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Review

Zebrafish as a tool in Alzheimer's disease research ☆

Morgan Newman [a,1], Giuseppe Verdile [b,1], Ralph N. Martins [b], Michael Lardelli [a,*]

[a] *Discipline of Genetics, School of Molecular and Biomedical Science, The University of Adelaide, SA 5005, Australia*
[b] *Centre of Excellence for Alzheimer's Disease Research and Care, School of Exercise, Biomedical and Health Sciences, Edith Cowan University, Joondalup, WA 6027, Australia*

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ABSTRACT

Alzheimer's disease is the most prevalent form of neurodegenerative disease. Despite many years of intensive research our understanding of the molecular events leading to this pathology is far from complete. No effective treatments have been defined and questions surround the validity and utility of existing animal models. The zebrafish (and, in particular, its embryos) is a malleable and accessible model possessing a vertebrate neural structure and genome. Zebrafish genes orthologous to those mutated in human familial Alzheimer's disease have been defined. Work in zebrafish has permitted discovery of unique characteristics of these genes that would have been difficult to observe with other models. In this brief review we give an overview of Alzheimer's disease and transgenic animal models before examining the current contribution of zebrafish to this research area. This article is part of a Special Issue entitled Zebrafish Models of Neurological Diseases.

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1. Alzheimer's disease

Alzheimer's disease (AD) is the most common form of neurodegenerative disease. Globally, the incidence of dementia was estimated in 2001 to be 24.3 million with an estimated increase to 81.1 million in 2040 [1]. Clinically, AD is characterised by progressive memory loss and can include impairment of speech and motor ability, depression, delusions, hallucinations, aggressive behaviour and, ultimately, increasing dependence upon others before death (recently reviewed by Voisin and Vellas [2]). The major neuropathological hallmarks of the disease were first described by Alois Alzheimer as “fibers” and “small military” foci (English translation, [3]) Today these are commonly referred to as neurofibrillary tangles (NFTs) and amyloid plaques. Neurofibrillary tangles are hyperphosphorylated forms of the tau protein, while the major protein component of amyloid plaques is a small peptide referred to as amyloid beta (Aβ). Other forms of lesion have also been found in AD brains (reviewed by Duyckaerts et al. [4]).

Alzheimer's disease can be classified broadly into two groups, late onset AD (LOAD, occurring >65 years) and early onset AD (EOAD, occurring <65 years). LOAD accounts for the vast majority of AD cases (95%) and is associated with many risk factors. It is well established that age and possession of the ε4 allele of the *APOLIPOPROTEIN* gene (*APOE ε4*) are the major risk factors for most of the population (reviewed by Martins et al. [5]). However, other risk factors such as hormonal changes associated with ageing, diet and lifestyle factors play very important roles. Cardiovascular risk factors such as hypercholesterolemia, increased LDL and reduced HDL levels, hypertension, obesity and type II diabetes make important contributions to the risk of developing AD (reviewed by Martins et al. [5]). Genome-wide association studies in humans have identified a number of possible additional loci associated with risk for LOAD (reviewed by Rademakers and Rovelet-Lecrux [6]. In particular, two recent studies identified overlapping sets of candidates (see [7,8] and Table1). Individuals carrying the *APOE ε4* allele also bear some risk of developing the early onset form of AD. Overall, EOAD accounts for only a small percentage of AD cases but is the most severe form of the disease. The majority of EOAD cases are familial forms of AD showing dominant inheritance.

Familial AD (FAD) is typically associated with an early age of onset as young as 25 years (reviewed by Verdile and Martins [20]). Most of our knowledge of the molecular events underlying the development of Alzheimer's disease comes from FAD since we can use genetic analysis to identify the genes and proteins involved. Mutations at only three loci are known to cause FAD although other loci may yet be found (see review by Rademakers and Rovelet-Lecrux [6]). One locus hosts the Amyloid Precursor Protein (APP) from which Aβ is cleaved by the action of two “secretases,” β-secretase and γ-secretase (see Fig. 1). Mutations in APP alter these cleavages so that Aβ production is increased or more aggregation-prone forms of Aβ are formed (reviewed by Verdile et al., [20]). The remaining two loci host the presenilin genes *PSEN1* and *PSEN2* with mutations in these genes accounting for 50–70% of EOFAD cases [21]. The presenilin proteins form the catalytic core of protein complexes with γ-secretase activity. Mutations in the presenilins also appear to alter the rate or form of Aβ production and many LOAD associated risk factors also regulate Aβ levels in the brain. Therefore, it is not surprising that the currently dominant hypothesis for the cause of FAD (and LOAD) is the “amyloid hypothesis” that postulates that toxic oligomerisation of Aβ is the initiating factor beginning a cascade of consequences such as synaptic

