Review TRENDS in Genetics Vol.22 No.5 May 2006

A decade of modeling Alzheimer's disease in transgenic mice

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It has been over a decade since the first Alzheimer's disease (AD) transgenic mouse models were reported. These models have enabled dramatic advances in our understanding of the pathogenic mechanism in AD and of potential therapeutic approaches to tackling the inexorable clinical progression of the disease. In this article, we discuss the current status of AD mouse models and focus on recent work that has examined the development of the neuropathological lesions observed in AD (plaques and tangles). The relationship between these lesions, neurodegeneration and development of the clinical syndrome will be explored.

Introdution

Alzheimer's disease (AD) is characterized clinically by progressive memory loss that leads eventually to dementia. The neuropathology of AD is characterized by neuronal and synaptic loss, and by the development of two lesions; the extracellular senile plaque, which is composed mostly of amyloid formed from the amyloid β (Aβ) peptide, and the intraneuronal neurofibrillary tangle (NFT), which is composed of hyperphosphorylated forms of the microtubule associated protein tau (MAPT) [1]. Several genes have been implicated in Alzheimer's disease in humans, most notably, those encoding amlyoid βA4 precursor protein (APP) and presenilin 1 (PSEN1) presenilin 2 (PSEN2) (Box 1). A mouse model that recapitulates all aspects of AD has not yet been produced, this probably reflects the limitations of using a rodent system to reproduce a human disease process that takes several decades and which primarily involves higher cognitive function. Nonetheless, there are transgenic mouse lines that offer robust and relatively faithful reproductions of a subset of AD features.

Transgenic mice that reproduce amyloid deposition – Aβ plaques and CAA

The first mouse models that developed amyloid plaque pathology were generated by expressing human APP containing mutations associated with early-onset AD. Games and colleagues [2] published the first transgenic mouse (PDAPP) that developed amyloid plaque pathology. PDAPP mice over-express a minigene construct encoding APPV717F and develop robust amyloid plaque pathology by 6–9 months of age [2]. Ashe *et al.* [3] published the Tg2576 model, which over-expresses a human APP cDNA transgene with the K670M/N671L double mutation (APPswe). The Tg2576 model developed amyloid plaque pathology in an age-dependent manner and was also shown to have correlative memory deficits by Morris water-maze testing. Additional lines of mice expressing mutant human APP transgenes have since been reported that develop similar amyloid pathology and cognitive deficits [4–6]. Crossing APP mutant mice with mice expressing mutant PSEN1 transgenes (PSAPP mice) dramatically accelerates amyloid deposition [7–9] (Figure 1) owing to the increase in Aβ42 production mediated by mutations in *PSEN1* [10,11]. The pathology in these APP and PSAPP transgenic mice includes diffuse amyloid deposits and dense cored (fibrillar) plaques that resemble the senile plaques in human AD [2,3,8,9]. The cored plaques in the transgenic mice are surrounded by dystrophic neurites and are associated with increased gliosis [12,13]. Despite the robust amyloid deposition observed in APP and PSAPP transgenic mice, none of these models develops widespread neuronal loss [12,14]. MAPT pathology (tangles and neuropil threads) is also absent in mice producing APP and PSAPP alone, although phospho-MAPT species accumulate in dystrophic neurites surrounding cored amyloid plaques [2,3,9].

Multiple lines of APP and PSAPP mice develop age-dependent amyloid pathology. However, there are significant differences in the nature and location of the deposits that largely reflect the APP mutation expressed in each line and the resultant Aβ42:Aβ40 ratio. In Tg2576, the APPswe double mutation increases the production of both Aβ40 and Aβ42, and most of the amyloid load consists of dense cored plaques with relatively few diffuse deposits [3]. By contrast, in PDAPP mice the APPV717F mutation selectively increases Aβ42, producing a greater proportion of diffuse plaques [2]. The extent of congophilic amyloid angiopathy (CAA) also differs between these two models [15]; abundant CAA was observed in Tg2576 but this was largely absent in the brains of PDAPP mice [15].

To further investigate the amyloidogenic properties of different Aβ isoforms and of the impact of altering the ratio of Aβ42 to Aβ40 *in vivo*, several transgenic models have recently been produced that either produce individual Aβ peptides using a fusion transgene strategy [16] or express APP mutations, such as the Dutch (E693Q) and Iowa mutations (D694N) associated with vascular deposition of amyloid and cerebral hemorrhage [17–20]. APP_{Dutch} mice expressing APP_{E693Q} develop progressive

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Available online 29 March 2006

 0168-9525/$ - see front matter doi:10.1016/j.tig.2006.03.007

Box 1. Genes associated with Alzheimer's disease

APP

APP maps to chromosome 21q21.3–21q22.05. Processing of APP by β and γ-secreatse generates Aβ; mutations in APP either increase the total Aβ or selectively increase Aβ42 levels. To date, 20 mutations in *APP* have been linked to the development of AD with age of onset from 45 to 65 years. Duplications of the APP locus have been recently described to cause early onset AD with CAA [74]. APP mutations represent <1% of all AD cases.

PSEN1

PSEN1 maps to chromosome 14q24.3. More than 150 reported mutations throughout *PSEN1* cause familial early onset AD. PSEN1 mutations account for ~8% of familial AD cases, with an age-of-onset of 28–50 years. Presenilins are part of the γ-secretase complex that processes APP; PS mutations generally selectively increase Aβ42 levels.

PSEN2

PSEN2 maps to chromosome 1q31-q42. *PSEN2* is a homolog of *PSEN1*. To date 20 mutations have been described. Mutations in *PSEN2* cause a small percentage (~1%) of familial AD.
Together mutations in *PSEN1*, *PSEN2* and *APP* account for >90% of familial autosomal dominant AD.

APOE

APOE maps to chromosome19q13.2. The E4 allele increases susceptibility to late-onset AD, with low penetrance but a high incidence. The E2 allele confers protection against AD.

MAPT

MAPT maps to chromosome 17q21.1. Although mutations in *MAPT* do not result in AD, they can cause the related dementia, FTDP-17 (frontotemporal dementia with parkinsonism linked to chromosome 17). More than 30 MAPT mutations have been identified. MAPT is a microtubule-associated protein and identification of mutations demonstrated that tau dysfunction alone is sufficient to cause neurofibrillary pathology and dementia.

For more information on mutations in AD, see the Alzheimer's research forum (http://www.alzforum.org/) and the Alzheimer disease and frontotemporal dementia mutation database (http://www.molgen.ua.ac.be/ADMutations/default.cfm?MT=0andML=0andPage=Home) maintained by Marc Cruts and Rosa Rademakers).

CAA that is first seen in leptomeningeal vessels and predominantly consists of Aβ40 [18]. Only occasional diffuse Aβ deposits were detected in the parenchyma, compact deposits were absent. Crossing the APP_{Dutch} mice with mice expressing the mutant $PSEN1_{G348A}$ favors Aβ42 production, thus changing the Aβ42:Aβ40 ratio. This shifts amyloid pathology from the vasculature to the parenchyma, resulting in the development of both fibrillar and large, diffuse amyloid deposits [18].

