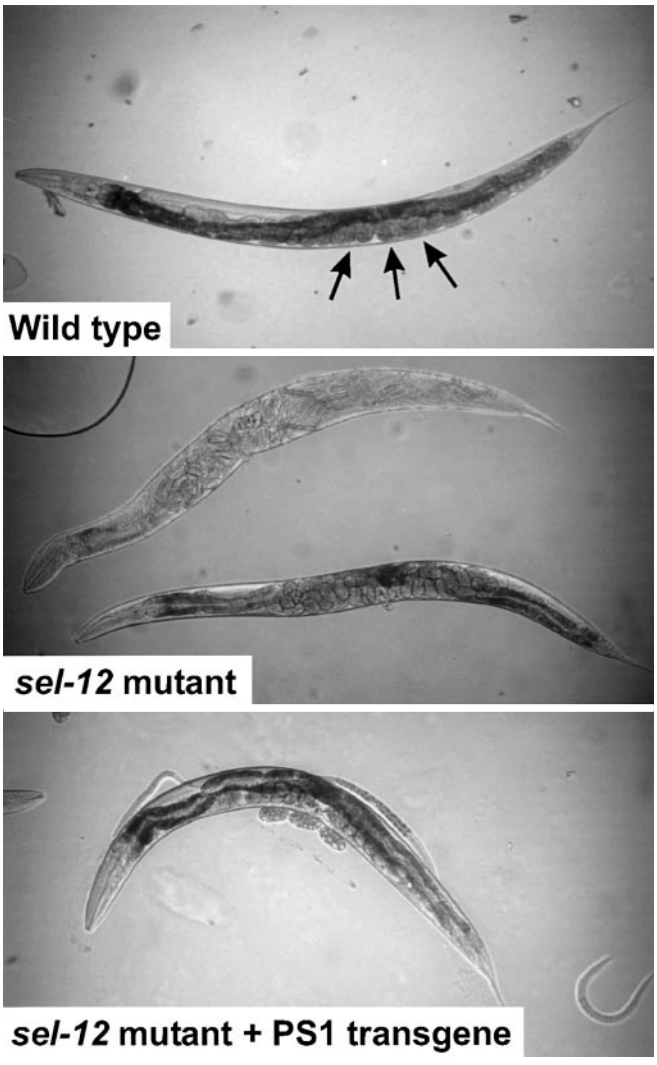

Fig. 2 PS1 function in *C. elegans*. A) A wild type *C. elegans* adult animal is self-fertilizing and lays 200 to 300 eggs over three days of adulthood. As a consequence, an adult animal constantly carries about 15 fertilized eggs in the uterus. Eggs are indicated by arrows. B) Egg laying, but not development of embryos, is severely disturbed in *sel-12*(ar171) mutants. This results in the accumulation of eggs in utero (lower animal). Eventually, an adult animal dies when the embryos hatch inside their mother (the "bag-of-worm" phenotype; upper animal). C) Expression of the human wild type PS1 gene, but not of FAD mutants, in a *sel-12* mutant animal fully rescues the egg-laying defective phenotype. This strongly suggests that human PS1 and *C. elegans* SEL-12 share identical functions. The picture shows freshly laid eggs, as well as several larvae

for human presenilins. These results, which were first presented at the 1996 Neuroscience Meeting (Marx 1996), were strongly supported by results from another group, who showed that even the expression of PS1 and PS2 from a heterologous promoter in a subset of the 959 cells of *C. elegans* is sufficient to rescue the *sel-12* egg-laying defect (Levitan et al. 1996).

Human presenilin proteins are processed in the large, cytoplasmic loop. Two cleavage sites, involving an as yet unidentified presenilinase (Thinakaran et al. 1996) and a caspase (Kim et al. 1997; Kim et al. 1997), have been identified. Strikingly, even this processing seems to be conserved in *C. elegans*, since the cleavage products of human PS1 can be detected upon expression in the nematode (Baumeister et al. 1997). However, neither the presenilinase nor the caspase cleavage seems to be a functional requirement for presenilin activity (Levitan et al. 1996; Baumeister et al. 1997; Brockhaus et al. 1998; Steiner et al. 1999).

Taken together, on the cellular level, human and *C. elegans* presenilins are obviously functionally equivalent, because the human presenilin can substitute for a defective *sel-12* in the worms. However, a presenilin defect in *C. elegans* manifests itself in a failure to lay eggs, whereas the consequence of FAD mutations in humans is an early onset of Alzheimer's Disease. What is the underlying mechanism involved in both cases?