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* Corresponding author. Tel.: +61 8 83033212; fax: +61 8 83034362.
E-mail address: michael.lardelli@adelaide.edu.au (M. Lardelli).
[1] Made equal contributions to this paper.

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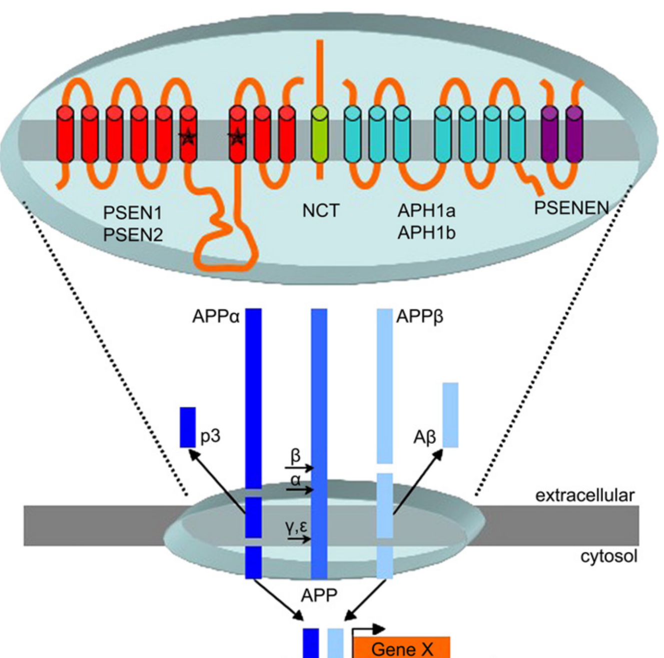

Fig. 1. The γ-secretase complex consists of four protein components including PSEN1 or PSEN2 that reside within lipid bilayers and cleave type 1 transmembrane domain proteins at a catalytic site dependent upon two aspartate residues (stars). APP is initially cleaved either by α- or β-secretase to produce APPα or APPβ. Only β-secretase cleavage permits Aβ peptide production after secondary cleavage by γ-secretase that also releases APP's intracellular domain that can enter the nuclear to contribute to gene regulation.

dysfunction, inflammation and oxidative damage causing neuronal dysfunction, degeneration and death (see review in [22]).

The amyloid hypothesis is an attractively parsimonious explanation for AD pathogenesis and finds support from numerous phenomena observed in EOFAD and LOAD brains. However, it fails to explain some observations in animal models and the outcomes of a number of human clinical trials of drugs intended to ameliorate Aβ toxicity. For example, the majority of transgenic mice harbouring FAD-causing mutations leading to accumulation of human Aβ in their brains do not show dramatic neurodegeneration [23] (see the discussion in the 2008 paper by Chen et al. [24]). However, these transgenic mice do show cognitive deficits in behavioural tests and show reduction in long-term potentiation, most likely indicating loss in synaptic activity [25]. To obtain significant neurodegeneration or neuronal loss in transgenic mice requires expression of three or more mutations in AD-associated genes. Examples of mouse AD models used currently include "3xTg-AD" mice bearing mutant alleles of *PS1*, *APP* and *MAPT* [26] and "5xFAD" mice bearing three *APP* and two *PS1* mutant alleles [27]. However, the co-existence of three or more mutations in AD-associated genes is not a situation that occurs in humans. A more physiologically relevant animal model is required that more closely mirrors human AD neuropathology including the observed neurodegeneration and neuronal loss (see the critical review by Buxbaum [28]).