Although the ratio of Aβ42 to Aβ40 influences the nature and location of Aβ deposition, traditional transgenic studies have not fully determined which Aβ species are responsible for initiating or seeding amyloid deposition in either the parenchyma or vasculature. To address this, mice were developed that express Aβ1-40 or Aβ1-42 in the absence of human APP over-expression by fusing Aβ42 and Aβ40 peptide sequences to the C-terminal end of the BRI protein [also known as integral membrane protein 2B (ITM2B)] [16]. Mutations in *BRI* are associated with familial British and Danish dementias [21,22]. Cleavage of the fused protein in BRI–Aβ40 and BRI–Aβ42 mice

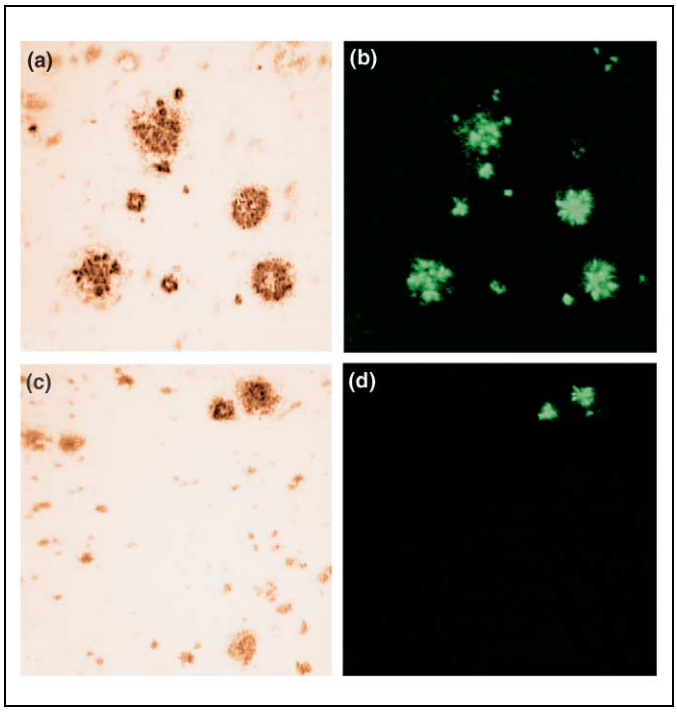

Figure 1. Mutant *PSEN1* accelerates amyloid deposition in mutant APP (Tg2576) mice. Representative sections from the entorhinal and piriform cortex of 17-month-old aged-matched PSAPP mice, expressing **(a)** mutant *PSEN1* and **(b)** mutant *APP* transgenes, and Tg2576 transgenic mice expressing **(c,d)** mutant *APP*, immunostained with a (a,c) pan- Aβ antibody or stained with (b,d) Thio-S Tg2576 mice develop plaque pathology from ~9-12 months of age. The addition of a mutant *PSEN1* transgene markedly accelerates the amyloid pathology in Tg2576 mice, increasing both diffuse amyloid and also the number of Thio-S positive (fibrillar) plaques (40× magnification).

results in the efficient production and secretion of Aβ40 and Aβ42, respectively. BRI–Aβ42 mice accumulate insoluble Aβ1-42 and develop compact amyloid plaques, diffuse Aβ deposits and extensive CAA with age [16] (Figure 2); by contrast, BRI–Aβ40 mice do not develop overt amyloid pathology at any age. Therefore, Aβ42 seems to be required to 'seed' both parenchymal and vascular deposits. Although Aβ42 is required to seed amyloid deposition, altering the Aβ42:Aβ40 ratio by increasing Aβ40 production favors amyloid deposition in the vasculature. The reason why the site of amyloid deposition is influenced by the Aβ42:Aβ40 ratio remains uncertain. Apolipoprotein E (ApoE) status also alters amyloid deposition in APP transgenic mice; ApoE4 promotes CAA over parenchymal deposition [15,23,24] and removing endogenous murine ApoE in mice expressing mutant APP reduces fibrillar plaque load and CAA [15,25,26]. As a result, it is clear that multiple factors (e.g. the Aβ42:Aβ40 ratio and ApoE status) contribute to the overall pattern of amyloid deposition in human AD.

Aβ and memory deficits in APP transgenic mice – the rise of the oligomer

Multiple lines of mutant APP and PSAPP transgenic have been shown to develop cognitive deficits in a variety of behavioral test paradigms, most commonly the Morris water maze [3,27–30]. To examine the relationship between amyloid deposition and memory function, Westerman *et al.* [27] analyzed the age-dependent memory

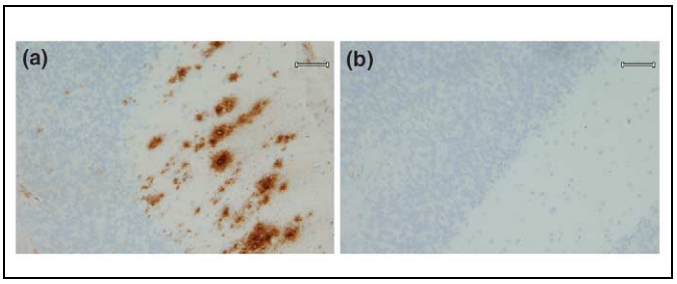

Figure 2. Aβ42 but not Aβ40 is necessary for pathology in transgenic mice. Mice engineered to express individual Aβ peptides (Aβ42 or Aβ40), through expression of a fusion transgene (BRI-Aβ), have elevated Aβ42 or Aβ40 levels in plasma and brain tissue. However, only Aβ42 mice develop compact and diffuse amyloid pathology. **(a)** Cerebellar sections from 14 month old BRI-Aβ42 mice **(b)** and BRI-Aβ40 mice immunostained with a pan-Aβ antibody. Cored and diffuse Aβ plaques were detected in the molecular and granule cell layers of the cerebellum in BRI-Aβ42 mice. No amyloid pathology was observed at any age in the brains of BRI-β40 mice. The scale bar is 60μm.

loss in Tg2576 in the Morris water maze. Spatial reference memory was shown to decline progressively from 6 months of age, corresponding with the development of detergent-insoluble aggregated Aβ. Crossing Tg2576 mice with mutant PSEN1 mice, which accelerates the rate at which insoluble Aβ is formed, also resulted in an earlier onset of memory decline in the double transgenic offspring [27]. These observations were consistent with Morris water maze studies in other APP mouse lines (PDAPP, TgCRND8 and PSAPP) that reported a correlation between deficits in spatial reference memory and increasing levels of insoluble Aβ aggregates [28,29,31]. However, analysis of Tg2576 mice over a broader age range (4–22 months) [27] failed to show any overall correlation between memory function and levels of insoluble Aβ, although a correlation was observed within individual age groups. These observations were interpreted as indicating that the level of insoluble Aβ is a surrogate measure for one or more small soluble Aβ assemblies that cause memory dysfunction in Tg2576 [27]. A role for soluble Aβ assemblies in memory dysfunction was further suggested by anti-Aβ immunization studies in APP and PSAPP mouse models. These studies demonstrated that either active immunization with Aβ or passive immunization with anti-Aβ antibodies could fully reverse memory deficits in APP and PSAPP mice, but resulted in minimal clearance of pre-existing amyloid plaque pathology [29,30,32,33].