Presenilins are involved in transport or processing of proteins

The expression patterns of presenilins in various organisms suggest that presenilins are expressed in many cell types, including the nervous system (Elder et al. 1996; Levitan et al. 1996; Baumeister et al. 1997; Wong et al. 1997; Boulianne et al. 1997; Hong and Koo 1997; Qian et al. 1998). In *C. elegans*, Drosophila, and the mouse, expression starts in embryogenesis and is visible long before phenotypic defects can be seen in the various mutants. A functional involvement in embryogenesis was revealed by mouse knock-out studies and by overexpressing *sel-12*, PS1, or PS2 in *C. elegans*, which resulted in sterility or early embryonic lethality (Baumeister et al. 1997; Shen et al. 1997; Wong et al. 1997).

The thorough analysis of the *spe-4* mutant defects had suggested that *spe-4* was involved in intracellular sorting or transport years before the first human presenilin was cloned (L'Hernault and Arduengo 1992). Could a similar function be impaired in *sel-12* and human PS mutants? Experiments in which the intracellular transport of cultured cells was blocked had shown that these cells accumulated a significantly higher amount of Aβ42, a proteolytic product of the amyloid precursor protein APP in the endoplasmic reticulum (Xia et al. 1997). Similarly, the most obvious phenotype of cells overexpressing human PS1 mutants was also an increase in Aβ42 production (Haass 1997). In contrast, the elimination of PS1 (by mouse knock-out experiments) resulted in a loss of gamma-secretase cleavage of APP, preventing one crucial step in Aβ42 generation (De Strooper et al. 1998). Taken together, these results suggested that in human cells presenilins are involved in the generation and/or processing of the APP protein, or in a sorting or transport step that is required to target this protein to a compartment in which processing occurs. Based on the functional similarity of PS1 and *sel-12*, as revealed by the *C. elegans* complementation analyses, one would expect that the SEL-12 protein fulfills a similar role.

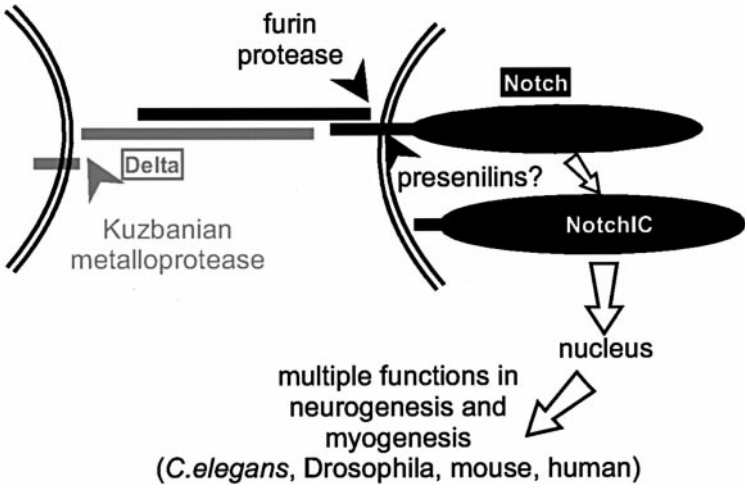

Fig. 3 Proteolytic processing of Notch. The various proteolytic steps involved in the processing of Delta (Kuzbanian metalloprotease) and Notch (furine protease) and activation of Notch (unknown transmembrane protease) are shown. Presenilin function affects the cleavage of the intracellular domain (NotchIC), which translocates to the nucleus and associates with a DNA-binding protein. Via transcriptional control, several activities in neurogenesis and myogenesis are controlled

Evolutionary conservation of Notch signaling in invertebrates and mammals

As indicated above, the *sel-12* gene was cloned because mutations in *sel-12* reduce the phenotypic defect caused by a hyperactive Notch receptor. In order to understand the contribution of the SEL-12 protein to Notch activity, I will now discuss some important aspects of Notch signaling in invertebrates and mammals. The Notch receptor gene for intercellular signaling was first identified in Drosophila melanogaster and subsequently was found in all multicellular organisms from *C. elegans* to humans (Artavanis-Tsakonas et al. 1995). There is currently one member of this family known in Drosophila (Notch), two in *C. elegans* with considerably different functions (glp-1 and lin-12), and at least four in mouse and humans (Chan and Jan 1999). The Notch genes encode a large, single trans-membrane (TM) protein in the cytoplasmic membrane (Fig. 3). Notch function is involved in various signaling pathways, and mutations in the human Notch genes have been implicated in a variety of diseases, including leukemia, cervical and colon carcinomas, and stroke (CADASIL: cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) (Joutel et al. 1996).

