Developmental control of Presenilin1 expression, endoproteolysis, and interaction in zebrafish embryos

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Developmental control of Presenilin1 expression, endoproteolysis, and interaction in zebrafish embryos

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Abstract

Dominant mutations in presenilin1 (PS1) and presenilin2 (PS2) are a major cause of early-onset Alzheimer’s disease. In this report we analyze the expression of the zebrafish presenilin1 (Psen1) and presenilin2 (Psen2) proteins during embryogenesis. We demonstrate that Psen1 and Psen2 holoproteins are relatively abundant in zebrafish embryos and are proteolytically processed. Psen1 is maternally expressed, whereas Psen2 is expressed at later stages during development. The Psen1 C-terminal proteolytic fragment (CTF) is present at varying levels during embryogenesis, indicating the existence of developmental control mechanisms regulating its production. We examine the codendency of Psen1 and Psen2 expression during early embryogenesis. Forced overexpression of psen2 increases expression of Psen2 holoprotein, but not the N-terminal fragment (NTF), indicating that levels of Psen2 NTF are strictly controlled. Overexpression of psen2 did not alter levels of Psen1 holoprotein, CTF, or higher molecular weight complexes. Reduction of Psen1 activity in zebrafish embryos produces similar developmental defects to those seen for loss of PS1 activity in knockout mice. The relevance of these results to previous work on presenilin protein regulation and function are discussed. Our work shows that zebrafish embryos are a valid and valuable system in which to study presenilin interactions, regulation, and function.

Keywords: Presenilin; Zebrafish; Embryo development; Somitogenesis; Notch; Alzheimer’s disease

Introduction

Alzheimer’s disease (AD) is a common, devastating neurodegenerative disorder of the brain. Accumulation of different forms of β-amyloid peptide (Aβ) in extracellular plaques is an early necessary step in the pathogenesis of the disease. Early-onset familial AD (FAD) has been linked to dominant mutations in the genes encoding amyloid precursor protein (APP), presenilin1 (PS1), and presenilin2 (PS2). These mutations are thought to alter the proteolytic processing of APP, resulting in overproduction of the highly amyloidogenic 42-amino-acid residue (aa) form of the amyloid-β peptide (Aβ) (for reviews, see [1,2]). Two proteases, the β- and γ-secretases, are responsible for the generation of Aβ. β-Secretase has been identified as a membrane-bound aspartyl protease, whereas the presenilins (PSs) have been linked to γ-secretase activity [3,4]. The PS proteins are thought to be involved in the normal proteolytic cleavage of the C-terminal fragments of APP and are speculated to be the actual γ-secretase [5].

The PSs are highly conserved hydrophobic multipass transmembrane (TM) proteins. Previous analyzes suggest that they possess eight TM domains [6,7]. They are found predominantly in the endoplasmic reticulum and Golgi apparatus [8–10], but they have also been detected in the plasma membrane, in the nuclear envelope, and in centrosomes and kinetochores [11–14]. The PS holoproteins are proteolytically cleaved to form N-terminal fragments (NTF) and C-terminal fragments (CTF) that assemble into a heterodimer [10,15]. It is suggested that these endoproteolytic derivatives are the functional units of PSs, based on evidence that the PS1 NTF and CTF accumulate to 1:1 stoichiometry in cell cultures and that there is a highly regulated and saturable level of PS derivatives [16,17]. Further, cross-
linking and coimmunoprecipitation data show that the NTF and CTF of either PS1 or PS2 can be coisolated [18]. Levitan et al. [19] have demonstrated that coexpressed PS1 NTF and CTF participate in APP processing and Notch signaling (see below), indicating that the PS1 fragments are the active components of the PS1 protein. Nevertheless, these authors also report that other components are necessary for full activity. Indeed, the presenilin derivatives have been shown to assemble into higher molecular weight (HMW) complexes [20] in which PS1 is associated with nicastrin [21].