Several studies have focused on identifying small Aβ assemblies that might underlie the memory deficits in Tg2576 and other mutant APP mouse models [34–37]. Walsh *et al.* [35] identified a population of stable Aβ oligomers, predominantly trimers, in the media of APP-transfected cells that produced deficits in long-term potentiation in rats *in vivo*, and further studies showed that the same type of oligomers were sufficient to induce memory deficits when injected into the brains of rats [36]. The memory deficits induced by Aβ oligomers were dissociated from any signs of neurodegeneration in the treated rats indicating that, as in Tg2576, memory loss was probably caused by reversible neuronal dysfunction. This suggests that early cognitive decline in pre-clinical AD might also reflect the impact of small oligomeric Aβ aggregates on neuronal function and might therefore be reversible. Recently a study by Lesne *et al.* [37] has gone further, by identifying one specific 56-kDa oligomeric Aβ species that consistently correlates with memory function in Tg2576 and also disrupts cognitive function in rats. However, despite these successes in understanding the molecular basis of the memory loss in the Tg2576 mouse model, existing mutant APP and PSAPP mice lack frank neurodegeneration and, as a result, these models probably only reproduce the earliest stages of cognitive decline in AD.

Neurodegeneration in APP and PSAPP mice

Unbiased, stereological neuronal counting has established that APP and PSAPP mouse models, despite extensive amyloid deposition in some lines, do not have widespread neuronal loss as seen in AD [12,14,38]. In the APP23 line, limited neuronal loss in the CA1 region of the hippocampus was associated with the localized impact of dense amyloid plaques [39–41]. Similar localized neuronal loss around thioflavin S (Thio-S)-positive amyloid plaques has also been reported in double transgenic PSAPP mice and in AD patients [42]. However, neurodegeneration caused by Thio-S-positive plaques is thought to account for only ~4% of the neuronal loss in the AD brain [42].

By contrast, evidence for progressive synaptic degeneration and dysfunction has been reported in multiple lines of APP and PSAPP mice, although marked variability is observed between different models. No evidence of synaptic degeneration, as indicated by staining for numerous pre-synaptic markers, has been reported in Tg2576 or PSAPP lines before robust amyloid plaque deposition (<12 months in Tg2576) [12,38,43]. However, recent studies have shown that in older Tg2576 mice (21–25 months) synaptophysin immunoreactivity is reduced only around dense cored amyloid plaques and this is associated with electrophysiological changes indicating abnormalities in synaptic function [44,45]. In addition, 3D multi-photon microscopy has been used to demonstrate altered neurite trajectory, dendritic spine loss and thinning of dendrites in a zone around the dense cored plaques in Tg2576 and PSAPP mice [44,46]. One of these studies also reported dendritic breakage and axonal abnormalities around amyloid plaques in PSAPP double transgenic mice (4–7 months) [46]. Loss of corresponding pre-synaptic markers also implied that the plaques impacted pre-synaptic boutons and post-synaptic structures [44,46]. Moreover, Aβ immunotherapy has also been shown to resolve at least some of the abnormalities in neuritic structure around plaques in Tg2576 [47]. These data imply that dense cored amyloid plaque formation is likely to contribute to the memory loss observed in AD and in older Tg2576 mice. However, as described earlier, plaque formation cannot completely account for the memory deficits in Tg2576 and it is likely that small Aβ assemblies causing reversible neuronal dysfunction underlie much of the memory loss in this model, especially in younger Tg2576 mice (<15 months) that lack widespread amyloid plaque pathology.

In addition to plaque-dependent synaptic loss reported in Tg2576 and PSAPP mice [44,46], there is evidence of

plaque-independent synaptotoxicity in other lines of APP mice [2,6,48]. Mucke *et al.* [6] reported a series of models expressing wild-type and mutant *APP* transgenes in which progressive decreases in the density of synaptophysin-immunoreactive presynaptic terminals was observed. The density of the pre-synaptic terminals was inversely correlated with the levels of Aβ produced in these lines but not with levels of APP produced or amyloid plaque load. The same group has also shown impairment in synaptic function in APP (V717F) mice through electrophysiological recording of evoked synaptic responses with >80% deficit in basal synaptic transmission by 8–10 months [48]. More recently, Buttini *et al.* [49] showed that active or passive Aβ immunotherapy can prevent the development of age-dependent synaptic deficits in the PDAPP mice [2]. This study demonstrated that plaque-independent synapse loss is also linked to the accumulation of toxic Aβ species.

It is currently uncertain how the synaptic degeneration observed in the APP and PSAPP relates to the process of neurodegeneration in human AD, and whether this synaptic loss could be a precursor of neuronal cell death given longer time periods than are possible in a mouse. However, it is clear, from the absence of neuronal loss in APP and PSAPP models, that physiological Aβ accumulation and amyloid plaque formation do not cause rapid neuronal cell death *in vivo*. This in turn suggests that Aβ species are unlikely to cause widespread neuronal loss in the absence of secondary pathological events in human patients. This is consistent with human neuropathological studies that show no correlation between overall levels of amyloid deposition and clinical progression of AD. One obvious possible explanation is that the glaring absence of NFT formation in the APP and PSAPP models is linked to the lack of neuronal cell loss.

Transgenic mice that reproduce MAPT pathology – a link to neuronal cell death

To examine the role of MAPT in AD and other tauopathies, mouse models have been developed, through expression of *MAPT* mutant and wild-type transgenes, that recapitulate most of the features of human neurofibrillary pathology (NFT and neuropil threads) [50–54] (see Box 2 for summaries of individual models). Transgenic mouse lines that develop robust neurofibrillary pathology, with both the structural and biochemical features of human NFT, also show significant neuronal loss in affected brain regions [50–54]. An important recent example is the

Box 2. Mouse models of Alzheimer's disease

This is not an exhaustive list of all AD transgenic models available but serves to highlight those models that are either in widespread use or have significantly advanced our understanding of AD pathogenesis. There are also many invertebrate models both in *Drosophila melanogaster* and *Caenorhabditis elegans* that have proved invaluable to AD research.

PDAPP: First mutant APP transgenic model with robust plaque pathology [2]. Mice express a human *APP* cDNA with the Indiana mutation (APP_{V717F}). Plaque pathology begins between 6–9 months in hemizygous PDAPP mice. There is synapse loss but no overt cell loss and no NFT pathology is observed. This model has been used widely in vaccination therapy strategies.