A number of therapeutic agents aimed at reducing Aβ production or accumulation/aggregation have failed human clinical trials. These include peptide mimetics that inhibit Aβ aggregation (i.e., Alzhemed™,) or agents that either inhibit γ-secretase activity (e.g. LY-411,575 and LY-450,139) or target the production of the more neurotoxic Aβ42 (e.g. the NSAID, Flurizan™ and refer to www.alzforum.org regarding information on AD drugs in clinical trials). This has raised questions over the validity of the amyloid hypothesis. However, issues surrounding penetration through the blood–brain barrier and the advanced state of neurodegeneration when these drugs were given may explain their failure. Apparently successful therapeutics currently in phase II/III trials such as the immunotherapeutics Bapinezuimab™ and Solabezumab™ or agents directed at neutralising Aβ toxicity (i.e. PBT2) are addressing such issues (reviewed by Wisniewski, 2009 [29]). There are also therapies not directed at Aβ that show promise including the anti-histamine Latrepirdine (formerly known as Dimebon™) which has completed phase IIIa trials and is currently in IIIb trials [30]. The mechanism(s) underlying the benefits of Latrepirdine is unclear but may include improving mitochondrial activity. Another agent that has shown some promise is Rember™ (methylthionium chloride) which reduces tau aggregation (see http://www.taurx.com/) although this drug was not seen to affect the histological or behavioural phenotypes of transgenic zebrafish expressing mutant human MAPT (see 2009 ICAD conference report at www.alzforum.org/new/detail.asp?id=2203). Methylthionium chloride is better known to those working with zebrafish as methylene blue which is used to inhibit fungal growth and to stain the yellow pteridine-containing pigment cells of fish, xanthophores [31]. Recently, the combination of Dimebon (Latrepirdine) and methylene blue has also been shown to inhibit aggregation of TDP-43 [32], a protein component of ubiquitinated inclusions found in the neurodegenerative diseases amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) (reviewed by Mackenzie [33]). The beneficial outcomes of these non-Aβ directed agents may provide further evidence for alternatives to the amyloid hypothesis, However, it is conceivable that these agents may be acting to ameliorate the consequences of Aβ toxicity e.g. by protecting neurons from insults such as aggregation of Aβ or other proteins.

2. Presenilin activity is involved in many aspects of AD pathology

The presenilins proteins are known to interact with both the APP and tau proteins [34–38] providing one possible link between Aβ production and changes in tau phosphorylation. Mice lacking expression of both *Psen1* and *Psen2* in forebrain tissue show a number of phenotypic effects resembling Alzheimer's disease, i.e. neurodegeneration such as hyperphosphorylation of tau, formation of intracellular tau inclusions, gliosis, expansion of brain ventricles and neuronal death all in the absence of any Aβ production [24]. Some characteristics of Alzheimer's disease, such as increased formation of glia, may result from changes in presenilin activity since γ-secretase complexes cleave not only APP but also a large collection of other transmembrane proteins such as NOTCH, E-cadherin, neuregulin-1 and syndecan (reviewed in Ref. [39]). Presenilins also function in a number of cellular processes, at least some of which appear independent of γ-secretase activity. For example, Kang et al. [40] showed that mouse Psen1 represents a second pathway for phosphorylation of β-catenin independent of Axin. Presenilins also appear to act as Ca^{2+} leak channels in the endoplasmic reticulum [41]. Presenilins are also found in interphase kinetochores [42] (although this observation has not been replicated) and changes in presenilin activity can cause aneuploidy ([43,44]) and dysregulation of cell cycle proteins [12,45]. Indeed, the activity in γ-secretase of at least some of the proteins comprising these complexes does not appear to be their original function. Homologues of presenilins exist in plants and this kingdom does not display γ-secretase activity [46].

The great majority of the mutations causing FAD are found in *PSEN1*. Currently over 180 different missense mutations are known (data on mutations are collated at Alzheimer Disease and Frontotemporal Dementia Mutation Database, http://www.molgen.ua.ac.be/ADMutations/). There is also evidence that oligomeric forms of Aβ can feed back onto the activity of γ-secretase ([47]). Thus it is possible that mutations that cause production of more Aβ, or more aggregation-prone forms of Aβ, may affect presenilin function and that changes in presenilin function may then affect tau phosphorylation, cell differentiation (e.g. via Notch signalling), calcium homeostasis, etc.