There is accumulating evidence that the presenilins physically interact with the Notch signaling pathway [22,23]. The Notch receptors are transmembrane proteins that interact with numerous signal transduction pathways and mediate embryonic cell fate decisions in both invertebrates and vertebrates (see [24] for a review). In vertebrates, Notch receptors are best known for their function in neurogenesis and the process of somitogenesis (for reviews, see [25,26]). In mouse embryos lacking Notch activity, excess neurons differentiate and the formation of boundaries between somites becomes irregular [27–29]. Mice lacking activity of both the PS1 and PS2 genes show somite disorganization similar to the Notch1 phenotype, whereas the phenotype of mice lacking only PS1 is less severe, and mice lacking only PS2 apparently develop normally [30–32].

The molecular mechanisms of Notch signaling are highly conserved in evolution. Notch receptor activation is thought to be initiated by binding of an extracellular ligand leading to proteolytic cleavage within the transmembrane domain. This is followed by translocation of the intracellular domain of Notch (NICD) to the nucleus and subsequent association with a CSL protein leading to activation of downstream genes that regulate cell differentiation [33]. A mutation of the intramembrane processing site of murine Notch1 was shown to mimic the Notch1 loss of function phenotype in mouse embryos, demonstrating that regulated intramembrane proteolysis of Notch1 is essential during embryogenesis [34]. Studies using presenilin-deficient mammalian cell cultures have shown that presenilins are obligatory for processing of Notch1 to release its intracellular domain [35–37] and indicate that γ-secretase activity is responsible for both APP and Notch processing [38–40].

The early embryos of zebrafish are numerous, large, easily accessible, and easy to manipulate genetically. They represent an excellent system for analysis of early biochemical and molecular biological events during embryogenesis that may be more difficult to investigate in mammalian systems. Zebrafish have become a popular model for investigation of human genetic diseases (e.g., [41]). Zebrafish possess two presenilin genes orthologous to the mammalian presenilins [42,43]. To test whether zebrafish embryos might serve to investigate presenilin function we set out to characterize the early embryological effects of changes in presenilin protein expression and to compare these to what is known about presenilin function in mice. We observed that Psen1 is maternally expressed, whereas Psen2 is expressed after zygotic transcription has begun. In addition, these proteins show varying levels of holoprotein and endoproteolytic fragment expression during development. Previous reports have found that forced high-level of expression of human PS2 inhibits the synthesis of murine PS1 NTF and CTF. In contrast, we do not detect any decrease in Psen1 CTF levels after overexpression of pse1. Furthermore, we show that zebrafish embryos microinjected with morpholino antisense oligonucleotides to inhibit pse1 mRNA translation exhibit defects similar to those seen in PS1 knockout mice. We discuss the implications of these results for the relevance to human biology of research on presenilin function in zebrafish.

**Materials and methods**

**Animal ethics**

This work was conducted under the auspices of The Animal Ethics Committee of The University of Adelaide.

**Production of antibodies**

A synthetic peptide corresponding to the C-terminal large loop epitope VDHQQHQLPGMOSPTE (aa328–342) of zebrafish Psen1 and a peptide corresponding to the N-terminal epitope PSYNQDNAMSLPQDT (aa26–41) of zebrafish Psen2 were conjugated with diphtheria toxoid through a cysteine residue added at the C-terminus of the Psen1 peptide and at the N-terminus of the Psen2 peptide. The peptides were used to immunize sheep and the serum was affinity purified using a Thiol Sepharose 4B column (Amersham Biosciences Ltd, London, UK) to which the high-performance liquid chromatography purified synthetic peptides were coupled following the manufacturer’s instructions.

**Western blot analyses**

Dechorionated and deyolked embryos were placed in sample buffer [2% sodium dodecyl sulfate (SDS), 5% β-mercaptoethanol, 25% v/v glycerol, 0.0625 M Tris–HCl (pH 6.8), and bromphenol blue], heated immediately at 100°C for 2 min, and then stored at −20°C prior to separation on 12% SDS–polyacrylamide gels. (We noted that failure to heat embryos immediately after dissection resulted in considerable presenilin protein degradation, especially for embryos at later stages of development.) Proteins were transferred to nitrocellulose membranes using a semi-dry electrotransfer system. For Western blotting with anti-