Notch signaling is crucial for many steps in the development of an organ and organism. In the fly, Notch functions in a process termed “lateral inhibition” to single out particular cells in a group of identical precursor cells. For this purpose, the Notch receptor is bound by a ligand (Delta) provided by a neighboring cell and subsequently ensures, through the activation of a signaling pathway, that this cell acquires a fate distinct from that of its neighbors (Fig. 3). This signaling pathway is used in Drosophila, for example, during the generation of mechanosensory organs that sense body touch, but also functions in early embryogenesis and in wing development (Jan and Jan 1995).

A similar pathway is used in *C. elegans* at multiple steps in development, most notably to single out precursor cells involved in vulva differentiation (Kimble and Simpson 1997). For this purpose, two cells that are initially functionally identical cross-talk through the activity of the lin-12/Notch gene product. The consequence of this interaction is that one cell will specialize in producing vulva cells, the other will specialize in generating uterine cells. Even without going into too many details, it is probably easy to imagine that any perturbation of such a lin-12 mediated cross-talk would significantly influence further development of both the uterus and the vulva (Fig. 4). A reduction of lin-12/Notch function causes an egg-laying defect that results from failures in vulva induction. In contrast, a hyperactivity of lin-12/Notch, as induced by specific mutations in the extracellular domain of the receptor, results in the transformation of epidermal cells into additional pseudo-vulvae (Muv or multivulva phenotype) as a consequence of inappropriate signaling in the epidermal tissues (Seydoux and Greenwald 1989). This pronounced phenotype was used to identify suppressors of lin-12 hyperactivity (Levitan and Greenwald 1995). The *sel-12* mutants represent such extragenic suppressors of the Muv phenotype, suggesting that a reduced or absent *sel-12* function may reduce the constitutive signaling of the hyperactive lin-12 (Fig. 4). Taken together, the *sel-12* wild type function seems to be required in the lin-12/Notch signaling pathway and seems to facilitate Notch signaling. This finding was later corroborated by the mouse PS1 knock-out phenotype that resembles to some extent a Notch loss-of-function phenotype. Indeed, it was shown that Notch expression was strongly reduced in PS1 -/- mice (Shen et al. 1997; De Strooper et al. 1998; Wong et al. 1997) and similar results were obtained in Drosophila (Ye et al. 1999).

Several proteases are involved in Notch maturation and function (Kidd et al. 1998; Schroeter et al. 1998; Lecourtois and Schweisguth 1998; Struhl and Adachi 1998) (Fig. 3). During its transport to the cellular membrane, Notch is cleaved in the trans-Golgi network by a furin protease, resulting in heterodimerization of the amino-terminus and the carboxy-terminus of the protein. Therefore, the membrane-bound Notch is a heterodimer,