3. Using zebrafish to study genes mutated in FAD

Mice are the dominant model used for modelling of Alzheimer's disease pathology (reviewed in Ref. [48]) but non-mammalian organisms have also

enlightened us. Mutation screening in the nematode, *Caenorhabditis elegans* first showed the connection between the presenilins and Notch signalling [49,50]. Expression of human Aβ in *C. elegans* [51] and *Drosophila* [52] has been seen to cause amyloid deposits in muscle and neurodegeneration respectively. Aβ toxicity can also be studied using yeast [53]. Use of these organisms has opened up possibilities for screening drug libraries to find compounds blocking Aβ toxicity.

As a model organism, zebrafish presents many advantages for the study of human disease. The vertebrate zebrafish genome and anatomy show only ~420 million years (Myr) of divergence from the human lineage [54] rather than the ~600 Myr of the ecdysozoans (*Drosophila* and *C. elegans*, [55]). Therefore, most human genes have clearly identifiable orthologues in zebrafish (however, a genome duplication early in the teleost (bony fish) lineage means that, in many cases, two zebrafish genes together perform the function of an orthologue). The numerous and externally fertilized embryos of zebrafish are easily amenable to methods for manipulating gene and protein activity such as injection of antisense oligonucleotides, mRNAs or transgenes and they can be arrayed in microtitre plates for screening of drug libraries. Cell labelling and transplantation for tracking the behaviour and interactions of living cells is also routine. A good indication of the potential utility of zebrafish for Alzheimer's disease research is the attempts to obtain patent coverage of wide areas of the field (e.g. WO/2006/081539).

Zebrafish possess genes orthologous to all the genes known to be involved in Alzheimer's disease (Table 1) The genes *appa* and *appb* [9] are duplicates of an ancestral teleost orthologue of human APP. The genes *psen1* [11] and *psen2* [16] are orthologues of human *PSEN1* and *PSEN2*, respectively. Our laboratory has also identified duplicates of an ancestral teleost orthologue of the *MAPT* gene encoding tau protein, *mapta* and *maptb* [56] (discussion of tau activities in zebrafish including the MAPT-based model of neurodegeneration of Paquet et al. 2009 [57] is to be found in an accompanying paper by Ed Burton).

The body of research literature examining the endogenous zebrafish *appa*, *appb*, *psen1* and *psen2* genes is still quite limited. Musa et al. [9] detected widespread and overlapping transcription of both *appa* and *appb* by whole mount *in situ* transcript hybridisation (WISH) from mid-gastrulation. Later in development these two genes show differing patterns of transcription but, notably, at 24 hpf both are expressed in the developing forebrain (telencephalon) and ventral diencephalon. In addition, *appb* is expressed in the ventral mesencephalon, a series of nuclei in the hindbrain and in the spinal cord. Joshi et al. [10] recently examined the effects of reduction of *appb* function through use of antisense morpholino oligonucleotides blocking translation (while they also attempted to reduce *appa* mRNA translation it is not clear that they were able to do this. Also, their *appb* analysis utilized only one morpholino so non-specific effects are not excluded). They observed defective convergent extension movements and reduced body length. Interestingly, injection into zebrafish embryos of mRNA encoding normal human APP was more effective at ameliorating the loss of *appb* translation than an Alzheimer's mutant form ("APP^{swe}" containing the "Swedish" double mutation K595N with M596L [58]). This demonstrates that zebrafish embryos can be exploited for analysis of differences in the activity of different mutant forms of APP [10].

Two papers examining transcriptional control of *appb* have been published [59,60]. Both emphasise the importance for expression of a regulatory element in the first intron of the gene. Approximately 14 kb of DNA from the promoter region and first intron of the gene can largely recapitulate the embryonic transcription pattern of *appb* [59].