1 Renamed from zfPS1 [42] and Pre2 [43] following recommendations from the Zebrafish Nomenclature Committee.
Psen1-antibodies, the membranes were blocked with 5\% goat serum in phosphate-buffered saline containing 0.1\% Tween 20 (PBT) for 3–4 h at room temperature and incubated with primary antibodies (1/5000 in PBT containing 2\% goat serum) o/n at 4°C. Filters were washed four times each for 15 min in PBT and incubated for 1 h in a 1/30,000 dilution of donkey antiseep IgG coupled to horseradish peroxidase (Jackson ImmunoResearch Laboratories, Inc., Baltimore, PA, USA) in PBT containing 2\% v/v goat serum. For Western blotting with anti-Psen2 antibodies, the membranes were blocked with 5\% w/v skim milk powder in PBT, incubated with a 1/1,000 dilution of primary antibodies in PBT containing 2\% w/v skim milk, washed in PBT, and incubated with a 1/20,000 dilution of donkey antiseep IgG (Jackson ImmunoResearch Laboratories, Inc.). For incubation with anti-\(\beta\)-tubulin antibodies (Antibody E7, Developmental Studies Hybridoma Bank, The University of Iowa, IA, USA), the conditions were the same as for Western blotting with anti-Psen1 antibodies except that the primary antibodies were diluted 1/200 and donkey antimouse IgG secondary antibodies (Jackson ImmunoResearch Laboratories, Inc.) were diluted 1/3,000. After incubation with secondary antibodies all the membranes were washed four times in PBT and visualized with luminol reagents (Sigma, St. Louis, MO, USA) by exposure to X-ray films (X-Omat-AR, Kodak Ltd, London, UK).

**Generation of expression plasmids**

Generation of the zebrafish psen2 cDNA was described in Groth et al. [43]. For cloning into the mammalian expression vector pCS2+ (kind gift of D. Turner, University of Michigan), the psen2 insert was excised from the Bluescript KS+ vector using XbaI and HindIII and amplified by polymerase chain reaction (PCR) using the following primers: 5’-CATCGATTCATCAGATTAAAGTTTCTC-3’ and 5’-GGCCTGTAAAAGTCTGTCAGATGTAG-3’. The PCR product was cloned as a Clal–Stul fragment into pCS2+. As a control, a psen2 expression vector containing a stop codon mutation close to the open reading frame was generated by PCR amplification using the following primers: 5’-TTCTTCTCATGGAATACCTAATGAGCTCGAGATC-3’ and 5’-GAGAGTCGCTCTGCATTTGTTATA-3’. The Quick Change site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA).

**RNA and morpholino injections**

RNA was transcribed from the pCS2+ expression vector containing the psen2 construct or the psen2 mutant construct using the mMessage mMachine kit (Ambion Inc., Austin, TX, USA). Approximately 1 nl of RNA at a concentration of 100 \(\mu\)g/\(\mu\)l was injected into one-cell embryos (for Western analysis) or into one cell of two-cell-stage embryos (for *in situ* transcript hybridization analysis). Morpholino oligonucleotides were obtained from Gene Tools (LLC, Corvallis, OR, USA). Two different anti-psen1 morpholinos were designed to block Psen1 translation by binding to the 5’ UTR or coding sequence, including the initiation methionine of psen1 mRNA, based on the manufacturer’s recommendations. The morpholino sequences were as follows: *MoPsen1A*, 5’-CAGCATTCTGCACTAAAATCAGCAT-3’; and *MoPsen1B*, 5’-ACTAAACCATCGCCATCGGAACTGTA-3’. The standard control morpholino sequence, also from Gene Tools, was as follows: 5’-CTCTTACCTAGTTACAAATTTATA-3’. Morpholino oligonucleotides were dissolved in distilled water at a concentration of 2 \(\mu\)g/\(\mu\)l and diluted to 1 \(\mu\)g/\(\mu\)l prior to injection. Oligonucleotides were injected into two-to four-cell stage embryos and incubated at 28.5°C until the desired developmental stages.