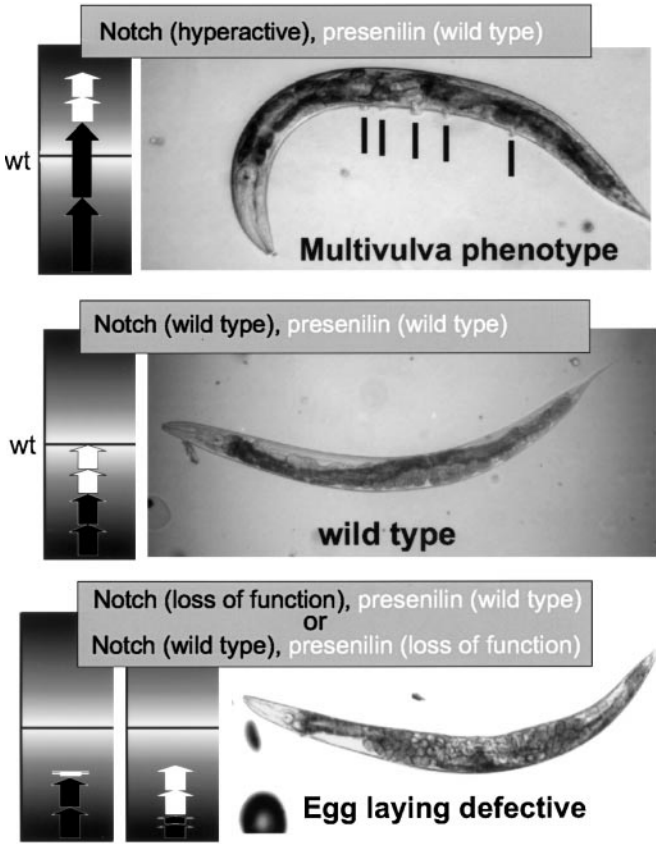

Fig. 4 LIN-12/Notch and SEL-12 activities contribute to Notch signaling. Schematic presentation of Notch signaling and the resulting phenotypes. In this diagram, the activities of Notch and SEL-12 are indicated by arrows. Middle: Wild type activities of LIN-12 (black arrow) and SEL-12 (white arrow) result in wild type signaling (white region of diagram, left side). The level of signaling is appropriate for correct vulva development and function (wild type animal at the right side). Top: Hyperactive Notch results in higher levels of signaling (dark region) causing a multivulva phenotype (indicated at right). This hyperactivity can be reduced by mutations in *sel-12* that reduce overall levels of signaling (data not shown). Bottom: Either a loss of *sel-12* or of lin-12 function reduces Notch signaling (dark region), resulting in an egg-laying defect (right side)

which can now be activated by interactions with its ligand, another trans-membrane protein called Delta. Delta is probably also processed via a proteolytic cleavage that releases the amino-terminus into the extracellular space. The metalloprotease Kuzbanian has been implicated in this step (Blaumueller et al. 1997). Upon binding of Delta to Notch, Notch is activated, although the details of this activation have not been fully resolved. It had been suspected that a first step involves reinternalization of the receptor-ligand complex, for which a dynamin protein encoded by the Drosophila shibire gene is required (Seugnet et al. 1997). A crucial step of the activation process involves the release of the intracellular domain (NotchIC) into the cytosol. NotchIC then shuttles into the nucleus and, together with transcription factors, directly regulates gene expression (Lecourtois and Schweisguth 1998; Struhl and Adachi 1998).

As we have already seen, the *C. elegans* presenilin *sel-12* faciliates Notch signaling. In order to determine where in the Notch pathway *sel-12* acts, Levitan et al. (Levitan and Greenwald 1998) overexpressed a truncated form of Notch consisting exclusively of the carboxy-terminal part of the protein. Overexpression of this NotchIC gene alone resulted in dominant activation of the Notch signaling, and this form of hyperactivity could not be suppressed by *sel-12* mutants. This strongly suggests that *sel-12* functions at an earlier step in the Notch pathway and, based on its intracellular localization, may affect transport or activation of Notch. Very recently, several elegant studies showed that upon inactivation of presenilins in *C. elegans*, Drosophila, mouse, and cell culture, the processing of Notch is indeed strongly impaired (Wolfe et al. 1999; Ye et al. 1999; Struhl and Greenwald 1999; De Strooper et al. 1999).

Are presenilins proteases?

Thus, both results from mouse and *C. elegans* indicate that presenilins are involved in the Notch receptor mediated signaling between adjacent cells. Where is the connection to Alzheimer's Disease? A functional link between APP and Notch receptor was first suggested, when several groups reported that inhibition of PS1 function did not only prevent APP processing by gamma-secretase, but also prevented the cleavage of the Notch C-terminus in the membrane. When Notch is activated, this cleavage results in the release of NotchIC and is a requirement for signal transduction (Struhl and Adachi 1998). This result immediately suggested that either presenilins are directly involved in cleaving both Notch and APP or mediate both cleavages in a more indirect way. Both the Notch and the APP cleavage can be prevented by mutating two transmembrane aspartate residues in TM6 and TM7 in presenilin 1 (Wolfe et al. 1999) and in PS2 (Steiner et al. 1999). In addition, these presenilin variants are also non-functional in *C. elegans* (Brockhaus et al. 1998; Steiner et al. 1999). Both aspartate residues are highly conserved in various presenilins across the animal kingdom (Baumeister and Haass 1998). Taken together, these results suggested that the identity of both residues is a crucial requirement for presenilin function, both in vitro (cell culture) and in vivo (*C. elegans*). It was, therefore, suggested that presenilins resemble aspartyl proteases in various aspects (Wolfe et al. 1999). Based on pharmacological data and on molecular modeling, it was also proposed that the APP cleaving gamma-secretase is an intramembrane-cleaving aspartyl protease (Wolfe et al. 1999). It remains to be determined whether presenilins are directly involved in Notch and APP processing (as proteases) or have a more indirect role, facilitating the transport and sorting of proteins into cellular compartments in which processing takes place. Results from the *C. elegans* presenilin *spe-4* (L'Hernault and Arduengo 1992; Arduengo et al. 1998), however, suggest that the latter may be more likely.