Attempts to model Aβ toxicity have, so far, proven difficult and no studies have yet been published. The existence of transgenic fish expressing human APP^{swe} in neurons in zebrafish the under the control of the promoter of the *elavl3* gene (previously known as *HuC*) was reported at the 6th European Zebrafish Development and Genetics Meeting in Rome in July 2009 (Hruscha, Teucke, Paquet, Haass and Schmid) but there is no data yet on any toxic effects. In our laboratories we have followed a number of approaches to analysing Aβ toxicity. Simple incubation of zebrafish embryos in media containing Aβ peptide appears to produce effects on neural development as monitored by changes in the patterns of neurons observed in non-transgenic zebrafish. It can also induce cell and embryo death (Frederich-Sleptsova et al. manuscript in preparation). We also attempted to generate a transgenic zebrafish model to facilitate screening for drugs suppressing Aβ toxicity by expressing the 42 amino acid residue form of human Aβ ($Aβ_{42}$) in the melanophores (melanocytes) that constitute the zebrafish's dark surface stripes. The hope was to create a highly visible but nevertheless viable phenotype in zebrafish larvae that could then be arrayed in microtitre dishes for exposure to various chemical compounds. For this we used a DNA fragment from the promoter of the zebrafish gene *mitfa* (previously *nacre*) that can specifically drive transcription in zebrafish melanophores [61]. This was coupled to DNA coding for a secretory signal fused to $Aβ_{42}$. Unfortunately, fish bearing this transgene only showed a disturbed pigmentation pattern at 16 months of age—too late for use in drug screening [81].

Analysis of presenilin function in zebrafish has tended to focus on *psen1* due to the more frequent role played by mutations of its human orthologue, *PSEN1* in AD. Zebrafish *psen1* sequence and activity were first analysed by Leimer et al. in 1999 [11] who noted extensive conservation of protein primary structure. Gene transcripts are

Table 1
Zebrafish genes orthologous to those involved in Alzheimer's disease.

	Zebrafish orthologue	Accession number	Zebrafish references
Loci for dominant mutations in FAD			
Amyloid Precursor Protein (APP)	*appa*	NM_131564	[9,10]
	appb	NM_152886	
Presenilin 1 (PSEN1)	*psen1*	NM_131024	[11–15]
Presenilin 2 (PSEN2)	*psen2*	NM_131514	[12,15,16]
Loci associated with risk for AD[a]			
Apolipoprotein E (APOE)	*apoeb*	NM_131098	
Microtubule-Associated Protein Tau (MAPT)	*mapta*	N/A	
	maptb	N/A	
Clusterin (CLU)	*Clu*	NM_200802	
Phosphatidylinositol Binding Clathrin Assembly Protein (PICALM)	*picalm*	NM_200927	
Complement Component (3b/4b) Receptor 1 (CR1)	*Unknown*		
Loci for γ-secretase complex members			
Nicastrin (NCT)	*ncstn*	NM_001009556	[17]
Anterior Pharynx Defective 1 Homolog A (APH1a)	*unknown*		
Anterior Pharynx Defective 1 Homolog B (APH1b)	*aph1b*	NM_200115	[18]
Presenilin Enhancer 2 (PSENEN)	*psenen*	NM_205576	[18,19]

[a] A large number of additional associated genes have been reported and are reviewed in Rademakers and Rovelet-Lecrux, 2009.

apparently present in all cells at all developmental times examined. When zebrafish Psen1 protein expression was driven at high levels in cultured human HEK293 cells the zebrafish protein (as expected) displaced human PSEN1 from γ-secretase complexes indicating sufficient structural conservation to interact with other complex components. However, the C-terminal fragment (CTF) of zebrafish Psen1 generated by the endoproteolysis of the holoprotein (required to activate γ-secretase activity [62]) is sufficiently different in size that it can be distinguished from the CTF of human PSEN1 on western blots. Also, zebrafish Psen1 possesses sufficient primary structural differences from human PSEN1 that its γ-secretase cleavage of APP was abnormal leading to increased production of $A\beta_{42}$ relative to production of the less aggregation-prone 40 amino acid residue form of Aβ ($A\beta_{40}$). These authors also demonstrated that mutation of one of the two critical catalytic aspartate residues in zebrafish Psen1 could abolish its γ-secretase activity.

As expected from the expression of the *psen1* transcript, we showed that Psen1 protein can be observed from the earliest stages of development [14]. Interestingly, this did not appear to be the case for zebrafish *psen2* that we identified in 2002 [16]. We detected the ubiquitous presence of *psen2* transcripts at all stages but an antibody raised against an oligopeptide corresponding to N-terminal fragment (NTF) sequence from Psen2 protein only revealed higher expression of Psen2 after 6 hpf [16]. However, there is evidence from humans [63,64] and from our own unpublished work of alternative splicing and phosphorylation of PSEN2 orthologous proteins so we presume that species of zebrafish Psen2 protein do, in fact, exist at the earliest developmental stages in this organism.