**In situ transcript hybridization on whole-mount zebrafish embryos**

Embryos were raised at 28.5°C and staged as previously described [44]. *In situ* transcript hybridization was performed as described [45] using single-stranded RNA probes labeled with digoxigenin-UTP (Roche Ltd, Basel, Switzerland). Riboprobes against *psen1*, *her1*, and *myoD* transcripts were synthesised from cDNA clones in the Bluescript SK vector (Stratagene) using T7 RNA polymerase after PCR amplification with M13 and M13R primers.

**Results**

**Expression of Psen1 in early-stage zebrafish embryos**

To examine the expression of zebrafish Psen1 protein during embryogenesis, zebrafish embryos at different stages of development were examined. Embryos at the one-cell stage (0.5 h postfertilization, hpf, at 28.5°C) and shield stage (6 hpf) and near the start and end of somitogenesis (12 and 24 hpf) were analyzed by Western blotting using an affinity-purified polyclonal anti-Psen1 antibody (AbPsen1). This antibody preparation is directed against residues 328–342 of the Psen1 C-terminal loop region. AbPsen1 detected a ~43-kDa holoprotein and a lower molecular weight C-terminal Psen1 derivative of between 25 and 32.5 kDa (Fig. 1). Both the Psen1 holoprotein and this C-terminal fragment show similar molecular weights to those previously reported for zebrafish [42]. In addition to the Psen1 CTF and holoprotein, Western blots revealed HMW aggregates containing Psen1. The appearance of the HMW complexes is consistent with previous observations [20]. These complexes can withstand SDS-PAGE [46]. The specificity of the Western blot signals was shown by competition experiments using the peptide against which the antibody was raised (Fig. 1, lane 5).

The Psen1 holoprotein, CTF, and the HMW complexes
expression of Psen2 in early-stage zebrafish embryos.

Overexpression of Psen2 and effects on Psen1 levels

Overexpression of human PS1 in mouse cell lines and transgenic mice is followed by a decrease in the steady-state levels of murine PS1 and PS2 derivatives. Likewise, the levels of PS1 derivatives are lowered in cultured cells overexpressing human PS2 [16]. The mechanisms underlying these findings are unknown although a model exists whereby the PSs are processed by limiting cellular factors. To investigate if this regulatory regime can be observed in our model system, we injected psen2 mRNA into zebrafish embryos at the one-cell stage, allowed them to develop until 6 hpf, and then analyzed the levels of Psen1 and Psen2 on Western blots. To control for any unrelated effects of mRNA injection, we also observed the effects of injection of a mutant psen2 mRNA containing a stop codon close to the start of the open reading frame. (This mRNA encodes a short nonfunctional oligopeptide and its injection produced no effects on presenilin protein levels, data not shown.) Experimental results were confirmed by complete replication at least twice. Consistent with the results of Thinakaran et al. [16], embryos injected with psen2 mRNA showed greatly increased expression of the Psen2 holoprotein. However, in contrast, we did not see an increased level of Psen2 NTF (Fig. 2A, Lane 2). Furthermore, the level of endogenous Psen1 holoprotein, CTF, and HMW complexes was not altered in these embryos (Fig. 2B, lanes 1 and 2).

We also analyzed the transcriptional activity of psen1 in zebrafish embryos overexpressing psen2. To achieve this we overexpressed psen2 unilaterally in embryos by microinjection of psen2 mRNA into one cell at the two-cell stage of development. The embryos were then fixed at 12 hpf and whole-mount in situ hybridization was performed using an antisense psen1 riboprobe. The levels of psen1 transcripts on the psen2-injected side were unaltered compared to those on the control sides of these embryos (data not shown). Thus, Psen2 activity does not appear to regulate transcription of psen1 in early zebrafish embryos.
zebra and mice, we designed two anti-Psen1 activity in zebra transcript translation. To examine whether reduction of morpholino oligonucleotides (morpholinos) that block bra morpholinos, targeted reduction of gene activity in zebrafish embryos has the same effect as in mice, we designed two anti-psen1 morpholinos, MoPsen1A and MoPsen1B, directed against nonoverlapping regions of the psen1 transcript. These were injected into fertilized zebrafish eggs at the two- to four-cell stages during which morpholinos can rapidly spread into all cells. To confirm reduction of Psen1 activity, we examined extracts taken from these embryos at 16 hpf on Western blots. The Psen1 CTF was greatly reduced in embryos injected with either MoPsen1A or MoPsen1B compared to uninjected embryos or embryos injected with a nonfunctional, control morpholino. Interestingly, Psen1 holoprotein and the HMW complexes persist in the morpholino-injected embryos. Presumably, the Psen1 holoprotein and the HMW complexes are already present when morpholino injection occurs. Alternatively, the apparent persistence of the Psen1 holoprotein and the HMW complexes may be due to continued translation of a Psen1 isoform(s) derived from an alternatively spliced mRNA(s) that does not bind our morpholino oligonucleotides.