Tg2576: Mice express mutant APP_{SWE} under control of the hamster prion promoter [3]. Plaque pathology is observed from 9 months of age. These mice have cognitive deficits but no cell loss or NFT pathology. It is one of the most widely used transgenic models.

APP23: Mice express mutant APP_{SWE} under control of the Thy1 promoter. Prominent cerebrovascular amyloid, amyloid deposits are observed from 6 months of age and some hippocampal neuronal loss is associated with amyloid plaque formation [39–41].

TgCRND8: Mice express multiple APP mutations (Swedish plus Indiana). Cognitive deficits coincide with rapid extracellular plaque development at ~3 months of age. The cognitive deficits can be reversed by Aβ vaccination therapy [29].

$PSEN1_{M146V}$ or $PSEN1_{M146L}$ (lines 6.2 and 8.9, respectively): These models were the first demonstration *in vivo* that mutant *PSEN1* selectively elevates Aβ42. No overt plaque pathology is observed [7].

PSAPP (Tg2576×$PSEN1_{M146L}$ [9], PSEN1-A246E+APP_{SWE} [8]): Bigenic transgenic mice, addition of the mutant *PSEN1* transgene markedly accelerated amyloid pathology compared with singly transgenic mutant *APP* mice, demonstrating that the PSEN1-driven elevation of Aβ42 enhances plaque pathology.

APP_{Dutch}: Mice express *APP* with the Dutch mutation that causes hereditary cerebral hemorrhage with amyloidosis–Dutch type in humans. APP_{Dutch} mice develop severe congophilic amyloid angiopathy [18]. The addition of a mutant *PSEN1* transgene redistributes the amyloid pathology to the parenchyma indicating differing roles for Aβ40and Aβ42 in vascular and parenchymal amyloid pathology.

BRI-Aβ40 and BRI-Aβ42: Mice express individual Aβ isoforms without *APP* over-expression [16]. Only mice expressing Aβ42 develop senile plaques and CAA, whereas BRI-Aβ40 mice do not develop plaques, suggesting that Aβ42 is essential for plaque formation.

JNPL3: Mice express 4R0N *MAPT* with the P301L mutation [53]. This is the first transgenic model, with marked tangle pathology and cell loss, demonstrating that MAPT alone can cause cellular damage and loss. JNPL3 mice develop motor impairments with age owing to severe pathology and motor neuron loss in the spinal cord.

Tau_{P301S}: Transgenic mice expressing the shortest isoform of 4R *MAPT* with the P301S mutation [51]. Homozygous mice develop severe paraparesis at 5–6 months of age with widespread neurofibrillary pathology in the brain and spinal cord and neuronal loss in the spinal cord.

Tau_{V337M}: Low level synthesis of 4R MAPT with the V337M mutation (1/10 endogenous mouse MAPT) driven by the promoter of platelet-derived growth factor (PDGF) [75]. The development of neurofibrillary pathology in these mice suggests the nature of the MAPT rather than absolute MAPT intracellular concentrations drives pathology.

Tau_{R406W}: Mice expressing 4R human MAPT with the R406W mutation under control of the CAMKII promoter [76]. Mice develop MAPT inclusions in the forebrain from 18 months of age and have impaired associative memory.

rTg4510: Inducible *MAPT* transgenic mice using the TET-off system [50,77]. Abnormal MAPT pathology occurs from one month of age. Mice have progressive NFT pathology and severe cell loss. Cognitive deficits are evident from 2.5 months of age. Turning off the transgene improves cognitive performance but NFT pathology worsens.

Htau: Transgenic mice expressing human genomic MAPT only (mouse MAPT knocked-out) [78]. htau mice accumulate hyperphosphorylated MAPT from 6 months and develop Thio-S-positive NFT by the time they are 15 months old.

TAPP (Tg2576×JNPL3): Increased MAPT forebrain pathology in TAPP mice compared with JNPL3 suggesting mutant APP and/or Aβ can affect downstream MAPT pathology [63].

3×TgAD: Triple transgenic model expressing mutant APP_{SWE}, $MAPT_{P301L}$ on a $PSEN1_{M146V}$ 'knock-in' background (PSEN1-KI) [68,79]. Mice develop plaques from 6 months and MAPT pathology from the time they are 12 months old, strengthening the hypothesis that APP or Aβ can directly influence neurofibrillary pathology.