The essential role of presenilin activity in Notch signalling during development and the resorption of dead embryos that occurs in mice has hindered the analysis of the roles of presenilin during vertebrate development. Resorption is not a problem in zebrafish so we have been keen to exploit morpholino antisense technology to block the activity of the zebrafish *psen1* and *psen2* genes. In mice, loss of *Psen1* activity is lethal during development and $Psen1^{-/-}$ embryos show a similar phenotype to those lacking mouse *Notch1* activity [65,66]. In contrast, mouse $Psen2^{-/-}$ embryos are viable with no obvious developmental defects [67,68] (although adults do display haemorrhaging and histological changes in lung tissue [69]. However, mice lacking both Psen1 and Psen2 activity have a more severe phenotype and loss of γ-secretase activity than when only Psen1 activity is absent [67]. When we blocked *psen1* translation in zebrafish we observed similarly irregular delineation of somites to that seen in $Psen1^{-/-}$ mouse embryos and the cyclic expression of the *her1* gene that controls somite formation was disturbed in the presomitic mesoderm [13]. Interestingly, blockage of translation of zebrafish *psen2* transcripts also produces significant changes in embryo development including expansion of brain ventricles at 48 hpf and decreased trunk neural crest formation with increased numbers of neurons at 24 hpf. The latter two defects at least can be attributed to decreased Notch signalling indicating that Psen2 in zebrafish plays a more prominent role in Notch signalling than its mammalian orthologue [15]. Indeed, in unpublished work, van Tijn et al. [70] have shown that zebrafish homozygous for null mutations in *psen1* are, nevertheless, viable and fertile indicating that *psen2* activity can satisfy all essential *psen1* functions, at least in a laboratory setting.

The viable homozygous null *psen1* mutant zebrafish of van Tijn et al. [70] show decreased cell proliferation and *de novo* neurogenesis. Such fish also present an opportunity for *in vivo* analysis of the effects on *psen1* function of mutations causing Alzheimer's disease [70,71]. Since presenilin proteins are ubiquitously expressed, simple ubiquitous transgenic expression of mutated *psen1* genes in fish lacking endogenous *psen1* activity can allow analysis of their function *in vivo*.

The ability to analyse the function of genes involved in FAD *in vivo* is important since gene regulatory and functional data from cultured cells and *in vitro* assays is sometimes not consistent with what occurs in the whole organism. This may be due to genetic and epigenetic changes that occur in cultured cells during successive passages and/or immortalisation. Also, cultured cells exist in a highly abnormal environment without the normal three-dimensional context of cellular contact and signalling. This may explain, to some extent, differences that we have observed while analysing the function of presenilins in zebrafish embryos compared to what has been observed from mammalian cell culture. For example, when we overexpressed Psen2 protein in zebrafish embryos at relatively high levels by mRNA injection we did not see a decrease in Psen1 levels [13] whereas transfection of constructs expressing presenilin proteins into cultured mammalian cells is known to reduce the levels of endogenous presenilin proteins [72].