Fig. 2. Overexpression of Psen2 does not inhibit synthesis of Psen1 in zebrafish embryos at 6 hpf. (A) Protein extracts from 20 psen2 mRNA-injected zebrafish embryos (lane 2) or 20 uninjected embryos (lane 1) were analyzed by immunoblotting using anti-Psen2 antibodies. Psen2 holoprotein is indicated. (B) Protein extracts from five psen2 mRNA-injected zebrafish embryos (lane 2) or five uninjected embryos (lane 1) were analyzed by immunoblotting using anti-Psen1-antibodies. (C) Western analysis of β-tubulin concentration in each sample as a gel loading control (protein from five embryos per lane).

Anti-psen1 morpholino injections disrupt somite formation

Targeted inactivation of the PSI gene in mice results in malformation of the axial skeleton and ribs. These abnormalities are preceded by mild defects in somite formation that are characteristic of mice heterozygous for null mutations in genes that contribute to the Notch signaling pathway [30,31,54]. Targeted reduction of gene activity in zebrafish embryos is possible by injection of antisense morpholino oligonucleotides (morpholinos) that block transcript translation. To examine whether reduction of Psen1 activity in zebrafish embryos has the same effect as in mice, we designed two anti-psen1 morpholinos, MoPsen1A and MoPsen1B, directed against nonoverlapping regions of the psen1 transcript. These were injected into fertilized zebrafish eggs at the two- to four-cell stages during which morpholinos can rapidly spread into all cells. To confirm reduction of Psen1 activity, we examined extracts taken from these embryos at 16 hpf on Western blots. The Psen1 CTF was greatly reduced in embryos injected with either MoPsen1A or MoPsen1B compared to uninjected embryos or embryos injected with a nonfunctional, control morpholino. Interestingly, Psen1 holoprotein and the HMW complexes persist in the morpholino-injected embryos. Presumably, the Psen1 holoprotein and the HMW complexes are already present when morpholino injection occurs. Alternatively, the apparent persistence of the Psen1 holoprotein and the HMW complexes may be due to continued translation of a Psen1 isoform(s) derived from an alternatively spliced mRNA(s) that does not bind our morpholino oligonucleotides.

her1 expression oscillates within the cells of the pre-somatic mesoderm (PSM) such that waves of expression emerge from the tailbud and progress through the PSM in a caudal-to-rostral direction. The most anterior wave fades just before morphological somites can be distinguished, and, at the same time, new waves of expression emerge from the tailbud [56]. Notch pathway signaling is required for her1 oscillation [57]. Notch receptor signaling requires presenilin activity for the cleavage and release of the active intracellular domain of the receptor [34]. To determine whether reduction of Psen1 activity affects Notch signaling in zebrafish embryos, we examined the pattern of her1 transcription during active somitogenesis (16 hpf) after injection of MoPsen1B morpholinos. As expected, loss of Psen1 activity resulted in loss of waves of her1 expression. The stripes of her1 expression became nearly uniform throughout the PSM (Fig. 3.89% of embryos, n = 18). This is consistent with the uniform her1 expression seen in embryos homozygous for the mutations deadly seven (des)/Notch1 and after eight (aei)/DeltaD [57]. We also analyzed myoD expression in MoPsen1B-injected embryos. This gene is expressed segmentally in the somitic mesoderm of zebrafish and so examination of myoD expression allows easy visualization of defects in somite formation. In MoPsen1B-injected embryos the somitic domains of myoD expression were less extensive laterally, less regular, and less well organized (Fig. 4, 96%, n = 24) than in un.injected or control morpholino-injected embryos. These changes in myoD expression contrast with the effects seen in Notch1 and DeltaD mutants, where only the first seven to nine somites are formed [58]. However, our somitic phenotype is consistent with that seen in mice lacking PSI1 activity that have a milder phenotype than Notch1 null mutant mice due to the partially redundant activity of murine PS2 [32]. Like mice, zebrafish embryos lacking Psen1 activity also show defective brain development (data not shown).