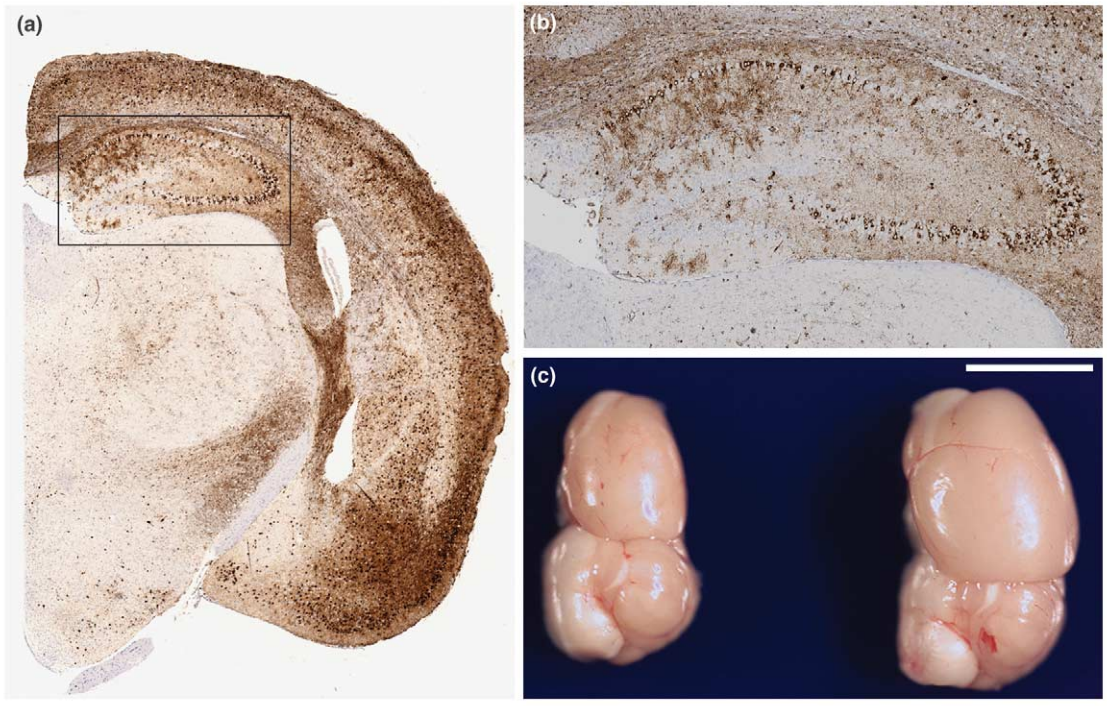

Figure 3. Neurofibrillary pathology is associated with neuronal loss and brain atrophy in inducible mutant MAPT transgenic mice (rTg4510). At 9 months of age there is widespread neurofibrillary pathology in rTg4510 mice in many forebrain structures, including the cortex, hippocampus, striatum and hypothalamus. Thy hypothalamus is highlighted in **(a)** and enlarged in **(b)**, to show the severe NFT pathology and massive loss of CA1 pyramidal neurons in the hippocampal formation. These sections were immunostained with the MAPT antibody, Ab39, which specifically labels mature NFT, and were counterstained with hematoxylin. rTg4510 mice have significant forebrain atrophy compared with non-transgenic age-matched control littermates, as shown in **(c)**, and have significant decreases in whole brain weight from an early age. Macroscopic photographs (c) show forebrain atrophy in a representative 10-month old Tg4510 mouse (left) compared with non-transgenic control (right). The images are at 4× and 20× magnification in (a) and (b), respectively; the scale bar in (c) is equal to 0.5 cm.

inducible rTg4510 model [50,55], which expresses MAPT with the FTDP-17 mutation P301L [56,57] (Figure 3). This mouse develops massive neurodegeneration in AD-relevant cortical and limbic structures to the point where global forebrain atrophy is observed and brain weight is significantly reduced. Furthermore, the progression of neurofibrillary pathology and neuronal loss is also correlated with the progression of deficits in spatial reference memory as assessed by the Morris water maze [50]. This and other *MAPT* transgenic mouse models have therefore shown that the development of neurofibrillary pathology and neurodegeneration are closely linked. The results in the MAPT mice are also consistent with the long established correlation between NFT pathology and the progression of memory deficits in AD [58].

Importantly however, analysis of the inducible rTg4510 mice demonstrated that the formation of NFT lesions could be dissociated from MAPT-induced neuronal loss and memory decline (Figure 3) [50,55]. In this model, onset of memory deficits (2.5–4 months) precedes the development of significant NFT pathology or neuronal loss (5.5 months). Furthermore, suppressing inducible transgene expression, with doxycycline treatment, after initial NFT formation has occurred (>4 months) did not halt the continuing increase in NFT numbers; treated and control mice had similar numbers of NFT, in cortex and hippocampus, after 80% transgene suppression for 6 weeks. By contrast, transgene suppression halted the loss of neurons and enabled at least partial recovery of spatial reference memory function. These findings suggested that NFT formation is not directly responsible for neurodegeneration and memory loss in rTg4510 and a toxic intermediate MAPT species is more likely to underlie these processes. This obviously parallels the finding that soluble oligomeric Aβ species, rather than insoluble amyloid, drives memory loss in APP mice [27,36]. In addition, the results imply that reversible neuronal dysfunction, as opposed to structural neurodegeneration, explains much of the early memory loss in rTg4510 and suggests that memory decline in early stage AD can also be partially reversible.

The mechanism of neuronal cell death in mouse models that develop NFT pathology is uncertain, but most of the data suggest that apoptosis is not the primary mechanism of neuronal cell death [51,59], and this is consistent with pathological findings in human tauopathies [60,61]. Recent studies in mice expressing mutant [53,59] and wild-type [62] MAPT transgenes have implicated axonal dysfunction and degeneration as an initiator of MAPT-induced neuronal loss. In particular, Zhang and colleagues [62] recently demonstrated that treatment with the microtubule-stabilizing drug paclitaxel restored deficits in fast axonal transport and reversed mild motor dysfunction in a transgenic model expressing the shortest wild-type MAPT isoform. In addition, in htau mice, which express a *PAC* transgene containing the entire human

MAPT gene and lack endogenous mouse *Mapt*, cortical neuronal loss is associated with evidence of incomplete, abnormal cell-cycle re-entry [54]. Despite these findings, it is clear that additional studies are required to determine how MAPT pathogenesis causes neuronal cell death in these models and in human tauopathy. In particular, it will be important to identify potentially toxic MAPT species and to determine whether loss of MAPT function has a role in the initiation of axonal dysfunction and neurodegeneration.

Transgenic mouse models that develop amyloid plaques and NFT

Given that mutations in *APP* and *PSEN1* cause AD with abundant NFT, it is unclear why mice expressing the mutant human *APP* and *PSEN1* transgenes fail to develop detectable NFTs. Therefore, it is important to understand the extent to which Aβ and MAPT pathologies interact during the development of AD.

Aggressive experimental approaches involving crosses of mutant APP, PSEN1 and MAPT mice have helped us to understand this interaction. Lewis and colleagues [63] crossed mutant APP and MAPT (JNPL3) mice, and demonstrated that the double-transgenic TAPP mice developed enhanced limbic neurofibrillary pathology, when compared with single transgenic littermates. Importantly, the enhanced NFT pathology in the TAPP mice was also associated with evidence of neuronal loss in the entorhinal cortex. More recent studies involving a similar cross of *APP* and *MAPT* transgenic mice yielded a similar enhancement in MAPT pathology [64,65]. Similarly, Gotz *et al.* [66] found that the intracranial administration of Aβ into mutant (P301L) MAPT mice resulted in the generation of NFTs within the amygdala. Results of these studies strongly suggested that Aβ accumulation accelerated the development of neurofibrillary lesions, although the exact mechanism of this interaction was unknown. Interestingly, cross breeding the mutant APP mice with transgenic mice over-expressing mutant synuclein (A53T) have also shown enhanced synuclein inclusion pathology, consistent with the hypothesis that Aβ accumulation is also linked to the Lewy body formation pathology commonly observed in AD patients [67].

Oddo and colleagues [68] extended these studies by generating a triple-transgenic model (3×Tg-AD), harboring $PSEN1_{M146V}$, APPswe and MAPT (P301L) transgenes. This model accumulates intraneuronal Aβ, and subequently forms amyloid plaques and MAPT lesions in an age-dependent fashion. The stage and extent of the MAPT pathology in this line has not yet been fully determined; however, it is clear that extracellular Aβ deposition precedes MAPT pathology by several months, suggesting that elevated Aβ enhances tauopathy development in this model. Consistent with this observation, mice expressing the *MAPT* (P301L) and mutant *PSEN1* transgenes did not develop MAPT pathology in the absence of APPswe overexpression and Aβ deposition. The 3×Tg-AD mice also develop age-dependent synaptic dysfunction, including long-term potentiation deficits, and memory deficits that correlate with the accumulation of intraneuronal Aβ [68,69]. An assessment of neuronal loss in these mice has not yet been reported.