A fertilized zebrafish egg represents a macroscopic single cell in which the activity of one or multiple endogenous genes/proteins can be subtly manipulated to obtain a phenotypic or biochemical readout. We attempted to model the molecular effects of mutations that alter human *PSEN1* splicing to cause FAD by altering the splicing of zebrafish *psen1* using injected morpholino antisense oligonucleotides. Mutations causing increased levels of a *PSEN1* transcript isoform lacking exon 8 or that cause loss of exon 9 can cause a form of FAD with a peculiar histology of plaques lacking neuritic cores and showing a "cotton wool" morphology [73,74]. Unfortunately, when morpholinos binding over the splice acceptor sites of zebrafish exons cognate to human exon 8 or exon 9 were injected we did not observe the desired exon loss from transcripts. Instead we saw inclusion of cognate introns 7 or 8, respectively, in a proportion of transcripts causing truncation of the open reading frames after exons 7 or 8. Interestingly this produced very strong dominant negative effects in the case of truncation after exon 7 sequence but not for truncation after exon 8 (which is very similar to a normal Psen1 NTF). The dominant negative effects included cross-inhibition of *psen2* activity [14]. Injection of mRNAs encoding Psen1 proteins truncated after exon 7 or exon 8 sequences produced similar results ([14] and unpublished data). Tests with further morpholinos and mRNAs have shown that transcripts producing dominant negative truncated proteins are those that code for part or all of sequence cognate to human PSEN1 exons 6 and 7 but not exon 8. (Curiously one of two nonsense mutations in zebrafish *psen1* currently under analysis in the laboratory of Dana Jongejan-Zivkovic falls in this region but there appears to be no dominant negative effect probably due to nonsense mediated decay of the transcript before it can be translated into a truncated protein. (Jongejan-Zivkovic personal communication). The discovery of potently dominant negative forms of truncated presenilin may be important since it has been suggested that a unifying feature of all the 180+ mutations in human *PSEN1* may be that they are hypomorphic with respect to γ-secretase activity [75]. It is possible that the truncated forms of numerous proteins that are seen to accumulate in the brains of LOAD individuals may represent a failure of RNA quality surveillance mechanisms such as nonsense mediated decay [76]. This, combined with increased rates of aberrant splicing, etc. seen in aged cells [77] may allow accumulation of dominant negative truncated forms of presenilin in ageing brains that could suppress γ-secretase activity and thus contribute to LOAD.

A zebrafish model of FAD or LOAD would be very useful for screening for drugs to prevent or ameliorate these diseases. However, even embryo development in non-transgenic zebrafish can provide assays for the actions of drugs relevant to Alzheimer's disease. The drug N-[N-(3,5-difluorophenacetyl)-l-alanyl]-S-phenylglycine t-butyl ester (DAPT, [78]) is now commonly used to inhibit γ-secretase activity in zebrafish [79]. Unfortunately, it is commonly and incorrectly assumed to be blocking only Notch signalling which, while very important, is not the only influential signalling pathway facilitated by γ-secretase. For example, as noted above, Joshi et al. [10] showed that inhibition of *appb* activity causes aberrant convergence-extension movements although it has not been shown that γ-secretase activity is required for this and treatment with DAPT [79,15] or simultaneous inhibition of translation of both Psen1 and Psen2 by morpholino injection [15] does not produce this effect. We have previously shown that numbers of

a particular spinal cord cell type, the Dorsal Longitudinal Ascending (DoLA) interneuron, appear dependent upon Psen2 but not Psen1 activity [14,15]. We used this as an assay for Psen2 activity to show that truncated Psen1 could suppress Psen2 activity [14]. Finally, in a publication from the Xia laboratory, Yang et al. [80] used zebrafish embryos to observe the differential effects on development of drugs inhibiting primarily the APP or NOTCH cleavage activities of γ-secretase. Thus, zebrafish embryos can provide a valuable tool for discovery of additional compounds that show differential inhibitory effects on γ-secretase. The Xia laboratory has also used injection of different combinations of morpholino antisense oligonucleotides to analyse the effects of inhibiting translation of additional protein components of γ-secretase complexes such as *aph1b* (orthologous to human *APH1B*) and psenen (orthologous to human *PSENEN* and previously named *PEN2*). In this way they demonstrated that zebrafish Psenen interacts with NF-kappaB [19] and the p53-dependent apoptosis pathway [18].

4. Concluding remarks

Despite many years of intensive research uncertainty still surrounds the fundamental mechanisms underlying AD pathology. There is considerable doubt about the relevance of existing transgenic mouse models of the disease. Our lack of understanding has slowed the development of therapies to treat or prevent AD. The vertebrate genome and neurobiology of zebrafish and our rapidly expanding ability to manipulate gene activity in this organism offer alternative pathways in which to probe the mysteries of AD pathology. In particular, zebrafish are well suited to biochemically-based approaches whereby gene activities are manipulated transiently and then the consequences examined in an otherwise normal cellular environment. As genome-wide association studies reveal additional genes involved with development of AD pathology these biochemically-based approaches can rapidly contribute to our understanding of the relevance of these genes while reducing the chance of being misled by experimental results from tissue culture systems that may not reflect *in vivo* reality.

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