Fig. 3. Reduction of Psen1 protein in immunoblots of Anti-psen1 morpholino-injected zebrafish embryos. (Lane 1–4) Detection of Psen1 in protein extracts of 16 hpf embryos (5 embryos per lane). (Lane 1) Uninjected embryos. (Lane 2) Embryos injected with standard control morpholino. (Lane 3) Embryos injected with MoPsen1A morpholinos. (Lane 4) Embryos injected with MoPsen1B morpholinos.
Discussion

The eggs of zebrafish are large and, unlike mammals, are fertilized externally. Thus, zebrafish embryos are easily accessible from the earliest stages of development. Because their food reserve is limited, these embryos do not grow extensively during development. This allows direct comparison of protein concentrations at different developmental stages simply by comparing extracts from equal numbers of embryos. The large embryos of zebrafish are also very amenable to forced overexpression or repression of gene expression by injection of mRNA or morpholino antisense oligonucleotides respectively. Since zebrafish are vertebrates and have presenilin proteins very similar to those in humans, we sought to characterize the expression and interactions of these proteins using zebrafish embryos.

We have shown previously that, despite the ubiquitous presence of psen2 mRNA from fertilization onward, Psen2 protein cannot be observed until after the onset of gastrulation. Thus, Psen2 expression is probably under posttranscriptional control [43]. Production of a polyclonal antibody against the C-terminal of Psen1 has allowed us to examine its developmental expression. Unlike Psen2, Psen1 protein can be detected before activation of the zygotic genome. However, Psen1 is similar to Psen2 in that the absolute levels of these proteins change during development. Thus, total Psen1 levels are lowest during early cleavage stages and steadily increase during embryogenesis up to 24 hpf.

Our observations indicate that presenilin activity is, apparently, regulated at the level of holoprotein endoproteolysis and/or the stability of the proteolytic fragments. During early development the level of Psen1 holoprotein appears constant but the relative concentrations of the CTF and the HMW complexes vary. This argues against an autocatalytic mechanism of presenilin endoproteolysis as suggested by Wolfe et al. [59]. Alternatively, the maternally provided Psen1 holoprotein may show differential stability relative to zygotically synthesized Psen1 or an alternatively spliced form of psen1 mRNA may exist encoding a holoprotein isoform not subject to endoproteolysis. Indeed, Psen1 ho-
holoprotein apparently persisted after blockade of translation of pse
l mRNA despite the loss of the CTF. Also, the phenotype produced by loss of the CTF following morpholino antisense oligonucleotide injection suggests that the holoprotein and HMW complexes have insufficient γ-secretase activity to facilitate Notch signaling in the embryo.

The relative stability of the Psen1 and Psen2 holoproteins is in contrast to observations using cultured mammalian cells and transgenic mice by Thinakaran et al. [47]. These authors reported that the holoproteins are immediately processed into N- and C-terminal fragments. In transfected cells, PS1 holoprotein has been shown to have a short half-life, whereas the cleaved derivatives are stable for over 12 h [10,60]. It has been suggested that excess uncleaved PS1 holoprotein is degraded [15,61]. However, apparently more stable presenilin holoproteins have been observed in other in vivo systems [49–51,53]. The fact that we have been able to observe changes in the ratio of the holoproteins and the proteolytic fragments over developmental time suggests that presenilin activity may be dynamically regulated in cells via protein stability, in particular the endoproteolysis of the holoprotein to the NTF and CTF. This would explain the wide variety of observations seen in many laboratories working with a variety of experimental systems.