It is noteworthy to emphasize that the functional consequence of mutating the aspartate residues in the cell cul-

ture system results in an opposite effect as compared to the FAD mutations: whereas expression of FAD mutations raises the level of Aβ42, presenilin variants in which these aspartates are mutated are dominant negative and behave like a presenilin knock-out. This suggests that the FAD mutations reveal a dominant-negative, pathological activity (increase of Aβ42) that is different from the wild type activity. The biological activity (and its modulation) by FAD mutants can be analyzed in the *C. elegans* model; however, there is currently no evidence for a pathological activity of these variants in *C. elegans* as seen in human cells. This may be due to the fact that a true APP homologue is missing in *C. elegans*. Although the *C. elegans* genome contains an APP related gene (*apl-1*; (Daigle and Li 1993)), this does not contain the Aβ sequence and therefore cannot produce toxic Aβ aggregates. However, overexpression studies of human APP and Aβ42 in *C. elegans* clearly indicate that the biochemistry of aggregation can be also studied in *C. elegans* (Link 1995; Fay et al. 1998), suggesting that the genetic determinants of Aβ aggregation (except for the Aβ molecule) are encoded in the nematode genome. It will be very interesting to see whether the generation of human Aβ42 deposits in *C. elegans* can be influenced by mutations in *sel-12* and/or *hop-1*.

A role for presenilins in nervous system function?

The biological role of the presenilins in the nervous system is unknown. Although the PS1 -/- mice develop hemorrhages of the brain during embryonic development, any biological consequences of functional defects in neurons, except for their effect on APP processing, are still speculative (Shen et al. 1997; Wong et al. 1997; De Strooper et al. 1998). The *C. elegans* presenilin *sel-12* is also strongly expressed in the nervous system. *C. elegans* should be ideally suited to analyze neural functions of presenilins, since the complete anatomy of the nervous system is known and the wiring of all neurons has been analyzed (White et al. 1986). In addition, a large repertoire of behavioral abnormalities can be linked to the malfunction/absence of particular neurons. However, until now no detailed analysis of neural defects has been reported for *C. elegans sel-12* mutants. The gross morphology, at least, does not seem to be affected in *sel-12* mutants.

Presenilins may cooperate in Notch signaling

How many presenilins does a particular genome need? In Drosophila and Zebrafish, one presenilin has been identified so far (Boulianne et al. 1997; Archer et al. 1998). Deletion of the Drosophila presenilin results in embryonic lethality, underscoring the importance of the gene (Ye et al. 1999). The human genome encodes at least two presenilins, PS1 and PS2, and in *C. elegans* three genes which encode proteins with significant sequence similarity to PS1 and PS2 are known. The third *C. elegans* presenilin, *hop-1*, was identified by the *C. elegans* genome sequencing project. Knock-out experiments with RNAi and the isolation of a large deletion in *hop-1* surprisingly did not reveal any strong phenotype (Li and Greenwald 1997; Westlund et al. 1999). Similarly, the mouse PS2 knock-out also did not result in a phenotype, whereas the inactivation of PS1 caused embryonic lethality (Steiner et al. 1999). This suggests that presenilins do not function redundantly in one organism, but rather that one factor is more crucial than the other. This could simply be the consequence of the significantly different levels of expression. Whereas PS1 is highly expressed, the PS2 expression levels are about a factor of 10 weaker. This would also explain why in *C. elegans*, both PS and PS2 can rescue the *sel-12* mutant phenotype, when expressed from the *sel-12* promoter. At least some synergy of presenilin function may be concluded from the double knock-out experiments in *C. elegans*: deletion of both *sel-12* and *hop-1* resulted in lethality not seen in either of the two single mutants (Westlund et al. 1999). The consequence of a double knock-out of PS1 and PS2 in mouse has not been reported yet. Based on the *C. elegans* results, I would propose that the respective deletion of both PS1 and PS2 would result in embryonic lethality, resembling the lethality observed in Notch -/- animals. Taken together, these data suggest that although presenilins may be involved in rather similar functions, they also reveal clearly distinct aspects of activity that have not been addressed experimentally.

Conclusion

A tremendous amount of information about presenilins, their functions, and malfunctions (resulting in AD) has accumulated in four years since the cloning of the human presenilins. Much data about the function of presenilins are derived from the *C. elegans* model and many new details will pop up as soon as the excellent genetic tools available for *C. elegans* (but also for Drosophila) are used to identify the first genetic modifiers. It will, for example, be extremely interesting to identify genetic suppressors of the *sel-12* egg-laying defect, which may identify new components of the functional pathway that the presenilins are involved in. The rapidly proceeding human genome sequencing project can then be exploited to identify and isolate human homologues of these genes. Recent results have already shown that invertebrate model organisms are very useful in understanding human gene functions, or, to cite Friedrich Nietzsche: “You have made your way from worm to man, but much in you is still worm” (F. Nietzsche, Thus spoke Zarathustra).

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