In subsequent studies, Oddo *et al.* [70,71] found that injection of anti-Aβ antibodies, or antibodies specific for oligomeric forms of Aβ, into the brains of 3×Tg-AD mice led to the rapid clearance of accumulated Aβ deposition and early MAPT lesions in the cell body. The removal of both Aβ and MAPT lesions proceeded in a hierarchical, time-dependent manner; clearance of accumulated Aβ occurred before a reduction in the MAPT pathology. Furthermore, after clearance of the injected Aβ antibody, Aβ pathology re-emerged before the appearance of MAPT lesions. However, later stage hyperphosphorylated MAPT (antibody AT8 and AT180 positive) lesions were resistant to clearance by Aβ immunotherapy [70]. These results suggested the existence of reversible and irreversible stages of MAPT pathology. The nature of this shift from reversible to irreversible pathological stages is unclear, but one obvious possibility is that MAPT hyperphosphorylation is associated with aggregation into filaments that renders the lesion resistant to clearance. These data are highly reminiscent of the findings of Santa-Cruz *et al.* who showed that in the rTg4510 inducible model of tauopathy NFTs were stable and increased in number after MAPT (P301L) transgene suppression [50].

Studies in mice expressing *APP* and *MAPT* transgenes have provided clear evidence that Aβ and MAPT interact in the pathogenesis of AD. Moreover, the results clearly support the hypothesis that Aβ can accelerate, if not initiate, the formation of MAPT neurofibrillary lesions. However, the close link between NFT formation and neuronal loss observed in multiple MAPT transgenic lines, and in the TAPP double transgenic mice, strongly suggests that in AD the development of pathological MAPT species is a major pathway to neurodegeneration.

Concluding remarks – the relationship between Aβ, MAPT and neurodegeneration

A complete mouse model of AD has proven elusive, probably because of the short lifespan of the mouse and the nature of the relationship between Aβ accumulation and other pathological features that contribute to the complex phenotype of AD. In particular, based on results from APP and PSAPP mice, it seems likely that Aβ accumulation does not lead directly to neuronal cell death in AD but usually requires the initiation of a secondary process. This provides a simple explanation of why APP and PSAPP mice fail to develop frank neurodegeneration despite expressing mutant forms of APP and presenilins that cause early-onset AD in humans and despite the abundant amyloid deposition in many of these models [12,14,38]. In addition, this explanation is completely consistent with human neuropathological studies that show no correlation between total amyloid deposition and memory loss in AD [58].

By contrast, mice that develop robust MAPT neurofibrillary pathology almost inevitably develop widespread neurodegeneration, regardless of whether the neurofibrillary lesions are generated through expression of a mutant [50–53], wild-type *MAPT* transgenes [54] or overexpression of a relevant MAPT kinase [72]. These results

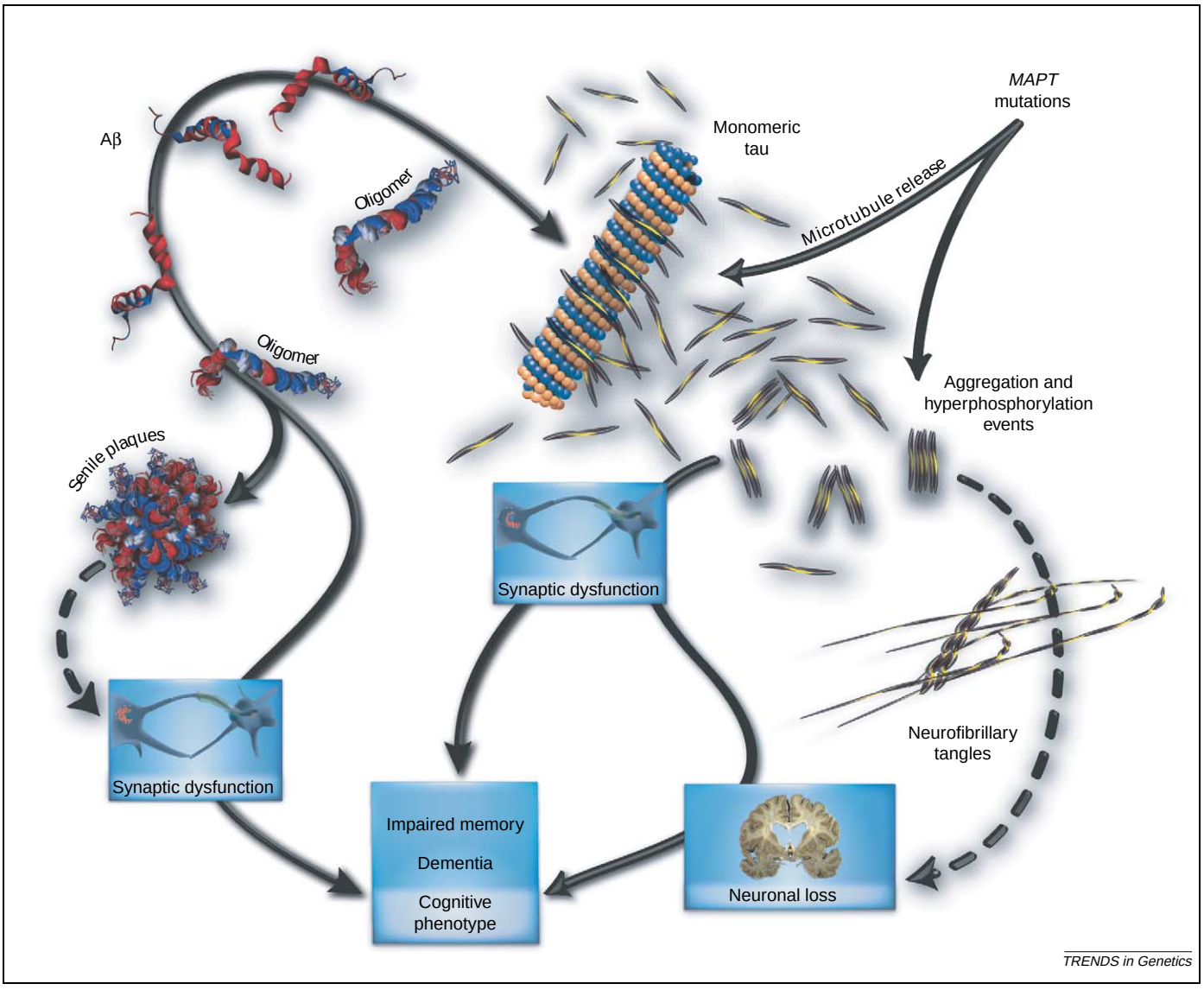

Figure 4. The interaction of Aβ and MAPT in AD pathogenesis. Transgenic mouse models have been used to examine the relationship between Aβ, MAPT and neurodegeneration in AD pathogenesis. In this proposed scheme, Aβ monomers can form soluble oligomeric species that cause synaptic dysfunction but do not lead directly to neuronal cell death [36]. Aβ oligomers also aggregate to form senile plaques, and these dense cored structures have been shown to cause synaptic degeneration directly [44,46]. In addition to their direct impact on synaptic activity, soluble Aβ oligomers can target MAPT pathogenesis, causing an acceleration of MAPT aggregation [63,71]. The effects of Aβ on MAPT are likely to be similar to the impact of FTDP-17-associated MAPT mutations causing an increase in unbound MAPT that is available for aggregation [73]. Soluble MAPT can aggregate to form NFT with associated hyperphosphorylation; however, the major MAPT toxic species are unlikely to be NFT but rather earlier stage aggregates or modified monomers, as shown in the rTg4510 mice [50]. These toxic MAPT species appear to cause reversible neuronal (perhaps synaptic) dysfunction initially; however, the accumulation of these pathogenic MAPT species leads to neuronal loss and permanent effects on cognitive phenotype [50].