Previously it has been assumed that presenilin NTF and CTF levels are coordinately regulated through the endoproteolytic cleavage event that generates the active, heterodimeric protein. However, recent data shows that the regulation is more complex. Kirschbaum et al. [62] reported that, after proteolytic cleavage of mammalian PS1 holoprotein, the level of CTF is regulated by glycogen synthase kinase-3β (GSK-3β). In addition, they found that PS1 CTF is produced in excess and that surplus heterodimer is degraded following its forced generation. Furthermore, cyclin-dependent kinase-5/p35 has also been found to regulate PS1 CTF levels [63], whereas casein kinase I and II regulation of PS2 has been reported by Walter et al. [64]. We observed that the concentration of Psen1 CTF appears to be regulated throughout zebrafish development independently of holoprotein concentration. This suggests that control mechanisms other than simple regulation of holoprotein cleavage are responsible for varying the concentration of the endoproteolytic fragments during development and supports the conclusion of others that the amounts of NTF and CTF are not regulated by proteolytic cleavage of the PS holoproteins. The relatively high levels of presenilin proteins in zebrafish embryos may ensure an adequate source of active fragments during the rapid cell divisions that occur during their early development.

We found that overexpression of pse
l in zebrafish embryos has no effect on Psen1 protein levels. This inability to see codependent regulation of Psen1 and Psen2 levels in developing embryos may be due to the tight developmental regulation of these proteins during early embryogenesis. We were unable to boost levels of Psen2 NTF despite a sever-

alfold increase in Psen2 holoprotein after mRNA injection. This contrasts with the observations of Thinakaran et al. [16], who saw increases in PS1 and/or PS2 holoprotein and endoproteolytic fragment levels after induced transcription in transgenic mice and cell lines. These authors observed that limits exist to overexpression of the endoproteolytic fragments but not the holoprotein. However, it is apparent that these limits in fragment concentration lie far above the normal endogenous levels of these fragments. It may be that, in the early zebrafish embryo, tight regulation of Psen1 and Psen2 activities is achieved by expression of their proteolytic fragments at the maximal levels allowable by limiting cellular factors. That Psen2 holoprotein overexpression does not lead to downregulation of Psen1 levels is not surprising considering the dramatic changes in endogenous Psen2 protein levels that normally occur during early development. It is difficult to conceive of any advantage that would be conferred by codependent regulation of Psen1 and Psen2 levels during these dramatic changes in Psen2 expression. Our results suggest that the levels of endoproteolytic fragments of Psen1 and Psen2 are regulated independently in early zebrafish embryos.

Anti-pse
l morpholino-injected zebrafish embryos show defects in somitogenesis consistent with partial loss of Notch signaling in the presomitic mesoderm. Similar defects are seen in PS1 knockout mice, confirming the orthologous activities of the murine PS1 and zebrafish pse
l genes. Expression of myoD is disrupted in embryos injected with morpholinos blocking pse
l mRNA translation. However, these embryos do not display the ubiquitous myoD expression seen in embryos treated with a γ-secretase inhibitor that is thought to block all presenilin activity [65]. This implies that, in zebrafish as in the mouse, there is some functional redundancy between Psen1 and Psen2.

Both PS1 and PS2 have been implicated in the control of cell fate decisions during mouse embryonic development through mediation of Notch1 receptor processing [34,66]. However, whereas PS1-deficient mice show disruption of somite formation and other developmental processes, similar effects have not been found in PS2-deficient mice, which appear to develop normally [30,32,66]. Nevertheless, redundancy in activity exists between the PS1 and PS2 genes because the PS1-deficiency defects are not as severe as those in mice lacking both presenilins [32]. Similarly, the defects that we have observed from loss of zebrafish Psen1 activity are not as severe as those seen in embryos treated with γ-secretase inhibitor [65] which closely resemble the Notch1-deficiency phenotype. Thus, loss of presenilin1 activity in mice and zebrafish does not completely abolish Notch signaling. These parallels in presenilin activity in the mouse and zebrafish systems further validate the use of zebrafish embryos to investigate human disease genes.
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