from the transgenic mice clearly confirm the close relationship between MAPT and neurodegeneration and are consistent with human studies that have reported a correlation between NFT pathology and the clinical progression of AD [58]. However, results from TAPP ($MAPT_{P301L}$×APPswe) and the 3×Tg-AD mouse models support the hypothesis that Aβ accumulation can accelerate, if not initiate, the formation of neurofibrillary pathology [63,68,70]. Thus, the transgenic mouse data are consistent with a scheme for AD pathogenesis (Figure 4) in which the accumulation of aggregated Aβ oligomers accelerates the parallel process of age-dependent formation of MAPT pathology. However, it is the accumulation of toxic MAPT species (again likely to be early aggregates rather than NFT) or other secondary pathological events (e.g. α-synucleinopathy) that initiates neurodegeneration and, by extension, accounts for most of the clinical syndrome.

Acknowledgements

E.M. is supported by NIA RO1 AG022595–01 and the Mayo Foundation. M.H. is supported by NIA RO1 AG020216–01A2, NIA PO1 AG17216 and the Mayo Foundation.

References

1 Selkoe, D.J. (1991) The molecular pathology of Alzheimer's disease. *Neuron* 6, 487–498
2 Games, D. *et al*. (1995) Alzheimer-type neuropathology in transgenic mice overexpressing V717F β-amyloid precursor protein. *Nature* 373, 523–527
3 Hsiao, K. *et al*. (1996) Correlative memory deficits, Aβ elevation, and amyloid plaques in transgenic mice. *Science* 274, 99–102
4 Chishti, M.A. *et al*. (2001) Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant

form of amyloid precursor protein 695. *J. Biol. Chem.* 276, 21562–21570
5 Janus, C. *et al*. (2001) New developments in animal models of Alzheimer's disease. *Curr. Neurol. Neurosci. Rep.* 1, 451–457
6 Mucke, L. *et al*. (2000) High-level neuronal expression of Aβ 1-42 in wild-type human amyloid protein precursor transgenic mice: synaptotoxicity without plaque formation. *J. Neurosci.* 20, 4050–4058
7 Duff, K. *et al*. (1996) Increased amyloid-β42(43) in brains of mice expressing mutant presenilin 1. *Nature* 383, 710–713
8 Borchelt, D.R. *et al*. (1997) Accelerated amyloid deposition in the brains of transgenic mice coexpressing mutant presenilin 1 and amyloid precursor proteins. *Neuron* 19, 939–945
9 Holcomb, L. *et al*. (1998) Accelerated Alzheimer-type phenotype in transgenic mice carrying both mutant amyloid precursor protein and presenilin 1 transgenes. *Nat. Med.* 4, 97–100
10 Borchelt, D.R. *et al*. (1996) Familial Alzheimer's disease-linked presenilin 1 variants elevate Aβ1-42/1-40 ratio *in vitro* and *in vivo*. *Neuron* 17, 1005–1013
11 Citron, M. *et al*. (1997) Mutant presenilins of Alzheimer's disease increase production of 42-residue amyloid beta-protein in both transfected cells and transgenic mice. *Nat. Med.* 3, 67–72
12 Irizarry, M.C. *et al*. (1997) APPSw transgenic mice develop age-related Aβ deposits and neuropil abnormalities, but no neuronal loss in CA1. *J. Neuropathol. Exp. Neurol.* 56, 965–973
13 Frautschy, S.A. *et al*. (1998) Microglial response to amyloid plaques in APPsw transgenic mice. *Am. J. Pathol.* 152, 307–317
14 Irizarry, M.C. *et al*. (1997) Aβ deposition is associated with neuropil changes, but not with overt neuronal loss in the human amyloid precursor protein V717F (PDAPP) transgenic mouse. *J. Neurosci.* 17, 7053–7059
15 Fryer, J.D. *et al*. (2003) Apolipoprotein E markedly facilitates age-dependent cerebral amyloid angiopathy and spontaneous hemorrhage in amyloid precursor protein transgenic mice. *J. Neurosci.* 23, 7889–7896
16 McGowan, E. *et al*. (2005) Abeta42 is essential for parenchymal and vascular amyloid deposition in mice. *Neuron* 47, 191–199
17 Miao, J. *et al*. (2005) Cerebral microvascular amyloid β protein deposition induces vascular degeneration and neuroinflammation in transgenic mice expressing human vasculotropic mutant amyloid β precursor protein. *Am. J. Pathol.* 167, 505–515
18 Herzig, M.C. *et al*. (2004) Aβ is targeted to the vasculature in a mouse model of hereditary cerebral hemorrhage with amyloidosis. *Nat. Neurosci.* 7, 954–960
19 Davis, J. *et al*. (2004) Early-onset and robust cerebral microvascular accumulation of amyloid β-protein in transgenic mice expressing low levels of a vasculotropic dutch/iowa mutant form of amyloid β-protein precursor. *J. Biol. Chem.* 279, 20296–20306
20 Davis, J. *et al.* Deficient cerebral clearance of vasculotropic mutant Dutch/Iowa Double Aβ in human AβPP transgenic mice. *Neurobiol. Aging* (in press)
21 Vidal, R. *et al*. (1999) A stop-codon mutation in the BRI gene associated with familial British dementia. *Nature* 399, 776–781
22 Vidal, R. *et al*. (2000) A decamer duplication in the 3′ region of the BRI gene originates an amyloid peptide that is associated with dementia in a Danish kindred. *Proc. Natl. Acad. Sci. U. S. A.* 97, 4920–4925
23 Holtzman, D.M. *et al*. (2000) Apolipoprotein E facilitates neuritic and cerebrovascular plaque formation in an Alzheimer's disease model. *Ann. Neurol.* 47, 739–747
24 Fryer, J.D. *et al*. (2005) Human apolipoprotein e4 alters the Aβ 40:42 ratio and promotes the formation of cerebral amyloid angiopathy in an amyloid precursor protein transgenic model. *J. Neurosci.* 25, 2803–2810
25 Bales, K.R. *et al*. (1999) Apolipoprotein E is essential for amyloid deposition in the APP(V717F) transgenic mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 96, 15233–15238
26 Bales, K.R. *et al*. (1997) Lack of apolipoprotein E dramatically reduces amyloid beta-peptide deposition. *Nat. Genet.* 17, 263–264
27 Westerman, M.A. *et al*. (2002) The relationship between Aβ and memory in the Tg2576 mouse model of Alzheimer's disease. *J. Neurosci.* 22, 1858–1867
28 Chen, G. *et al*. (2000) A learning deficit related to age and β-amyloid plaques in a mouse model of Alzheimer's disease. *Nature* 408, 975–979
29 Janus, C. *et al*. (2000) Aβ peptide immunization reduces behavioural impairment and plaques in a model of Alzheimer's disease. *Nature* 408, 979–982
30 Morgan, D. *et al*. (2000) Aβ peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature* 408, 982–985
31 Gordon, M.N. *et al*. (2001) Correlation between cognitive deficits and Aβ deposits in transgenic $APP^{+}PS1$ mice. *Neurobiol. Aging* 22, 377–385
32 Kotilinek, L.A. *et al*. (2002) Reversible memory loss in a mouse transgenic model of Alzheimer's disease. *J. Neurosci.* 22, 6331–6335
33 Dodart, J.C. *et al*. (2002) Immunization reverses memory deficits without reducing brain Aβ burden in Alzheimer's disease model. *Nat. Neurosci.* 5, 452–457
34 Lambert, M.P. *et al*. (1998) Diffusible, nonfibrillar ligands derived from Aβ1-42 are potent central nervous system neurotoxins. *Proc. Natl. Acad. Sci. U. S. A.* 95, 6448–6453
35 Walsh, D.M. *et al*. (2002) Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*. *Nature* 416, 535–539
36 Cleary, J.P. *et al*. (2005) Natural oligomers of the amyloid-beta protein specifically disrupt cognitive function. *Nat. Neurosci.* 8, 79–84
37 Lesné, S. *et al.* A specific amyloid-β protein assembly in the brain impairs memory. *Nature* (in press)
38 Takeuchi, A. *et al*. (2000) Age-related amyloid beta deposition in transgenic mice overexpressing both Alzheimer mutant presenilin 1 and amyloid beta precursor protein Swedish mutant is not associated with global neuronal loss. *Am. J. Pathol.* 157, 331–339
39 Sturchler-Pierrat, C. *et al*. (1997) Two amyloid precursor protein transgenic mouse models with Alzheimer disease-like pathology. *Proc. Natl. Acad. Sci. U. S. A.* 94, 13287–13292
40 Calhoun, M.E. *et al*. (1998) Neuron loss in APP transgenic mice. *Nature* 395, 755–756
41 Calhoun, M.E. *et al*. (1999) Neuronal overexpression of mutant amyloid precursor protein results in prominent deposition of cerebrovascular amyloid. *Proc. Natl. Acad. Sci. U. S. A.* 96, 14088–14093
42 Urbanc, B. *et al*. (2002) Neurotoxic effects of thioflavin S-positive amyloid deposits in transgenic mice and Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 99, 13990–13995
43 Calon, F. *et al*. (2004) Docosahexaenoic acid protects from dendritic pathology in an Alzheimer's disease mouse model. *Neuron* 43, 633–645
44 Spires, T.L. *et al*. (2005) Dendritic spine abnormalities in amyloid precursor protein transgenic mice demonstrated by gene transfer and intravital multiphoton microscopy. *J. Neurosci.* 25, 7278–7287
45 Stern, E.A. *et al*. (2004) Cortical synaptic integration *in vivo* is disrupted by amyloid-beta plaques. *J. Neurosci.* 24, 4535–4540
46 Tsai, J. *et al*. (2004) Fibrillar amyloid deposition leads to local synaptic abnormalities and breakage of neuronal branches. *Nat. Neurosci.* 7, 1181–1183
47 Lombardo, J.A. *et al*. (2003) Amyloid-β antibody treatment leads to rapid normalization of plaque-induced neuritic alterations. *J. Neurosci.* 23, 10879–10883
48 Hsia, A.Y. *et al*. (1999) Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc. Natl. Acad. Sci. U. S. A.* 96, 3228–3233
49 Buttini, M. *et al*. (2005) β-amyloid immunotherapy prevents synaptic degeneration in a mouse model of Alzheimer's disease. *J. Neurosci.* 25, 9096–9101
50 Santa-Cruz, K. *et al*. (2005) Tau suppression in a neurodegenerative mouse model improves memory function. *Science* 309, 476–481
51 Allen, B. *et al*. (2002) Abundant tau filaments and nonapoptotic neurodegeneration in transgenic mice expressing human P301S tau protein. *J. Neurosci.* 22, 9340–9351
52 Lewis, J. *et al*. (2000) Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat. Genet.* 25, 402–405
53 Zhang, B. *et al*. (2004) Retarded axonal transport of R406W mutant tau in transgenic mice with a neurodegenerative tauopathy. *J. Neurosci.* 24, 4657–4667
54 Andorfer, C. *et al*. (2005) Cell-cycle re-entry and cell death in transgenic mice expressing nonmutant human tau Isoforms. *J. Neurosci.* 25, 5446–5454

55 Ramsden, M. *et al*. (2005) Age-dependent neurofibrillary tangle formation, neuron loss, and memory impairment in a mouse model of human tauopathy (P301L). *J. Neurosci.* 25, 10637–10647
56 Hutton, M. *et al*. (1998) Association of missense and 5′-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 393, 702–705
57 Poorkaj, P. *et al*. (1998) Tau is a candidate gene for chromosome 17 frontotemporal dementia. *Ann. Neurol.* 43, 815–825
58 Braak, H. and Braak, E. (1991) Neuropathological stageing of Alzheimer-related changes. *Acta Neuropathol. (Berl.)* 82, 239–259
59 Zehr, C. *et al*. (2004) Apoptosis in oligodendrocytes is associated with axonal degeneration in P301L tau mice. *Neurobiol. Dis.* 15, 553–562
60 Migheli, A. *et al*. (1994) A study of apoptosis in normal and pathologic nervous tissue after *in situ* end-labeling of DNA strand breaks. *J. Neuropathol. Exp. Neurol.* 53, 606–616
61 Ferrer, I. *et al*. (2001) Phosphorylated map kinase (ERK1, ERK2) expression is associated with early tau deposition in neurones and glial cells, but not with increased nuclear DNA vulnerability and cell death, in Alzheimer disease, Pick's disease, progressive supranuclear palsy and corticobasal degeneration. *Brain Pathol.* 11, 144–158
62 Zhang, B. *et al*. (2005) Microtubule-binding drugs offset tau sequestration by stabilizing microtubules and reversing fast axonal transport deficits in a tauopathy model. *Proc. Natl. Acad. Sci. U. S. A.* 102, 227–231
63 Lewis, J. *et al*. (2001) Enhanced neurofibrillary degeneration in tr